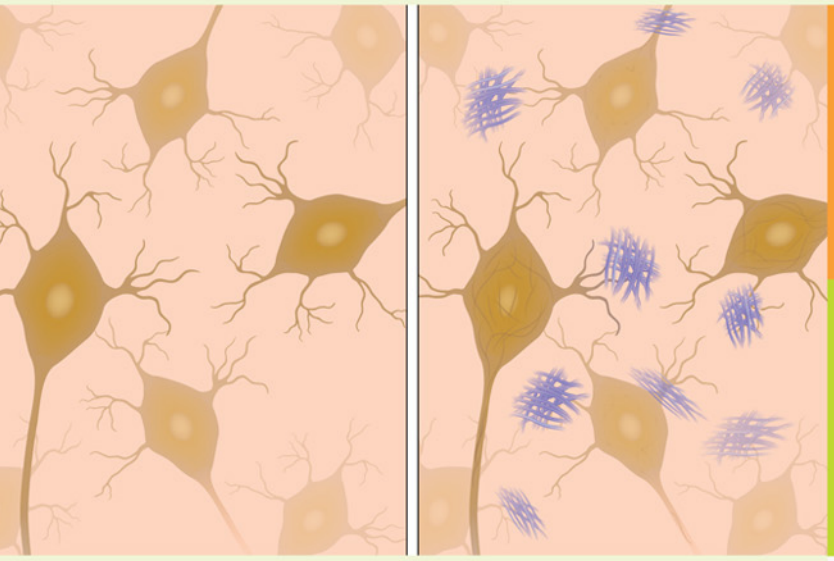


Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults




Edited by

Ronald Ross Watson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Preface

This book and its focus are broadly divided into four sections. The first, **Part 1: Non-Nutritional Components in Diet and Supplements, Nutraceuticals and their Role in Health Promotion in the Mature Adult**, looks at constituents of foods and herbs in common problems of aging. For example, *Ferreira, Palmer, Gendron and McKenna* evaluate the many plants unique to the New World for their benefits as anti-aging dietary agents. *Rani, Vissavajjhala and Reddy* review nutrients as potential therapies in one of the major and rapidly growing plagues of seniors in the 21st century, Alzheimer's Disease. *Mathai, Tonse, Kalekhan, Colin, Prabhu, Rao and Baliga* continue the focus on anti-aging by reviewing the actions of amla and its correlation with ethno-medicinal claims, asking if current research supports historical uses and information. *Candow and McLeod* begin chapters relating to specific dietary supplements by looking at the literature supporting the use of creatine in *Sarcopenia* treatment and prevention. *Baliga, Prabhudev, Thimothy, Thilakchand and Kalekhan* write about research, historic and forward-looking, on another major health problem of seniors, rheumatoid arthritis. Its autoimmune components and other features are modified by spices with well-established effects. *Kaur, Saxena, Fayad, Haniadka, Saldanha, D'Silva, Ponemone and Baliga* review medicinal benefits of ginger in the therapy of gastrointestinal ailments. Ginger is a traditional root used in many geriatric conditions. Finally, neurological effects of foods and components of several diseases discussed by other authors are reviewed by *Hosseini and Mostafavi.*

Part 2: Nutraceuticals in Chronic Disease and Cancer Therapy in Seniors applies herbs and dietary supplements to chronic diseases, including a major killer of seniors, cancer. *Head* delves into understanding the mechanistic effects of changes made to mitochondria to produce a more functional and healthy brain in the aging adult. *Nakajima, Xiaofang, Naito, Kitamori and Yetti* review the progression of nonalcoholic fatty liver disease and lifestyle intervention in older adults. *Shivashankara, Kumar, Ravi, Simon, Rai, Francis and Baliga* evaluate potential protective and therapeutic effects of tea use on such liver disease. They document the potential benefits of this common food via its bioactive components, even though tea can have adverse effects due to caffeine. *Sowmya, Kalekhan, Kamath and Baliga* review cataracts that occur to a major extent in many seniors and which are frequently treated with expensive surgery. They target the role of fruits in prevention, applicable during the early aging process by modification of aldose reductase as a future therapy. *Baliga, Kalekhan, Chacko, Fazal, Latheef, Prabhudev and Haniadka* combine two items, ginger and arthritis—separately reviewed in previous chapters—for the health benefits of ginger on this autoimmune disease. *Szliszka and Krol* review polyphenols targeting the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling pathway for cancer chemoprevention. *Baliga, Meera, Rai, Saldanha, Pais, Jayachander and Palatty* review traditional Ayurvedic plants for the first of three chapters on this traditional Indian therapy and medicine. They review the usefulness of the Ayurvedic drug triphala in medical conditions afflicting older adults. Then the Indian experts *Baliga, Meera, Rai, Saldanha, Pais, Jayachander and Palatty* review these plants' historical uses as immunomodulators in geriatric diseases. Finally, *Beluga, Meera, Shivashankara, Palatty and Haniadka* provide an overview of Ayurvedic medicine in the health promotion and disease prevention of seniors. *Shivashankara, Venkatesh, Bhat, Palatty and Baliga* complement a review above on alcoholic liver disease. They document the role of phytochemicals present in foods as therapies. *Srivastava and Gupta* continue a theme of this book by reviewing chamomile. It is a component in foods that is showing promise to modify various diseases and conditions of aging.

Part 3: Nutritional Approaches to Therapy in Clinical Medicine in Old Age looks at a variety of health problems of older adults and the roles of foods and their components as therapies. *Delattre, Staziaki and Ferraz* review an extremely promising dietary supplement supported by clinical, epidemiology and animal model studies, omega-3 fatty acids. Their focus is on neurodegenerative diseases and stroke. *Zhao and Castonguay* continue the theme of the importance of small molecules in foods. They review selenium binding protein 1: a moonlight protein and its mechanistic role in health. *Wu and Cheng* further investigate the mechanism of selenium in seniors. Selenium supplementation plays a role in the health and function of the genome and maintenance of gene structure. *Calvani, Landi, Collamati, Serafini,Bernabei and Marzetti* build upon and support the above review on sarcopenia

by providing a broad review of how nutritional strategies affect its development during aging. *Doley* continues the theme of this section by broadly reviewing essential minerals in the health and activity of seniors. *Wishart, Maggini and Wintergerst* support the focus on vitamin D, a deficiency of which is frequently found in people who receive little exposure to the sun, a common issue for seniors. Supplementation can provide a variety of benefits to their health. The same authors then combine a bioactive plant and vitamins by reviewing micronutrients and ginseng for immune support in older adults. *El-Kadiki* finishes this section with an interesting discussion of micronutrients in boosting resistance to various infectious diseases.

The final section, **Part 4: Food and Supplements in Chronic Heart Diseases, Obesity and Stroke**, focuses on traditional and major chronic diseases of older age. *Robine and Bernstein* recognize and review the various actions of dietary protein on stroke. *Carlsson and Karin and Hagg* continue this theme by reviewing problems in eating and thus getting the benefits of bioactive foods due to damage caused by stroke. *Ntaios'* expertise is applied to understanding homocysteine, B vitamins and cardiovascular risk. Many factors affect blood pressure and thus cardiovascular disease risk. *Ferreira-Filho* reviews those related to diet, supplements and food. *Chow and de Keizer* describe the need for diet modification after acute events. *Bayir* describes the widely recognized effects of vitamin B12 and folic acid deficiencies on stroke. He then summarizes the benefits of vitamin B12 and folic acid supplements to prevent stroke or its recurrence in seniors. Other nutrients are known to have beneficial effects on heart disease. Therefore, *de Céniga, Bravo, Izagirre and Aramendi* review the data on several nutrients in the therapy and prevention of arterial disease. *Zittermann and Ernst* expand upon reviews by others in the book on the role of vitamin D in the health of seniors. They review its role in anemia and heart failure and the benefits of supplementation with vitamin D. Finally, *Marx* reviews major changes in macronutrient intakes leading to the metabolic syndrome: diet, obesity and chronic inflammation. In summary, the book reviews macronutrient intake, individual nutrients and non-nutrient bioactive components in foods in promoting health of aging biological systems in people.

Ronald Ross Watson, PhD

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PART I

NON-NUTRITIONAL COMPONENTS IN DIET AND SUPPLEMENTS, NUTRACEUTICALS AND THEIR ROLE IN HEALTH PROMOTION IN THE MATURE ADULT

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CHAPTER

1

A Traditional Elder's Anti-Aging Cornucopia of North American Plants

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1.1 INTRODUCTION

Phytotherapy is a common form of medicine for many North American Aboriginal people, and a wide variety of plants are used as food and medicine to maintain health. Plants are a rich source of natural compounds whose various biological activities have provided medicinal value to traditional healers for centuries. About 80% of the world's population still relies on traditional medicines for their primary health care [1]. Despite their cultural value, only 6% of the plants have been studied for their biological activity [2]. Although some pharmaceutical agents developed in labs are synthetic, many drugs originate from natural products such as those found in fungi, bacteria, animals, protists, and plants. Plant extracts and their derivatives have received considerable attention as therapeutic agents for preventing and treating health problems. Since the 1940s, for example, most molecules involved in cancer treatment have been of natural source, with almost half being either natural products or their transformed products [3]. The promising biological activities of these molecules warrant more research. Newman and Cragg strongly recommend continued exploration of these natural products to find much needed novel medicines [3].

It is important to appreciate and understand the knowledge possessed by traditional healers and Elders from North America, a vast continent with many biomes and many indigenous people. Many ethnomedicine research projects originate from collaboration of Western scientists with Aboriginal communities, to enhance appreciation regarding indigenous science. For example, several plant species used by traditional healers in the boreal regions of Canada have been examined for antioxidant activity and treatment of the symptoms of diabetes [4]. Findings from such studies corroborate the traditional land and plant knowledge of Aboriginal plant gatherers to effectively select plants with specific medicinal value. In the Native culture, traditional foods are seen as sacred and may have spiritual and medicinal value above provision of food energy. Traditional foods are rich in bioactive molecules that may have medicinal value. It is important to recognize that many plants utilized in Native North American culture fall on the continuum of foods and medicines.

Turner points out that a description of traditional plants in the Aboriginal culture is not complete without talking about the tools used to harvest, process, and prepare them (such as digging sticks), their names in different languages, and their connection to the land [5]. This information is conveyed by oral traditions, such as when younger generations spend time on the land with their families, especially the Elders. Unfortunately, the younger generation today spends less time with Elders. There is a need to find new ways to share traditional knowledge about plants with the Aboriginal and scientific communities, and to explore their documented biochemical properties.

In this chapter, we collaborate with a female Elder (Betty McKenna) to explore how specific plants can be used for the health of older adults. Elder Betty is an Anishinabe First Nation woman who has been teaching

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and practicing traditional knowledge for the past 45 years. We are interested in knowing more about selected plants she uses with older adults, and the cultural teachings centered on these plants. Plants presented in this chapter are Indian breadroot (*Pediomelum esculentum* (Pursh) Rydb.), gumweed (*Grindelia squarrosa* (Pursh) Dunal), Labrador tea (*Ledum* spp.), and blueberry (*Vaccinium* spp.). For each plant, both traditional knowledge and Western-based scientific knowledge are examined through meetings with Elder Betty, and review of the peer-reviewed published literature.

1.2 INDIAN BREADROOT (*PEDIOMELUM ESCULENTUM* (PURSH) RYDB. FORMERLY *PSORALEA ESCULENTA* PURSH)

(Anishinabe name for Indian breadroot: *pahkwe sikun ocheh pic*)

Elder Betty:

> We harvest both Indian breadroot (*P. esculentum*) and silverleaf root (*P. argophyllum*). The whole plant is picked and cut at the base of the stem. The root is chopped and used as flour in meals. The top part of the plant with the leaves, stems, and flowers are laid out on a cloth in my kitchen to dry. Once dried, I chop them with a knife or I use a coffee bean grinder to chop them finer. I harvest enough plants to keep me for the full year. I make a tea with the top part. The tea has a hearty taste. The tea is good for women going through menopause. At menopause, the hair, the nails and the skin get drier and lose their elasticity. The tea helps slow down this effect. It also serves to smooth the skin. The tea prevents bone fractures. My grandmother used to say that it is like "glue for your bones." When I grew up, I did not drink milk after I was breastfed so we ate bone marrow as our source of calcium. The bone marrow is not good enough for women going through menopause so we drink the tea which would hold the bones stronger. The tea also helps to reset sleep patterns and reduces night terrors and panic attacks in menopause. Women go through stages in their life and one of them is when we go from life-giver to grandmother. The tea helps with a smooth transition from Mother Earth to Grandmother Moon. Women in their menopause could not afford to have their sleep disturbed because they have to be spiritually well to receive the message from the ancestors and Grandmother Moon. This is why it is important to have a natural dream–wake cycle that is not disturbed and the tea helps with that. It also helps balance the spiritual and mental health, especially in winter when a lot of older people might fall in depression. I drank this tea three times a week.

Pediomelum esculentum is a low, bushy herb of the Fabaceae (Leguminosae; pea or bean) family, with a strong fleshy taproot, often greatly enlarged as a bulbous tuber-like structure. Its stem is covered with bristly hairs, and its hairy leaves are palmately compound with five gray-green pubescent leaflets. The flowers are formed in a spike of bluish-purple flowers with five petals and five sepals in typical legume arrangement. After flowering the flowers rapidly wither and become brittle, causing them to detach and blow away in the wind. It is then much more difficult to locate the underground taproot. For this reason, the root is usually harvested from May to July across the prairies [6]. In Saskatchewan, roots are dug up between the middle of June and the middle of July. If left too long the root increases in size and the interior becomes woody, with lignified and inedible tissue [7,8]. It is native to central Canada and the USA [9], and is found in prairie grassland. The Lewis and Clark expedition of 1804–1806 collected specimens that were identified botanically, but it was Frederick Pursh who first published its description in 1814 [10].

Indian breadroot was known as *pomme blanche* ("white potato") by the French Canadian voyagers, and as prairie potato by the early American settlers. *Pediomelum esculentum* has long been a reliable and plentiful staple of the Plains Aboriginals' diet during spring and early summer [6]. In fact, it might have been the most important wild plant food regularly harvested by Aboriginal nomadic buffalo hunters in the northern plains during the fur trade era. It was used as a key ingredient in dishes that were prepared during gatherings, and not only as a mean of subsistence [10]. Gatherers used a digging stick with a sharpened end to extract the underground roots [6]; it has also been reported that women and children were the main gatherers, and used fire-hardened digging sticks [10]. Preparation traditionally involved cutting the roots into thin slices, or braiding them whole to let them dry. The dry roots were ground into flour to thicken soups [6]. The dry roots and flour were easily preserved, and thus could be used during the wintertime [6].

Pediomelum esculentum is a valuable food source, and its nutritional value has been examined. The tuber has 16.3% crude fiber, up to 70% starch, and between 4.22% and 6.7% protein content, which is higher than in a potato (2%). It is a good source of calcium, magnesium, iron, zinc, and potassium [7,10–12]. Large and separate deposits of starch and proteins are found in the xylem parenchyma of the root [13]. Its high starch content makes it an ideal source of energy. It has between 0.38 (fresh weight) and 17.1 (dry weight) milligrams of vitamin C per 100 grams, which is slightly lower than in citrus fruits (25–30 (dry weight) mg/100 g) [10,11].

In addition to its nutritive value, *P. esculentum* contains bioactive compounds with medicinal value. The isoflavones genistein and daidzein are found in *P. esculentum*. Genistein is found in various parts of the plant, with the leaves and stems having the highest concentration (3–11 mg/g dry weight), followed by the flowers and the rind of the root (0.6–1.2 mg/g dry weight), with the bulb having the lowest concentration (0.2–0.7 mg/g dry weight) [14]. The concentration of genistein, however, varies during the growing season, and is at its

lowest later in the season [14]. Daizden concentrations in the rind are higher than genistein [14]. Genistein and daidzein are common bioactive components in the leaves of the *Pediomelum* genus, and are also found in the seeds [15]. Commercial soy bean (*Glycine max* (L.) Merr.) products are the main source of genistein and daidzein, with concentrations of 0.74 mg and 0.47 mg/g soy, respectively [16]. *Psoralea corylifolia* Linn. is a legume species widely used in Asian traditional medicine, and is related to *P. esculentum*. Its genistein and daidzein concentrations are 2.15 mg/g and 0.099 mg/g of leaves, respectively [15]. It is clear from these statistics that *P. esculentum* may be a promising avenue for supply of beneficial isoflavones from native North America.

Isoflavones have high antioxidant capacity. Free-radical damage and oxidative stress are correlated with chronic diseases and disorders [17]. Free radicals are atoms or molecules that contain an unpaired electron in an outer shell, making them highly reactive. A free radical is capable of destroying a biomolecule, such as an enzyme, or a cell. Oxidative stress in humans is associated with the build-up of highly reactive free radical species that damage biomolecules, and/or the decrease of defense mechanisms to protect against biological damage by free radicals. Once a cell is badly damaged the DNA can be altered, resulting in diseases such as cancer [17]. Isoflavones such as genistein and daidzein have demonstrated antioxidant capacity [16].

Genistein is part of the diet of people who regularly consume soy products. It is linked to a decreased risk of mortality from several types of cancer [18], and is especially associated with decreased numbers of breast and prostate cancers [1]. Genistein is involved in several steps during cancer progression: it inhibits cancer metastasis because of its roles in the cell cycle and apoptosis, it alters cell adhesion, and it decreases the rate of cell migration and cell invasion [18]. Genistein and daidzein decrease the production of angiogenic growth factors [19]. Angiogenesis is the development of blood vessels that supply oxygen and nutrients, allowing for the formation of new tumors. Genistein also upregulates the expression of the non-steroidal anti-inflammatory drug activated gene 1 (NAG-1) [20]. One of the roles of the NAG-1 protein is to promote the programmed death of cells, known as apoptosis.

Genistein and daidzein are called phytoestrogens because they are structurally similar to the mammalian hormone estrogen and are able to bind to the estrogen receptor. During aging, especially in menopause, there is a decrease in the production and concentration of estrogens. Low sex-steroid concentrations have a negative impact upon bone health. Reduced bone mineralization can lead to fractures such as hip fractures in older people, especially women. Soy-derived genistein and daidzein are beneficial supplements as they may reduce bone loss by binding to the estrogen receptors when the concentration of estrogen is low, such as in postmenopausal women [21]. Hot flashes and vaginitis, common symptoms associated with menopause, are reduced when women take these phytoestrogens [1,18]. We speculate that Indian breadroot, as a whole food or a natural health product, is a source of bioactive isoflavones and other molecules from native North America that is worthy of further study.

1.3 GUMWEED (*GRINDELIA SQUARROSA* (PURSH) DUNAL)

(Anishinabe name for gumweed: *Pusakwan wapa gwanese*)

Elder Betty:

> Before I harvest gumweed, I say my prayer specific to that plant. We can put the yellow sticky flower on the cut; it is like a band-aid. There is no need for stitches as the stickiness will hold the skin together. We can pick the flowers, let them dry and store them in a glass jar for later use. The plant helps with headache and sore eyes. We never boil gumweed because it would ruin the pot by sticking to it. Instead, we crush it in a cloth and put it around the head of the people we are treating. The cloth has a strong smell. Once the flowers are ground, we can put it on cuts. It is an antibiotic so the infection will not set in. Older people usually keep ground gumweed in their home for cuts. It is very upsetting when we see someone bleeding, but older people would know exactly what to do in case of bleeding. They would not try a lot of different plants, they would know right away which one to use. They would use the "tried and true" method first by applying the stored ground gumweed to the cut.

Gumweed has several branched stems with a deep taproot. It is a member of the Aster family or Asteraceae, and has both yellow ray and yellow disc florets. Its numerous branches end with a yellow flower head. The plant gets one of its names, curlycup gumweed, from the overlapping rows of stiff backward-curling resinous bracts subtending the flower. The plant is sticky (or "gummy") to the touch because the surfaces of the leaves, stems, and involucres of the flower heads are pitted with resin glands. *Grindelia* produces a high yield of resin, ranging from 5% to 20% of the plant's dry weight depending on the species [22,23]. The generous resin may be an ecological adaptation to the arid and semi-arid environments in which these plants grow [22]. *Grindelia squarrosa* is native to Western Canada and most of the United States, with the exception of the lower eastern areas [9], and is commonly found on arid prairies. The related *Grindelia* species *G. camporum* Greene and *G. camporum* Greene var. *camporum*, formerly known as *G. robusta* [9], have also been examined for their medicinal properties. In the following section, these plants are identified according to how they were identified in the referenced paper(s).

The genus name *Grindelia* originates from the name of the German botanist David Grindel (1766–1836) [23]. It has been used by the indigenous North American people to treat bronchial problems such as asthma, as well as skin afflictions of all kinds [23,24]. For example, Native Americans would make an infusion from the leaves and roots of the *G. squarrosa* plant and use that as a wash for measles [25]. In southern Alberta, indigenous people brew the flower heads into a tisane to treat migraine headaches and venereal disease [26].

Several compounds have been isolated and identified in *G. squarrosa*, including diterpene acids such as grindelic acid, 17-hydroxygrindelic acid, 7-8-epoxygrindelic acid, and 17-acetoxygrindelic acid [22]; phenolic acids [27]; flavonoids; and triterpenoid saponins [23]. Flowers contain higher amounts of phenolic acids than leaves [27,28]. Hexane extracts from *G. squarrosa* show increases during the summer, and this increase is correlated with greater resin production and higher content of diterpene acids, possibly to deter insects from feeding on the flower heads [29].

Phenolic acids are associated with the anti-inflammatory, antibacterial, spasmolytic, and antioxidant effects of *G. squarrosa* [27]. Inflammation is implicated in many chronic diseases that occur in older adults. It acts in the pathogenesis of diseases such as metabolic disorders, arthritis, cardiovascular disease, and cancer [30]. *Grindelia robusta*'s plant methanol extracts significantly reduce the production of nitric oxide and the pro-inflammatory cytokines IL-1β and IL-12, and the expression of the pro-inflammatory proteins iNOS and cyclooxygenase-2 (COX-2) *in vitro* [30]. Nitric oxide, cytokines, and COX-2 are all implicated in the inflammation process. For example, the enzyme COX-2 is necessary for the production of prostaglandins, which contribute to inflammation and tissue damage. This enzyme is a drug target for cancer prevention and inflammation reduction because it is a required mediator for pro-inflammatory prostaglandin production [30]. Bacterial lipopolysaccharide-induced macrophage cells were treated with methanol whole plant extracts of *G. robusta*. The results show a reduction in the expression of the enzyme COX-2, therefore suppressing pro-inflammatory prostaglandin production [30]. It is reported that *G. robusta* extracts inhibit the secretion of these pro-inflammatory mediators by inactivating the molecule that regulates their transcription in the nucleus: nuclear factor-kappa B (NF-κB) [24,30]. NF-κB is a molecule present in the cytoplasm that moves to the nucleus in response to various stimuli (e.g., free radicals, bacterial lipopolysaccharides, tumor promoters, and ultraviolet radiation). Once in the nucleus, it controls the transcription of DNA and regulates the expression of nearly all genes involved in inflammation [20]. Plant extracts with the capacity to regulate transcriptional factor NF-κB activity may be a promising avenue to explore in halting the inflammatory cascade implicated in so many chronic diseases.

As mentioned, there is an increase in the production of nitric oxide during inflammation [30]. Nitric oxide is a free radical, which can cause nucleic acid and cellular damage, contributing to the pathogenesis observed in several diseases. For nitric oxide production to occur, nitric oxide synthase must be expressed. Verma and colleagues showed *in vitro* that *G. robusta* methanol extract is associated with a significant reduction in the amount of nitric oxide synthase, thus inhibiting the production of nitric oxide [30].

Grindelic acid demonstrates *in vitro* antitumor activity against human breast, cervix, lung, and colon solid-tumor cell lines [2]. While this compound is naturally abundant in the *Grindelia* genus, it is also possible to synthesize compounds derived from this diterpene. These synthetic derivatives are also effective *in vitro* as antitumor derivatives, sometimes being even more effective than the original grindelic acid found in plants [2]. With cancer treatment it is imperative to find a drug that destroys the cancerous cells but is not toxic to the healthy cells, thereby causing further damage to the body. Although the studies were performed using a cell-based assay further study is encouraged, because even at a high concentration the alcohol based-extract from *G. robusta* plants is not found to be cytotoxic [24,30]. As previously discussed, NF-κB complex activation leads cancer cells to multiply rapidly. The *G. robusta* methanol extracts are able to suppress the activation of NF-κB in LPS-induced macrophages [30]. Further testing in animal models is needed to determine if *Grindelia* species extracts are antitumorigenic.

Essential oils give the flavor and aroma of many herbs and spices, as well as scents used in perfumes. Depending on the plant species, the functions of essential oils range from attracting pollinators to discouraging herbivores. These volatile oils are produced in specialized cells or glands. Essential oils are important compounds when examining the medicinal properties of plants. Essential oil yield is much greater in *G. squarrosa* flowers (0.10% of the dry weight, g/g) compared with their stems (0.01%, g/g) and their leaves (0.05%, g/g) [31]. The monoterpenes limonene and α-pinene are the major components identified in essential oils in *G. squarrosa* [31,32]. The essential oil of a related plant, *G. robusta*, demonstrates relevant antioxidant activity *in vitro* using the DPPH and 5-lipoxygenase assay [28]. Although monoterpenes are usually assumed to contribute to the anti-inflammatory, antimicrobial, and expectorant activities associated with *Grindelia* plants, some research reports that other compounds may also play a role. For example, quercetin-3-methylether, an abundant flavonol found in *G. robusta*, is very active at inhibiting human neutrophil elastase activity *in vitro* [33]. Elastases are also linked to a variety of inflammatory diseases.

The *Grindelia* genus is used successfully as a source of antimicrobial substances. Methanol extracts from *Grindelia camporum* display significant antifungal activity against several pathogenic and toxinogenic fungal species *in vivo* [34]. *Grindelia squarrosa* methanolic extracts show fungal and antibacterial effects [25].

1.4 LABRADOR TEA (*LEDUM* SPP.)

(Anishinabe name for Labrador tea: *mashkiigobag*)
Elder Betty:

> This is our most important tea and we drink it every day to keep us healthy. We can drink other teas but this one has all the nutrients and it also has other benefits. When I was a kid, my mom would give it to us before a canoe trip. It has a sedative effect and it would calm us down so that we would not jump in the canoe. It helps with constipation, which is common in older people. It also helps older people so that they don't get confused. When I was young, older people had to remember where things were. For example, they had to know where the birch trees were for medicines, where the strawberry patch was, where not to go like muskeg holes that were deep and kids could sink in them, and where to hunt. We were hunters and gatherers and older people knew where everything was. They had to remember the landmarks. We could not always take people to these locations and so we had to tell them where it was: "You go to the second bend on the river," and so on. This tea helps with keeping a keen memory. Today, the more store-bought food we eat the more we lose our culture! Drinking tea helps us keep our connection to Mother Earth.

Another group with interesting medicinal properties includes plants from the Ericaceae (heather) family. Commonly known in North America as Labrador tea, it is classified as *Ledum* or *Rhododendron* species [9]. Labrador tea is a small shrub, anywhere between 30 and 150 cm high, with flattened and matted twigs, hairy leaves, and white flowers on top. This shrub grows best in peaty soils, bogs, and wet pine forests, throughout Canada [35,36]. The plant is very strong and is able to survive fire by rapidly resprouting from the roots. It is powerful not only in survival but also in fragrance – for example, the fragrance of *R. tomentosum* is said to be so intense that it can cause a headache [36].

Labrador tea is commonly called muskeg tea in Saskatchewan. This tisane is used in traditional medicine for numerous ailments including, but not limited to, skin problems, cold and flu symptoms, inflammatory diseases, asthma, tuberculosis, stomach ache, diarrhea, rheumatism, arthritis, chest pain, swollen limbs, burns, and liver and kidney diseases [36,37].

There are many uses for Labrador tea, but one of the more common methods is to make a steeped beverage by utilizing the leaves [35]. Drinking excessive amounts of Labrador tea is not recommended, as it is very strong and can lead to serious side effects, including intestinal disturbances, drowsiness, and a strong diuretic effect [36]. *Rhododendron tomentosum* has a high content of toxic volatile compounds in the essential oil, so it is considered a poisonous plant [36]. Therefore, the internal use of methanol and water extracts from this plant is not advised, as there is a danger in consuming the toxic compounds. One of the more toxic compounds involved is the sesquiterpenoid ledol, which has an effect upon the nervous system. The compound can cause psychomotor stimulation, leading to seizures, paralysis, and even death [36]. However, essential oil from this plant has been used in Russia as a cough medicine, with the only side effect recorded being an allergic reaction [36]. It is possible that there is something else in the plant extract that diminishes the effect of the toxic compound's severe side effects upon the central nervous system when it is consumed as a weak infusion. Serious reactions only seem to occur when there is chronic exposure to ledol, causing an overdose [36].

The role of reactive oxygen species in damaging cellular molecules and contributing to subsequent pathogenesis was outlined above. Dietary antioxidants from foods and phytomedicines may prevent the oxidation of molecules, and thus inhibit the dangerous effects of free radicals in the body. *In vivo* and *ex vivo* tests have determined that the methanol extracts of the leaves and twigs from *L. groenlandicum* Retzius show a strong antioxidant potential [35]. It is possible that this is due to phenolic compounds, as there is a strong correlation between antioxidant properties and total phenolic capacity when assessed using the ORAC assay [35]. This antioxidant activity found in the Labrador tea plants is noteworthy. The time of year in which the plant is harvested may have an effect upon the antioxidant activity. There is a negative correlation between antioxidants and daylight hours, impacting its ability to inhibit free radicals in a tumor necrosis factor-α (TNF-α) assay [36]. The antioxidant activity *in vitro* of Northern Labrador tea, *R. tomentosum* ssp. *subarcticum*, reaches a peak when the leaves turn red in September [38].

Labrador tea is a promising plant medicine to treat symptoms of diabetes, a common metabolic disease [39]. A person suffering from untreated diabetes fluctuates between very high and very low levels of glycemia. Cells are vulnerable to these extremes, and hyper- and hypoglycemia may initiate apoptosis and abolish peripheral nerve regeneration. Ethanol extracts of *R. tomentosum* leaves increase cell viability *in vitro* [39]. The cytoprotective activity of *R. tomentosum* leaves offers an interesting avenue for the treatment of symptoms of diabetes.

A study discovered that the methylene chloride extracts from *L. palustre* show 99% anticancer activity in mouse leukemia cells [40]. Unfortunately there are some downsides to this extraction as a future anticancer agent, in that it might cause necrosis of healthy cells [40]. There

is a safe compound found in the Labrador tea extract called ursolic acid; this acid is found in the twigs of the plant *L. groenlandicum* and has many beneficial properties, including its anticancer properties [35]. In Japan, ursolic acid has been used and recommended for skin cancer treatment, [35] although it is not clear whether this was recommended in traditional medicine or modern Western medicine. Ursolic acid is also moderately effective against colon- and lung-cancer cell lines [35]. This alcohol extract of ursolic acid does not cause genetic toxicity, so it is safe at acute levels in cells [36].

Labrador tea has anti-inflammatory potential, but it seems to have its greatest effect upon cancer cells *in vitro*. The Labrador tea extract is so strong that it results in damage to the healthy cells, and therefore further study is warranted. There is promise in the ursolic acid found in the Labrador tea extract, however. It is not as dangerous, yet is effective in inhibiting tumor growth *in vitro*.

1.5 BLUEBERRY (*VACCINIUM* SPP.)

(Anishinabe name for blueberry plant: *miinan*)
Elder Betty:

> When picking blueberry, I put down tobacco as an offering and I say my prayer. I am from the Bear Clan so I ask the bear if I can use its food. We used to let the berries dry to store them but now we freeze them and make juice throughout the year. We drink blueberry juices for different reasons. It is a blood cleanser, it prevents gout by cleansing the blood, and it helps with eyesight and glaucoma. Older people are at a greater risk of developing eye problems and the blueberry helps with that. You can eat blueberries or drink blueberry juice, but it has more benefits if you drink it because you can drink more juice than you can eat berries.

Blueberries are the fruit of the plant genus *Vaccinium*, which also is a member of the Ericaceae family [9]. Many species of blueberries grow in different parts of North America, and they are all native to the continent [9]. Blueberry bushes range in size depending on the species, and have long, leaf-filled stems, with the fruit itself growing at the end of the stem. For blueberry bushes to thrive, a more acidic soil is recommended but not required [41]. This helps to explain why they grow throughout all of North America, as the boreal forests of Canada have more acidic soils. Blueberry consumption is beneficial in the prevention of cardiovascular disease, diabetes, neurodegenerative diseases, and cancer [42]. Cardiovascular disease risk can be reduced by the consumption of polyphenol flavonoids, in particular anthocyanins [43]. Blueberries have high anthocyanin concentrations, and this helps explain their health benefits [44]. Anthocyanins have a beneficial effect upon inflammation, lipid metabolism, endothelial function, and oxidative damage [43,44]. Blueberries have strong antioxidant capabilities, and this is mostly explained by the 27 different anthocyanin pigments present in the fruit itself [45].

One of the risk factors for cardiovascular disease is high cholesterol, especially low density lipoproteins (LDLs) [46]. It is believed that cholesterol is partly disposed of through biliary excretion by conversion to bile acids. By adding blueberry anthocyanins to the diet of hamsters at 0.5% and 1.0%, the fecal excretion of bile acids significantly increases by 37% and 66%, respectively [42]. There is no observed impact upon gene expression of CYP7A1 and LXRx – enzymes that are involved in bile acid synthesis. The anthocyanins do not have an impact at the gene level by upregulating these enzymes; therefore, there may be an interaction between anthocyanins and bile acids in the intestine [42]. There is also an observed increase in excretion of neutral sterols by 24–30%, which is another mechanism by which serum cholesterol is lowered [42]. These two mechanisms together are associated with dose-dependent decreases in the total cholesterol concentration in plasma [42].

A reduction in total, LDL, and high-density lipoprotein (HDL) plasma concentrations is observed in pigs that were fed blueberries as part of their diet [43]. The most effective dosage in lowering total cholesterol is 2% (weight per weight) blueberry powder [43]. In a different experiment performed by Çoban and associates, some guinea pigs were fed a control diet with or without blueberries, while others received a high cholesterol diet with or without blueberries [47]. An 8% w/w (weight per weight) of fresh blueberries was chosen for the guinea pigs [47]. Significant decreases in serum cholesterol (and specifically LDL) levels were found in the guinea pigs fed a high cholesterol diet with blueberries [47]. The HDL cholesterol and triglycerides remained unchanged for that group of guinea pigs [47]. There were also some histopathological changes observed in the high dietary cholesterol and blueberry intervention group. There was a decrease in hepatic retention of lipids and an improvement in the aortic atherosclerotic lesions [47]. This suggests that blueberries ameliorate or prevent the hardening of the arteries that high dietary cholesterol causes.

The fruit itself is not the only part of the blueberry bush that has been studied. Recent findings include lowering of cholesterol levels by use of the leaves of the blueberry bush. In rats, liver triglycerides are reduced upon consumption of blueberry leaves [48]. When fed 3% (weight per weight) freeze-dried blueberry leaf powder in the diet, there was a 46.8% reduction in liver triglyceride levels [48]. Serum and liver total cholesterol levels reduced in a dose-dependent manner when increasing the dietary levels of blueberry leaves [48]. The best reduction in total cholesterol levels was 21%,

observed with 3% (weight per weight) blueberry leaf powder being added to the rats' diet [48]. When the rats in this study were fed a control diet, there was a fatty infiltration of hepatocytes [48]. This infiltration was nullified when the rats were fed the blueberry leaf diet [48]. Lastly, there was a reduction in the retention of lipids in the liver when rats were fed the diet rich in blueberry leaves, as observed in previous studies with blueberry fruit [48]. The mechanisms for these reductions in cholesterol are proposed to be decreased hepatic cholesterol synthesis, as well as increased excretion of cholesterol from the body [47]. While humans do not eat blueberry leaves, further study of aqueous and alcohol extracts of the leaves is suggested. Moermann lists several uses of infused blueberry leaves, including for gynecological purposes and blood purification [49].

Determining the mechanisms by which anthocyanins have a cholesterol lowering effect has been problematic because of their instability, unclear metabolism, and low bioavailability [50]. Bioavailability is defined as the rate at which a molecule becomes available and reaches the target tissue where it exerts a biological action [50]. Studies that have looked at anthocyanin bioavailability in animals and humans indicate that the highest levels are in the gastrointestinal tract, and there are also low levels absorbed from the circulation [50]. Theoretically, using a computer-controlled gastrointestinal model of the human upper gastrointestinal tract, it was noted that if blueberries were bound to a protein-rich matrix there would be greater amounts of anthocyanins delivered to the colon for further metabolism [50]. This suggests that anthocyanins are protected during transit when bound to a high protein-rich matrix, allowing for bioavailability to colonocytes [50]. This was confirmed in another study, where blueberry juice concentrate was bound to defatted soybean flour and added to the diet of mice [45]. The defatted soybean flour concentrated the anthocyanins and other polyphenols, but did not have a high sugar concentration after dilution and centrifuging, so only the beneficial phytonutrients were available [45].

High blood pressure, or hypertension, is another risk factor for cardiovascular disease [46]. Hypertension and the development of atherosclerosis are linked to deficits in endothelial nitric oxide synthase [51]. Although nitric oxide is a free radical and can cause damage to cells, it also has some benefits for the body. Nitric oxide is a vasodilator, which means it widens the blood vessels and helps prevent hypertension [51]. Flavonoids have the ability to increase nitric oxide bioavailability, and foods rich in flavonoids show an ability to decrease blood pressure [51]. This effect of lowering blood pressure can be observed in rats fed a high fat, high cholesterol diet for 10 weeks [51]. The systolic blood pressure was elevated to 140 mmHg by Week 4 [51]. The rats fed the non-blueberry diet had systolic blood pressures that fluctuated slightly but finished at 140 mmHg, the same as the Week 4 measurement [51]. In the rats that were given the high fat and high cholesterol diet with blueberries, systolic blood pressure was reduced by 14% by Week 10 [51]. Although a positive effect was seen with the blueberry intervention in these rats, there is a nonlinear dose–response relationship, in that the more flavonoids were consumed, the less potent were the effects upon the vascular system [51].

The renin–angiotensin system regulates blood pressure and water balance. The final product of the renin–angiotensin system pathway is angiotensin II [52], which is what raises blood pressure and also plays a role in fluid and electrolyte balance [52]. A diet with 3% blueberries (weight per weight) results in a reduced plasma angiotensin converting enzyme activity in a hypertensive stroke-prone rat strain, meaning angiotensin I does not convert to angiotensin II as quickly [52]. Angiotensin II is also a vasoconstrictor, so blueberries have a potential vasorelaxation effect [52].

Consumption of blueberries and their leaves has the ability to lower blood pressure and cholesterol by a variety of possible mechanisms demonstrated by *in vitro* and *in vivo* studies. If these pathogenic factors are reduced, the risk of cardiovascular disease is also reduced. The findings from animal studies regarding beneficent impacts of blueberry consumption are especially promising, but human studies are needed to confirm these results.

1.6 CONCLUSION

Indian breadroot (*Pediomelum esculentum* (Pursh) Rydb.), gumweed (*Grindelia squarrosa* (Pursh) Dunal), Labrador tea (*Ledum* spp.), and blueberry (*Vaccinium* spp.) comprise an anti-aging cornucopia of medicinal plants used by Native/Aboriginal Elders of North America. An overview of each plant has been provided here, from both indigenous science and Western science perspectives. These plants have been chosen to highlight their potential use by older adults as foods or ethnomedicines. More collaborative research between indigenous and Western scientists should be undertaken for greater therapeutic and economic development regarding these North American plants.

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CHAPTER

2

Alzheimer's Disease: Current Perspectives – Animal Models, Drugs Under Development, and Potential Nutritional Intervention

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2.1 INTRODUCTION

The onset of senility involves the impact of deteriorating physical and physiological efficiency of all the systems in the body, leading to degenerative disorders. One such disorder of the brain is dementia, a Latin term originally meaning "madness" (de-, "without" + ment, the root of *mens*, or "mind"), which is characterized by a serious loss of global cognitive ability in a previously unimpaired person. Identified as one of the types of senile dementia, the disease was first described by the German psychiatrist and neuropathologist Alios Alzheimer in 1906 and later became commonly known as Alzheimer's disease (AD). AD is a slowly progressive old-age disease of the brain characterized by the impairment of memory and, eventually, disturbances in reasoning, planning, language, and perception.

2.1.1 Dementia

While AD is one of the most common types of dementia, it is a challenge to diagnose. The term "dementia" encompasses many mental disabilities, disorders, or malfunctions of the brain.

- Symptoms of dementia must include decline in memory *and* in at least one of the following cognitive abilities:
  - to speak coherently or understand spoken or written language
  - to recognize or identify objects, assuming intact sensory function
  - to perform motor activities, assuming intact motor abilities and sensory function and comprehension of the required task
  - to think abstractly, make sound judgments, and plan and carry out complex tasks.
- The decline in cognitive abilities must be severe enough to interfere with daily life.

Different types of dementia are associated with distinct symptom patterns and brain abnormalities, and include AD, vascular dementia, dementia with Lewy bodies, frontotemporal lobar degeneration, mixed dementia, Parkinson's disease, Creutzfeldt-Jacob disease, and normal pressure hydrocephalus. AD and other types of dementias are irreversible, with no cure, and current available treatments help only in improving quality of life for AD patients.

2.1.2 Symptoms of Alzheimer's Disease

While AD may affect people in different ways, the most common symptom pattern begins with a gradually worsening ability to remember new information. This occurs as the neurons that begin to die and malfunction

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are the neurons involved in forming new memories. As neurons in other parts of the brain malfunction and die, individuals experience other difficulties. The following are common symptoms of Alzheimer's:

- Memory loss that disrupts daily life
- Challenges in planning or solving problems
- Difficulty completing familiar tasks at home, at work or at leisure
- Confusion with time or place
- Trouble understanding visual images and spatial relationships
- New problems with words in speaking or writing
- Misplacing things and losing the ability to retrace steps
- Decreased or poor judgment
- Withdrawal from work or social activities
- Changes in mood and personality.

As the disease progresses, the individual's cognitive and functional abilities decline. Individuals progress from mild AD to moderate and severe disease at different rates. In advanced AD, people need help with basic activities of daily living, such as using the bathroom, bathing, dressing, and dining.

2.1.3 Diagnosis of Alzheimer's Disease

A diagnosis of AD is typically made by the physician, who obtains a medical and family history, including psychiatric history and history of cognitive and behavioral changes. The physician may also often ask a family member or other person close to the individual for further information. In addition, the physician conducts cognitive tests and physical and neurologic examinations, and may request that the individual undergoes magnetic resonance imaging (MRI) scans that reveal changes in brain.

2.1.3.1 Differences between the Original and New Criteria in Diagnosing Alzheimer's Disease

The 1984 diagnostic criteria and guidelines by the Alzheimer's Association (AA) and the National Institute of Neurological Disorders and Stroke [1] were based mainly on physicians' judgment, as described above. The new criteria and guidelines proposed by the National Institute of Aging (NIA) and the AA in 2012 [2] incorporate two notable changes to help pathologists describe and categorize the brain changes associated with AD and other dementias:

1. They identify three stages of AD: preclinical AD, mild cognitive impairment (MCI) due to AD, and dementia due to AD with the first occurring before symptoms such as memory loss develop.
2. They incorporate biomarker tests. Levels of β-amyloid (Aβ1–42), total tau, and phospho-tau-181 are "validated" biomarkers for AD in cerebrospinal fluid (CSF).

2.1.3.2 The Three Stages of Alzheimer's Disease Proposed by the New Criteria and Guidelines

Using the new criteria, an individual with early brain changes would be recognized as having preclinical AD or MCI (mild cognitive impairment) due to Alzheimer's, and those with symptoms would be identified as having dementia due to AD. Dementia due to Alzheimer's would encompass all stages of AD, from mild to moderate to severe.

- *Preclinical AD*. In this stage, individuals have measurable changes in the brain and CSF that indicate the earliest signs of disease, but they have not yet developed symptoms such as memory loss.
- *MCI due to AD*. Individuals with MCI have mild but measurable changes in thinking abilities that are noticeable to the person affected and to family members and friends, but that do not affect the individual's ability to carry out everyday activities. Studies indicate nearly half of all people who have visited a doctor about MCI symptoms will develop dementia in 3 or 4 years [3].
- *Dementia due to AD*. This stage is characterized by memory, thinking, and behavioral symptoms that impair a person's ability to function in daily life, and that are caused by AD-related brain changes.

2.2 INCIDENCE AND PREVALENCE: GLOBAL AND US STATISTICS

Dementia, including AD, is one of the biggest global public health challenges of the present generation that is age-dependent, generally occurring in people aged 65 years and above. Estimates from selected studies on the incidence, prevalence, and characteristics of people with Alzheimer's and other dementias may vary depending on how each study was conducted.

2.2.1 Global Incidence of Dementia

The global incidences of the AD subtype of dementia were systematically reviewed in 2008 [4]. Of the 27 studies, only 7 were conducted outside of North America and Europe: 3 from Japan, and 1 each from China (Province of Taiwan), India, Nigeria, and Brazil. Hence, only three studies were performed in low- and middle-income countries (LMICs). Incidence at 80 years of age was higher in North America (20.6/1000 person-years [where person-years = number of persons × time in years]) and Europe (15.1) than in other countries (8.3). However, the doubling time was shorter in other countries (5.0 years)

than in North America (6.0) or Europe (5.8). Incidence was slightly higher among women (13.7 per 1000 person-years) than in men (10.6/1000 person-years). Incidence in Europe increased from 9 per 1000 person-years at ages 60–64 to 180 per 1000 person-years at ages 90–94. A new review was conducted to estimate the annual incidence rates and expected annual numbers of new cases in 21 global burden-of-disease regions. Details of the methodology are available at www.who.int/mental_health/publications/dementia_report_2012.

2.2.2 Global Prevalence of Dementia and Alzheimer's Disease

According to the 2013 report of AD International (ADI) [5] and the WHO bulletin [6], over 35 million people worldwide live with the condition of one or another type of dementia, including AD. As the world population is aging, the total number of people with dementia worldwide in 2010 was estimated at 35.6 million and has been projected to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050.

In Europe and the Americas peak incidence is among those aged 80–89 years, in Asia it is among those aged 75–84 years, and in Africa among those aged 70–79 years. The researchers estimated nearly 7.7 million new cases of dementia each year worldwide, implying one new case every 4 seconds. Some 3.6 million (46%) would impact in Asia, 2.3 million (31%) in Europe, 1.2 million (16%) in the Americas, and 0.5 million (7%) in Africa.

2.2.3 Prevalence of Alzheimer's Disease and Other Dementias in the USA

An estimated 5.2 million Americans of all ages had AD in 2013, including an estimated 5 million people age 65 and older [7], and approximately 200,000 individuals under age 65 with younger-onset Alzheimer's [8].

- One in every nine people aged 65 and older (11%) has AD.
- About one-third of people aged 85 and older (32%) have AD [7].
- Of those with AD, an estimated 4% are under age 65, 13% are aged 65–74, 44% are aged 75–84, and 38% are 85 years or older [7].

2.2.3.1 New Study Ranks Alzheimer's as Third Leading Cause of Death in the USA

It is difficult to determine how many deaths are caused by AD each year because of the way causes of death are recorded. According to final data from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), 83,494 people died from AD in 2010 [9]. Hence, based on these data, the CDC projected AD as the sixth leading cause of death in the USA [10].

However, a very recent report [11] suggests that the current system of relying on death certificates for causes misses the complexity of dying for many older people, and underestimates the impact of Alzheimer's. Indeed, it says an estimated 503,400 deaths in Americans aged 75 years and older were attributable to AD dementia in 2010, rather than the 83,494 reported on death certificates. Hence, the publication ranks AD as the third leading cause of death in the USA, behind heart disease and cancer.

2.3 ECONOMIC IMPACT AND HEALTHCARE COSTS

Dementia is one of the major causes of disability in old age, and accounts for 11.9% of the years lived with disability due to a non-communicable disease [12]. It is the leading cause of dependency (i.e., need for care) and disability among older persons in both high-income countries and LMICs. The total estimated worldwide cost of dementia was US$604 billion in 2010, or 1% of the aggregated worldwide Gross Domestic Product.

Regarding the economic impact of dementia, direct medical care contributes 16% of the global costs. In high-income countries, informal care (45%) and formal social care (40%) account for the majority of costs. In LMICs, direct social care costs are small but informal care costs (i.e., unpaid care provided by the family) are significant [13].

- Of total worldwide costs, 80% are incurred in high-income countries, reflecting the dominance of informal care in less developed countries and their much lower average wages (used to estimate informal care costs).
- Costs will increase at least in line with the increase in numbers of people with dementia, assuming that the age-specific prevalence of dementia, patterns of service use, and unit costs of care remain the same. On this basis, the ADI predicted a near doubling in worldwide societal costs from 2010 to US$1117 billion by 2030.

Total payments for healthcare, long-term care and hospice care for people with AD and other dementias are projected to increase from $203 billion in 2013 to $1.2 trillion in 2050 in the USA. This dramatic rise includes a six-fold increase in government spending under Medicare and Medicaid, which is currently 70%, and a five-fold increase in out-of-pocket spending.

While the numbers and the costs are daunting, the impact on those with the illness and on their caregivers and families is extreme – medically, psychologically, and emotionally. The behavioral and psychological symptoms

linked to dementia profoundly affect the quality of life of people with dementia and their caregivers.

2.3.1 The Need for Intense R&D in Alzheimer's Disease

No treatments are currently available to cure or even alter the progressive course of AD, although numerous new therapies being investigated are at various stages of clinical trials, the status of which will be discussed in subsequent sections. Currently there is, however, much that can be offered to support and improve the lives of people with dementia so that they can carry on with their daily activity. The principal goals for dementia care are:

- Early diagnosis
- Optimizing physical health, cognition, activity, and well-being
- Detecting and treating behavioral and psychological symptoms
- Providing information and long-term support to caregivers.

Research in terms of consolidating and validating more biomarker tests for early diagnosis would, ultimately, prevent the onset of dementia, resulting in improved quality of life not only for patients but also for their caregivers/family members.

2.4 MOLECULAR BASIS

Although it was discovered more than 100 years ago, the etiology of AD has yet to be elucidated. Having relied for many years mostly on autopsies, researchers have only recently been able to launch into an era of imaging live AD patients with invasive tools, thus bringing insights into AD pathology. Owing to the technological advances, a myriad of events culminating in synaptic irregularity, neuronal loss, and neurotransmitter dysfunction are now recognized as characteristics of AD that are often speculated to lead to the loss of cognitive function. These constant themes seen in AD pathology leave crucial memory structures to experience regional atrophy [14,15]. Acetylcholine, Aβ, glutamate, and intraneuronal tau are believed to be key players in the pathogenesis of AD. Several hypotheses have been proposed to rationalize the interaction(s) of these molecules in the AD-affected brain. The most prevalent, such as the involvement of acetylcholine, Aβ, and intraneuronal tau, and other AD hypotheses, are summarized in Table 2.1 [16–19].

TABLE 2.1 Other Known Hypotheses of Alzheimer's Disease

Hypothesis	Description
Vascular	Identifies age and a critical threshold of cerebral hypoperfusion (CATCH) as the two events responsible for the development of AD. Epidemiological data indicate that once CATCH is achieved endothelial nitric oxide production is interrupted, with dysregulation resulting in capillary atrophy.
Cholesterol	Intracellular cholesterol regulates secretase activity and modulates APP processing (synthesis). Manipulation of cellular cholesterol levels has been shown to modulate γ-secretase and amyloidogenic pathway activity. Evidence supports that cholesterol and lipid metabolism (ApoE) play a key role in generation, deposition, and clearance of Aβ [16].
Metallobiology	Metal ions like copper (Cu), iron (Fe), and zinc (Zn) in high levels are found in amyloid plaques. Aβ, through its binding to Cu^{2+} and Fe^{3+}, converts them to Cu^{+} and Fe^{2+}, respectively, ameliorating oxidative stress through the generation of highly reactive hydroxyl radicals. As the metallothionein system and genetic factor proteins mediate the traffic of metal ions, abnormalities in either could result in metal ion aggregation [17].
Insulin	Insulin and insulin-like growth factor I (IGF-I) prompt the neuronal release and later clearance of brain Aβ, respectively. A loss in sensitivity to IGF-I and, later, insulin is responsible for brain accumulation of Aβ. This desensitization perpetuates the irregular levels of insulin, IGF-I, and available insulin degrading enzyme (IDE) found in the brain of AD patients [18].
Cell cycle	AD-affected neurons experience a re-entry into the S-phase of the cell cycle, as seen by the ectopic expression of cdc2 and cdk4. APP-mediated activation of cell cycle proteins has been reported to be dysfunctional in AD-affected neurons. Subsequent activation of key cell cycle proteins like GSK3, PPARγ, and Pin1 seem to support the phenotypic observations in AD neurons, including memory impairment, tau hyperphosphorylation, increased Aβ production, and activation of inflammatory responses [19].

2.4.1 Cholinergic Hypothesis

In AD the modulation of neurotransmitters is known to lead to an imbalance of acetylcholine (ACh), a critical neurotransmitter involved in new memory formation [20]. Hence, the atrophy displayed in basal forebrain nuclei, which release ACh, is a crucial neurochemical event in AD pathology. This degeneration leads to a deficiency in central cholinergic transmission and a loss in nicotinic receptors. Evidence in support of correlating the lack of these receptors with abnormalities in cognition and memory loss has been proposed [21].

Some disagreement surrounds details of the emergence of these deficits during the pathogenesis of AD – i.e., early- vs late-stage AD. Previously, it was thought

that the deficits in ACh and other cholinergic markers that occurred early in AD development were associated with naïve memory impairment [22]. This has been challenged by the evidence that concentrations of enzymes essential to the synthesis and degradation of ACh do not experience deficits until late-stage AD. In fact, early-stage enzymatic loss may be due to abnormalities in cholinergic signal transduction. More prominently, the hypothesis is distinguished as the only approach that has, to date, produced clear proof of efficacy in well controlled multicenter trials, and hence different cholinesterase inhibitors have also been approved by the FDA for therapeutic use in AD patients. This will be discussed in later sections.

2.4.2 Amyloid Cascade

Most research efforts focus on the "amyloid cascade" hypothesis, owing to the persistent observations of plaques in AD patients' brains, which were thought to be the cause of cognitive decline. The AD-affected brain experiences an increase in Aβ production and a deficit in its clearance [23–25]. In the amyloidogenic pathway, amyloid precursor protein (APP) of the neuronal transmembrane is enzymatically cleaved by β-secretase and γ-secretase. Though cleavage by β-secretase is the first step in this pathogenic process, the next cleavage site of γ-secretase dictates the generation of either less toxic Aβ38–40 or more toxic Aβ42, with 95% of total Aβ production resulting in Aβ38–40. Discovery of a third player, α-secretase, which cleaves APP at a site that impedes Aβ42 formation, has led to the suggestion that it could be a key player in the non-amyloidogenic pathway. Cleavage by α-secretase is the predominant route of amyloid metabolism in healthy neurons. Evidence suggests that the resulting soluble segment of APP, sαAPP, may contain both neurotrophic and neuroprotective properties, and be non-pathogenic. The exact mechanism by which the balance between the amyloidogenic and non-amyloidogenic pathways is distorted is yet to be explored; however, an illustration of the proposed mechanism can be seen in Figure 2.1. Current knowledge notes that the aggregation, oligomerization, fibril formation, and deposition of Aβ42 perpetuates a cascade of events that culminates in synaptic dysfunction, excitotoxicity, or neuronal death. These events include generation of free radicals, cellular inflammatory responses, oxidative stress, and hyperphosphorylation of intracellular tau – all of which are hallmarks of an AD-affected brain [14].

2.4.3 Tau Hypothesis

In addition to Aβ plaques the most frequently observed other anatomical feature in AD patients' brains, and that is often believed to have a role in cognitive decline, has been neurofibrillary tangles (NFTs). Central to the tau hypothesis is the abnormal or exaggerated phosphorylation of a soluble microtubule-associated protein, tau, which results in the formation of paired helical filamentous tau (PHF-tau) and NFT. Normally used to stabilize microtubule assembly through its interaction with tubulin, the formation of PHF-tau and NFTs can damage cytoplasmic functions and obstruct axonal transport, and often results in cell death (apoptosis) [26,27].

2.5 MEDICATION

Currently, no drug is available to reverse AD. FDA-approved pharmacological treatments for AD presently include ACh esterase inhibitors (AChEIs) that help prevent the breakdown of ACh. These prescription drugs effectively increase cholinergic tone and only provide moderate and temporary improvements in cognitive abilities. Unfortunately, although they show symptomatic efficacy they do not slow or halt AD progression.

Drug name	Brand name	Approved for	FDA approved
1. Donepezil	Aricept®	All stages	1996
2. Galantamine	Razadyne®	Mild to moderate	2001
3. Memantine	Namenda®	Moderate to severe	2003
4. Rivastigmine	Exelon®	Mild to moderate	2000
5. Tacrine	Cognex®	Mild to moderate	1993

Three AChEIs are approved for AD therapy: donepezil (Aricept) to treat mild, moderate, and severe Alzheimer's, and rivastigmine (Exelon) and galantamine (Razadyne) to treat mild to moderate Alzheimer's. Exelon is also now available as a skin patch, so that the medication is slowly released throughout the day. Aricept is available as tablets either to swallow or to dissolve in the mouth. Cognex is the first of these drugs to be approved by the FDA, but is used less commonly than the other medications. Razadyne is also approved for mild to moderate Alzheimer's dementia, and is available as an extended-release capsule, an immediate-release tablet, and in liquid forms. Common side effects are usually mild for these medications, and include diarrhea, vomiting, nausea, fatigue, insomnia, loss of appetite, and weight loss. Cognex use may cause liver damage, so it is essential to monitor liver function.

Memantine (Namenda) is approved to treat moderate-to-severe AD. Namenda works by a different mechanism than other Alzheimer's treatments; it is thought to

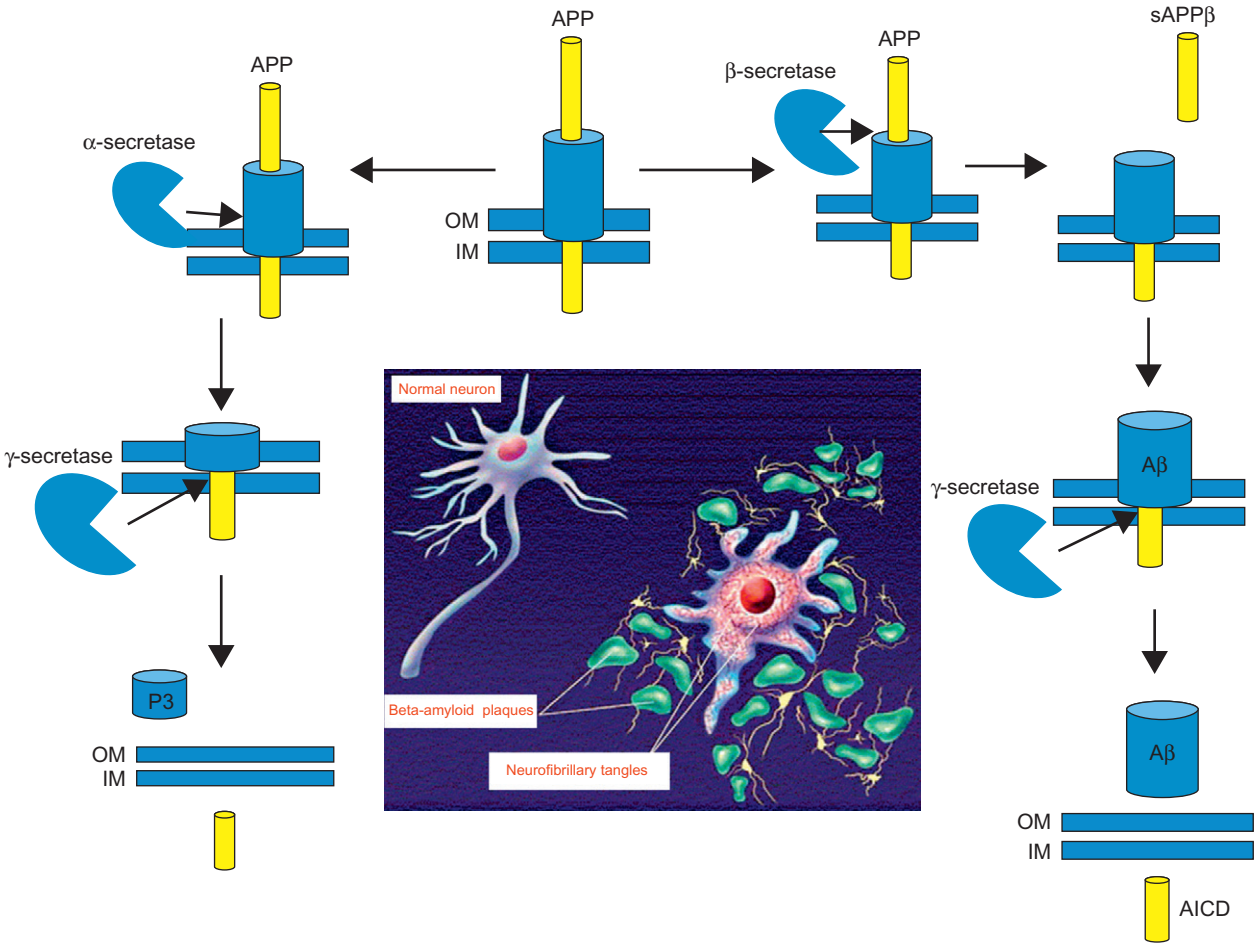

FIGURE 2.1 **Secretase proteolytic processing of APP.** APP can be proteolytically processed by two different pathways: amyloidogenic and non-amyloidogenic. In the amyloidogenic pathway the first cleavage of APP is by β-secretase followed by γ-secretase. β-secretase cleaves APP within the ectodomain, near the transmembrane domain, resulting in the shedding of soluble APP and the formation of C-terminal fragment 99 (C99). Later, the cleavage of C99 by γ-secretase results in Aβ release and APP intracellular domain (AICD) formation. Alternatively, in the non-amyloidogenic pathway, APP cleavage is sequentially conducted by the metalloprotease α-secretase and then γ-secretase. APP is cleaved by α-secretase approximately 12 amino acids from the transmembrane domain, secreting soluble sαAPP and forming C-terminal fragment 83 (C83). C83 is further processed by γ-secretase to release the p3-peptide.

play a protective role in the brain by regulating the activity of glutamate. Brain cells in people with AD release more glutamate than normal, leading to excitoneurotoxicity and neuronal death. Namenda helps regulate glutamate release/activity. It may improve mental function and performance of daily activities for some people. Namenda may have enhanced benefits when used in combination with Aricept, Exelon, or Razadyne.

A few AD therapies treat behavioral problems, such as agitation, aggression, and sleeplessness, and psychiatric symptoms that may be related to the disease, including hallucinations, depression, etc. However, none of these drugs is FDA-approved as an Alzheimer's therapy. Healthcare providers may also use other drugs to help manage other symptoms of AD, such as inflammation, oxidative stress, etc. Planning and medical/social management can help ease the burden on both patients and family members. Exercise, healthy nutrition, activities, and social interaction are important. A calm, structured environment may also help the person with AD to continue functioning as independently as possible.

2.6 EXPERIMENTAL ANIMAL MODELS AND THERAPEUTIC APPROACHES

While the limitations of AChEI drugs are widely known, new therapeutic approaches are under consideration for the treatment of AD in an effort to understand the effects of disease-modifying drugs. These drugs help to either alter or halt AD progression, mostly by targeting pathological manifestations such as Aβ plaques,

NFT, inflammation, oxidative damage, iron deregulation, and cholesterol metabolism – hence the name "disease-modifying drugs." Testing their efficacy in appropriate experimental animal models of AD is a common as well as key strategy prior to clinical trials in humans. Many animal models are available for the study of new drugs and their effects on specific pathways associated with AD; thus, a complete understanding of the limitations and roles of animal models in therapeutic testing is crucial. Ultimately, the role of animals in therapeutic development is two-fold: (1) to implicate causal mechanisms in disease state; and (2) to test the effects of new drugs in improving the disease condition.

To date, a wide range of species, including dogs, flies, fish, guinea pigs, mice, rats, and non-human primates, has been employed to study AD. This diversity of animal models utilized in AD research is likely to facilitate novel therapeutic discoveries and provide new insights into AD etiology. Transgenic murine models are regularly employed to study AD, and will be the main focus in this section.

2.6.1 Animal Models with Aβ Pathology

Many animal models have been generated to express one or more of the pathological hallmarks of AD; namely, Aβ deposition and tau hyperphosphorylation. Several of these models express cognitive decline, which can be used to provide insight into core deficits associated with learning and memory. The available transgenic murine lines engineered for the study of AD contain APP or tau mutations, PS mutations, or a combination of two or more mutations. Table 2.2 [28] lists the most common mouse models with Aβ pathology [29–40].

The transgenic mouse models listed in Table 2.2 have provided valuable information about Aβ pathology in AD; specifically, in correlating the abnormalities in behavior and neuronal function to plaque deposition. However, animal models with mutations in APP, PS, or a combination of alternations in both genes do not develop NFTs and fail to exhibit many of the neurological changes characterized in AD. Thus, these models alone do not provide a complete illustration of AD pathogenesis, and thus can be described as incomplete models best used for anti-Aβ therapies [29].

TABLE 2.2 The Most Common Mouse Models with Aβ Pathology

Model	Description
PDAPP	First animal model used to view Aβ aggregation and plaque development. Carrier of the Indiana APP mutation (V717F), these mice begin to develop plaques between 6 and 9 months of age. This model does not display NTF, but does exhibit an increase in hyperphosphorylated tau protein [29].
Tg2576	These mice contain the Swedish double mutation (K670N and M671L). At 9 months of age, the Tg2576 model exhibits cognitive deficits and plaque formation [30], although Aβ deposition is not as extensive as in humans. Due to the substantial neuronal loss, this model is often employed for use in drug intervention studies.
APP23	Expresses an alternatively spliced isoform of APP in the Swedish double mutation [31]. At 6 months of age these mice display cerebral amyloid antipathy (CAA) and tau hyperphosphorylation. Severe cognitive impairments are observed at 3 months of age, well before plaque formation [32]. The progressive age-related cognitive deficits may be a result of increased levels of soluble Aβ. At 14–18 months of age, these mice exhibit extensive neuronal loss within their hippocampal regions [33].
J20	J20 is a triple transgenic model made from the insertion of the Swedish double mutation into PDAPP mice. These models exhibit fast, aggressive Aβ accumulation and plaque formation at 6 months of age, followed by losses in synaptic plasticity and hyperexcitability of the neuronal network [34,35].
PS1	These mice contain a PS gene mutation (M146L) resulting in increased Aβ production with enhanced production of Aβ42. A heightened level of Aβ42 is attributed to increased Aβ aggregation, neurotoxicity, and impaired intracellular calcium regulation. These mice do not appear to exhibit any cognitive deficits associated with models containing APP mutations [36,37].
PSAPP	These mice are the result of a cross between Tg2576 and PS1 transgenic mice. Common features of this line are cognitive deficits before the appearance of amyloid pathology, increased Aβ42 levels, and earlier Aβ plaque deposition [38,39].
5XFAD	Engineered with one APP and two PS gene mutations, this triple transgenic model exhibits increased Aβ42 levels and displays plaque formation at 2 months of age. As a result of rapid plaque formation, severe synaptic deficits, increased neural degeneration, and behavioral abnormalities have been observed in 5XFAD mice [40].

2.6.2 Animal Models with Tau Pathology

The discovery that tau mutations resulted in frontotemporal dementia signified that tau does not rely on the presence of Aβ to induce cognitive defects and neurodegeneration. P301L tau mutation was widely studied, and was first inserted into JNPL3 mice. JNPL3 mice demonstrated NFT development between 4 and 6 months of age in both the brain and spinal cord [36]. Later, a doxycycline-suppressible model of P301L mutation expression was generated: the rTg4510 mouse model [41]. These mice experience NFT formation, and learning and memory abnormalities, at 4 months of age. The later-stage events, at 5–10 months, have been attributed

to soluble tau and not NFT formation as the reason for impaired hippocampal function and cell loss [42].

Geneticists have taken endogenous tau-knockout mice and inserted the human tau (hTau) gene to create an hTau model. hTau mice exhibit NFT distribution in the neocortex and hippocampal regions similar to that found in human AD [43,44]. Overall, a variety of tau models have been developed with altered levels of tau expression. Some models do exhibit tangle inclusions and levels of cell loss similar to those found in AD with no amyloid pathology. Thus, these models are also incomplete and are suited only to the study of tauopathies and tau-associated drug interventions.

2.6.3 Animal Models with Aβ and Tau Pathologies Combined

To overcome some of the limitations of the above two models, mice engineered to exhibit both Aβ and tau pathologies carrying mutations in either APP or PS in tandem with alterations in tau were generated. In this bid, the crossing of the Tg2576 line with the JNPL3 mouse model was one of the most well-known early attempts. The resulting line was termed TAPP [45]. TAPP Aβ pathology remained aligned with the observations made for the parental Tg2576 line in terms of deposition, progression, and severity; however, differences were found in TAPP tau pathology when compared with JNPL3 models. This led to the discovery that Aβ can ameliorate tau dysfunction [46].

Another model created to illustrate tandem Aβ and tau pathology was the 3 × Tg-AD model. This triple transgenic model contained the PS1 knockin construct, the Swedish double mutation, and the P301L tau mutation, allowing for a model capable of both plaque and tangle formation [36]. The 3 × Tg-AD mouse model contained soluble Aβ in both the neocortex and the hippocampus by 3–6 months of age. Deficits in long-term potentiation (LTP) and synaptic plasticity due to Aβ deposition were observed by 6 months of age. However, cognitive impairments correlate with the presence of intraneuronal Aβ observed in both the hippocampus and the amygdala [47].

It is pertinent that these animal models mirror the autosomal dominant mutations in APP and PS1, and thus raise questions regarding generalization. Subsequently, these animal models may not reflect the intricate, extensive cognitive or pathological disruptions characterized in AD-affected brains. Investigations of both double and triple transgenic models have unveiled many important interactions between the emergence of Aβ and tau pathology, which have laid the foundations for investigating new drug candidates for AD.

2.6.4 Non-transgenic Animal Models

Many non-transgenic animal model systems for AD have been investigated. Takeda and colleagues [48] developed a senescence-accelerated mouse (SAM) strain to investigate the physiological mechanisms of aging and neurodegeneration in AD. SAMP8 mice specifically exhibit visible age-dependent Aβ deposition at around 6 months of age. By the age of 10 months, advanced stage neurodegeneration and abnormalities in hippocampal neurogenesis are also observed [49,50].

The progressive loss of cholinergic neurons in the basal forebrain during the early stages of AD was paramount in the earliest AD studies [51,52]. Wiley and colleagues engineered an immunotoxin specific to nerve growth factor (NGF) receptors in rats [53]. Monoclonal 192 immunoglobulin G (IgG), an NGF-receptor specific antibody, was conjugated to saporin, a ribosome-inactivating protein. When the 192IgG–saporin solution is centrally administered, it results in a dose-dependent loss in cholinergic basal forebrain neurons. This dose-dependent deficit in cholinergic neurons also results in cognitive impairments. In rats, treatment with this complex can exaggerate APP expression [54,55]. In rabbits, treatment can lead to vascular Aβ deposition [56]. Further, 192IgG–saporin lesions can increase the amount of glycogen synthase kinase-3β (GSK-3β)-mediated tau hyperphosphorylation, also found in AD [57].

2.6.5 Therapeutic Approaches

A number of other promising treatments have been evaluated in AD animal models, but have met with unanticipated difficulties in human testing. For example, vaccine targeting full-length Aβ prevented plaque formation in 6-week-old PDAPP mice and reduced plaque burden in 11-month-old mice [58]. However, when AD patients were administered the same vaccine in clinical trials, 6% of the participants developed meningioencephalitis [59], likely facilitated by a T cell-mediated autoimmune response.

The anti-Aβ antibody m3D6 binds to aggregated Aβ that induces microglial activation in PDAPP mice [60]. A humanized version of this antibody, bapineuzumab, got as far as Phase III clinical trials in AD patients, but trials were terminated due to it showing no clinical benefit. CAD106 (Aβ1–6), a newly developed second-generation Aβ immunotherapy, reduces amyloid burden when administered to APP23 mice. *In vitro* studies have also shown that CAD106 reacts with Aβ monomers and oligomers, and interferes with Aβ aggregation [61]. CAD106 is now in the early stages of clinical trials.

ELN318463 and ELN475516 are γ-secretase inhibitors, selective for the APP substrate and effective for reducing

amyloid pathology in PDAPP mice. JNJ-40418677 reduced the formation of Aβ in human neuroblastoma cells and rat primary neurons without affecting Notch signaling. Although the γ-secretase inhibitors produce desirable results in animal models, this has not been reproduced in clinical trials with semagacestat. An alternate approach, using inhibition of Aβ production through interference with β-secretase 1(BACE) signaling, has been undertaken with several BACE inhibitors, currently in clinical trials.

Although decreasing Aβ production or aggregation would be expected to help AD treatment, the following findings indicate that other factors are probably equally important. Neurodegeneration continued unabated in some AD patients after almost total clearance of Aβ plaques following Aβ immunization [62], and reduction of Aβ and plaque load in aged canines also failed to rescue cognitive deficits [63]. Taken together with the failure of semagacestat in clinical trials, it appears that therapeutic AD targets in addition to Aβ should be pursued. More specifically, agents that are used prior to Aβ production or aggregation might be useful. Regardless, the chances of producing an effective treatment for AD should be enhanced by diversifying therapeutic targets beyond Aβ.

Therapies to disrupt neurotoxic Aβ production or aggregation that is promoted by interactions of Aβ with metal ions such as copper and zinc have recently met with some success in animal models and AD clinical trials. PBT2 is a second-generation 8-hydroxy quinoline complex that has significantly reduced Aβ levels and tau phosphorylation, restored Aβ-induced LTP and synaptic deficits, and rescued learning and memory impairments in transgenic mice. It also modestly improved cognitive performance and reduced Aβ concentration in the CSF of AD patients in Phase II clinical trials [64,65]. Disease-modifying drugs and trials with various transgenic mice models available to date are listed in Table 2.3 [66–86].

2.7 RECENT DRUG DEVELOPMENT EFFORTS

A number of anti-Aβ aggregation agents are currently in clinical testing. They prevent fibril formation and facilitate soluble Aβ clearance. The most studied is tramiprosate (Alzhemed®), a glycosaminoglycan (GAG) mimetic designed to cross the blood–brain barrier. GAGs bind to soluble Aβ, promoting fibril formation and deposition of amyloid plaques. GAG mimetics compete for GAG-binding sites, thus blocking fibril formation and reducing soluble Aβ [87,88]. Baseline CSF Aβ levels declined by up to 70% after 3 months in patients randomly assigned to the 100-mg or 150-mg twice-daily group. However, no differences were observed in cognitive functions between the tramiprosate and placebo groups. A Phase III study was then carried out in the US in 1052 patients with AD to test the tolerance, efficacy, and safety of the drug, but it unfortunately failed to show efficacy.

Another potential disease-modifying drug for AD is clioquinol (PBT2; Prana Biotechnology). It inhibits zinc and copper ions from binding to Aβ, thus promoting the solubilization and clearance of Aβ. Early clinical studies showed a reduction in the rate of cognitive decline [65,89], but clinical trials were halted due to toxic impurities inherent in the formulation. A novel metal–protein attenuating compound named PBT2 has recently been tested in a Phase II trial, where 78 patients with mild AD were randomly assigned to PBT2 50 mg, PBT2 250 mg, or placebo (in addition to acetyl cholinesterase inhibitors) for 12 weeks. No serious adverse events were reported by patients on PBT2. Patients treated with PBT2 250 mg had a dose-dependent and significant reduction in CSF Aβ42 concentration compared to those treated with placebo [65]. Cognitive efficacy was, however, restricted to two measures only, therefore future larger and longer trials will be necessary to test the efficacy of this drug on cognition.

Solanezumab is a monoclonal antibody against Aβ and, like flecanide, may also remove Aβ. Lilly was not alone in its attempt to target Aβ. Bapinezumab, funded by Pfizer and by Johnson & Johnson, failed in Phase III trials. Other failed attempts targeting Aβ include Elan's Aβ vaccine AN-1792; Axonyx's phenserine, which decreased Aβ production; Myriad Genetics' Flurizan®, which decreased Aβ through a direct effect on γ-secretase; and another Lilly drug, semagacestat, a γ-secretase inhibitor.

PBT2 has an entirely different mechanism of action. It is an ionophore, taking metals such as copper and iron from areas of excess (around the amyloid plaques) to areas of deficiency (the other neurons in the brain). These metals are required for normal neuronal function but become trapped and form toxic oligomers in patients with Alzheimer's dementia. By liberating the metals from these areas, PBT2 decreases their toxicity and helps promote normal neuronal function by returning them to where they are required.

Nitroflurbiprofen (HCT-1026; NicOx) is a nitric oxide-donating derivative of the non-steroidal anti-inflammatory drug flurbiprofen. It improves cognitive function in the rat following chronic lipopolysaccharide infusions [90] and reduces the plaque burden in mice [91]. In humans, this compound was shown to cross the blood–brain barrier. In addition, it reduces the rate of gastrointestinal ulcers by 60–80% compared with flurbiprofen.

TABLE 2.3 Disease-modifying Drugs for AD and Trials with Various Transgenic Mice Models

Details of effector molecule	Cellular target/pathway mechanism	Study outcome
Monoclonal antibody against synthetic Aβ1–28-2C1, 3A3	Monoclonal antibody/passive immune therapy	Reduced total Aβ levels in transgenic PDAPP mice [66]
Polyclonal antibody against Aβ1–42	Passive immune therapy/polyclonal antibody	Reduced plaque burden in PDAPP mice [67]
Lenti-apoE2	Gene delivery	Reduced brain Aβ burden PDAPP transgenic mice overexpressing a human APP mutation (V717F) [68]
Lithium	Metal ion	Directly or indirectly inhibited GSK3β and reduced plaque burden in PD-APP [69]
Rapamycin	mTOR inhibitor	Prevented AD-like cognitive deficits, decreased Aβ42 levels in the PDAPP transgenic mouse model [70]
4-O-methylhonokiol	Natural therapeutic/lignan	Prevented memory impairment, decreased β-secretase activity; inhibited Aβ deposition in Tg2576 mice [71]
Bryostatin-1	Natural therapeutic/PKC activator	Prevented and/or reversed the loss of hippocampal synapses, prevents the memory impairment and learning and memory deficits in Tg2576 [72]
CHF5074	Amyloid pathway modulation/γ-secretase inhibitor	Reduced the area occupied by plaques and the number of plaques in cortex and hippocampus, reduced total brain Aβ40 and Aβ42 and rescued a contextual fear learning deficit in Tg2576 mice [73]
Diosmin	Natural therapeutic/semisynthetic flavonoid/GSK3β inhibitor	Inhibiting amyloidogenic-secretase cleavage of amyloid precursor protein and reduced plaque burden in Tg2576 mouse model [74]
DP-109	Metal chelator	Reduced the burden of amyloid plaques and the degree of cerebral amyloid angiopathy in transgenic Tg2576 mice brains [75]
Galantamine	Natural therapeutic/ChEI/alkaloid/cholinergic system modulation	Increased the cortical levels of synaptophysin and improved spatial memory in Tg2576 transgenic mice [76]
Huprine X	Cholinergic system modulators/ChEI	Increased hippocampal α7 nicotinic acetylcholine receptors (nAChRs) in Tg2576 and 3 × Tg-AD mouse models [77]
Luteolin	GSK3β inhibitor/natural therapeutic/flavonoid	Reduced cerebral Aβ levels and reduced cognitive deficits in Tg2576 mouse model [74]
Memantine	Glutamatergic system modulation/NMDA antagonist	Reduced the total cortical levels of membrane-bound APP and improved cognitive performance in 3 × Tg-AD mice [78]
MRK-560	Amyloid pathway modulation/gamma-secretase inhibitor	Attenuated the appearance of amyloid plaques in the Tg2576 mouse [79]
Nicotine	Nicotinic acetylcholine agonist/alkaloid/natural therapeutic	Reduced guanidine-soluble Aβ in Tg2576 transgenic mice [76]
PBT2	Second-generation 8-hydroxyquinoline analog	Improved cognitive function, decreased tau phosphorylation, restored Aβ-induced LTP and synaptic deficits in PSAPP and Tg2576 mice [80]
Rosiglitazone	Antidiabetic/thiazolidinedione	Improved spatial learning and memory abilities in Tg2576 mice [81]
Simvastatin	Lipid metabolism modulators/statin	Reversed learning and memory deficits in the Tg2576 mice [82]
Carvedilol	α/β-Adrenergic receptor blocker	Increased basal synaptic transmission and LTP in TgCRND8 mice [83]
Clioquinol (CQ)	Antifungal/hydroxyquinoline	Significant reduction of amyloid-β plaque burden, both in the cortex and in the hippocampus in the TgCRND8 mouse model [84]
Donepezil	Cholinergic system modulators/reversible ChEI	Improved cognitive function and spatial memory in APP23 mice after chronic treatment [32]
Rivastigmine	Cholinergic system modulation/ChEI	Reduced cognitive deficits in the APP23 mice [85]
Calpastatin (CAST)	Endogenous calpain inhibitor	Decreased amyloid plaque burdens, prevented tau phosphorylation and loss of synapses in the APP/PS1 mice [86]

Preclinical studies with the various transgenic mouse models published in the last decade are summarized here. There are individual animal models based on acetyl choline esterase, Aβ or tau, but a combined animal model where all the targets could be targeted is not available and is difficult to develop because multiple pathways are implicated in AD pathology.

However, nitroflurbiprofen did not show clinical efficacy in AD.

Recent epidemiologic evidence has suggested that diabetes mellitus significantly increases risk for the development of AD, independent of vascular risk factors. Moreover, even patients who are insulin resistant, without diabetes, have been shown to share this elevated risk for the development of AD. Several potential mechanisms for the interaction of insulin resistance or diabetes with AD have been suggested, such as decreased cortical glucose utilization, particularly in the hippocampus and entorhinal cortex; increased oxidative stress through the formation of advanced glycation end products; increased tau phosphorylation and NFT formation; and increased Aβ aggregation through inhibition of insulin-degrading enzyme. The future treatment of AD might involve pharmacologic and dietary manipulations of insulin and glucose regulation. NIC5-15 is a single, small, naturally occurring molecule [92]. Animal studies and some clinical trials have shown NIC5-15 to be safe, and a potent insulin sensitizer at doses equivalent to 800–2000 mg per day. In preclinical studies, at doses higher than those previously studied in clinical trials, it was observed that NIC5-15 interferes with the accumulation of Aβ – an important step in the development of Alzheimer's. However, clinical trials did not show any positive results with AD.

CERE-110 (NGFvirus), Trx-237-005, tau vaccine, and many other agents are being tested in AD animal models and in clinical trials.

New research findings are giving reason for hope, and several drugs are being studied in clinical trials to determine whether they can slow the progress of the disease, or improve memory or other symptoms, for a period of time.

2.8 SCOPE OF NUTRITIONAL INTERVENTION

In the quest to improve the quality of life for AD patients, not only aspects of AD pathology but also the consequences of the disease are being targeted. One such prognostic approach is nutritional intervention. Though unclear whether it is the cause or the effect, considerable evidence exists to suggest that brain tissues in AD patients are exposed to oxidative stress during the development of the disease [93].

Oxidative stress is generated by an imbalance between the production of reactive oxygen species (ROS) and the antioxidative defense system. Both systems are critical in the process of age-related neurodegeneration and cognitive decline. Age-dependent memory impairments also correlate with a decrease in brain and plasma antioxidant defense mechanisms. Intracellular glutathione concentration decreases with age in mammalian brain regions, including hippocampus [94], which may lead to the situation where the rate of ROS production exceeds that of the radical detoxifying enzymes, thus causing oxidative stress in AD.

High-level oxidation of proteins, lipids, and glycoxidation end products; formation of toxic substances such as peroxides, alcohols, aldehydes, free carbonyls, ketones, and cholestenone; and oxidative modifications in nuclear and mitochondrial DNA are the main manifestations of oxidative damage occurring during the course of AD. Elevated levels of those oxidized products mentioned above have been described not only in brain, but also in the CSF, blood, and urine of AD patients [95].

Some of the antioxidant intervention studies using animal and/or human subjects have been summarized in Table 2.4 [96]. The scope of and hope for antioxidant agents is encouraging. As of now, they seem to hold a certain degree of potential and promise – if not as therapeutic agents, certainly as agents improving the quality of life of patients, and retarding the progress of AD.

From the nutritional perspective, other forms of phytonutrients have also captured attention. A phytosterol, stigmasterol, has shown promise of interfering with the advancement of AD. It reduced Aβ generation by (1) directly decreasing β-secretase activity, (2) reducing expression of all γ-secretase components, (3) reducing cholesterol and presenilin distribution in lipid rafts implicated in amyloidogenic APP cleavage; and (4) decreasing BACE1 internalization to endosomal compartments, involved in APP-β-secretase cleavage. Mice fed with stigmasterol-enriched diets confirmed protective effects *in vivo*, suggesting that dietary intake of phytosterol blends mainly containing stigmasterol may be beneficial in preventing AD [97].

2.9 INSIGHTS AND THE FUTURE

While relentless efforts are ongoing in search of new drugs for AD, both at experimental and clinical levels, there are other aspects to focus on regarding how to limit the prevalence of AD. The numbers of under-diagnosed dementia sufferers, and especially of those with AD, should be reduced by adopting the new criteria of diagnosis of AD and other dementia. Though the new criteria and guidelines for diagnosing AD have suggested two biomarker categories, this needs vast expansion. New sets of promising biomarkers, such as circulating microRNA for AD [98], are emerging. More research is needed to validate the accuracy of potential biomarkers and better understand which biomarker test, or combination of tests, is most effective in diagnosing AD. After all, the most effective test or combination of tests may differ depending on the stage of the disease and the type of dementia.

TABLE 2.4 Antioxidant Intervention Studies in AD Models

Antioxidant intervention	Cells or animal models of AD	Human trials	Outcome
Vitamin E (α-tocopherol)	Aβ-induced rat model		Attenuated toxic effects of Aβ and improved cognitive performance
		Treatment with α-tocopherol (2000 IU a day) in patients with moderately severe impairment from AD	Reduced neuronal damage and slowed progression of AD
	APP Tg2576 mice		Suppressed brain lipid peroxidation, reduced Aβ levels, and senile plaque deposition
	Drosophila		Suppressed tau-induced neurotoxicity
		AD patients whose regimens included vitamin E	Longer survival rate than those taking no drug or a ChE1 alone
Vitamin C	Methionine diet-induced hyperhomocysteinemia rats		Decreased oxidative stress *in vivo*, enhanced NO bioavailability, restored regulation of shear stress in arterioles, and normalized systemic blood pressure
Vitamin B_{12}	Cats		Increased choline acetyl transferase activity
		AD patients	Improved cognitive function
MnSOD	APP Tg2576 mice		Deficiency of MnSOD increases Aβ levels and accelerates the onset alteration
LA (α-lipoic acid)	APP Tg2576 mice		Decreased expression of lipid peroxidation markers of oxidative modification but not β-amyloid load within the brains; improved performance in Morris water maze but not effective at altering cognition in the Y-maze test
MitoQ and Szeto Schiller (SS) peptide 31	APP Tg2576 mice and mouse neuroblastoma (N2a) cells incubated with the Aβ peptide		Prevented Aβ toxicity in mitochondria on neurons
Caffeine	Cholesterol-fed rabbit model system for late onset sporadic AD		Decreased Aβ production and accumulation; reduced phosphorylation of tau; attenuated ROS and 8-Iso-PGF2α levels and reduced glutathione depletion; and protection against cholesterol-induced ER stress
Curcumin	APP Tg2576 mice		Reduced carbonyls and facilitated disaggregation of Aβ and reduction in AD-associated neuropathology
Silibinin	Aggregated Aβ25–35-induced AD model mice		Prevented memory impairment and oxidative damage induced by Aβ

(*Continued*)

TABLE 2.4 (Continued)

Antioxidant intervention	Cells or animal models of AD	Human trials	Outcome
Ginkgo biloba	APP Tg2576 mice		Improved cognitive function but without any effects on Aβ levels or senile plaque
		Post mortem brain ELISA measurement	No significant effects on senile plaque size or Aβ levels
Melatonin	APP695 transgenic mouse model		Improved learning and memory deficits
	AD cell models such as mouse microglial BV2 cells, rat astroglioma C6 cells, and PC12 cells		Attenuated Aβ-induced apoptosis, inhibited Aβ-induced mitochondria-related bax increase
	Microglia exposed to Aβ1–42		Inhibited phosphorylation of NADPH oxidase via a PI3K/Akt-dependent signaling pathway
	APP Tg2576 mice		Decreased Aβ burden in young mice; no effects on F2-IsoPs or Aβ burden in older plaque-bearing mice
Selegiline (L-deprenyl)		Treatment with selegiline (10 mg a day) in patients with moderately severe impairment from AD	Reduced neuronal damage and slowed progression of AD

Advanced imaging technology, especially of the brain, may be considered simultaneously in order to complement the diagnosis and predict the onset of the disease early. In addition to the clinical biomarkers from body fluids, new imaging techniques may help toward a better correlation and understanding and, thereby, an early and accurate diagnosis of AD. Needless to say, an early diagnosis of AD can make life easier not only for the patients but for the caregivers as well. It paves the way to early modification/blocking of progress of the pathological steps leading to AD that are responsible for the clinical symptoms [99,100].

Dependence, a consequence of chronic disease disability, will increasingly dominate the health and social care agendas in many countries. The proportions of dependent persons who are aged 60 years and over will increase between 2000 and 2050 from 29% to 45% overall; 21% to 30% in sub-Saharan Africa; 23% to 44% in India; 23% to 47% in Latin America; 30% to 60% in China; and 45% to 61% in high-income countries [101].

There is a lack of awareness and understanding of dementia, at some level, in most countries. It is often considered to be a normal part of aging, or a condition for which nothing can be done. This affects people with dementia, their caregivers and families, and their support structure, in many ways. Low awareness levels contribute to stigmatization and isolation. Poor understanding creates barriers to timely diagnosis and to accessing ongoing clinical, medical, and social care, leading to a large delay and gaps in treatment.

On the other hand, research identifying modifiable risk factors of dementia is in its infancy. Primary prevention should focus on targets suggested by current evidence, which include countering risk factors for vascular disease, insulin resistance, diabetes, hypertension, obesity, smoking, and physical inactivity. Today, the importance of proper and adequate nutrition cannot be emphasized enough, as it not only keeps the majority of the health-threatening conditions away but also lessens the burden on relatives and, in turn, on the society. Poorly and improperly organized dietary habits can increase the likelihood of succumbing to several of the diseases mentioned above. The subsequent inflammation and oxidative stress may also lead to exposing the individual to neurodegeneration early. Hence, maintaining and inculcating healthy food habits and an active lifestyle is currently almost the need of the hour in order for people to avoid the agony of degenerative disorders.

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CHAPTER

3

Amla in the Prevention of Aging: Scientific Validation of the Ethnomedicinal Claims

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3.1 INTRODUCTION

Phyllanthus emblica (syn. *Emblica officinalis*), commonly known as the Indian gooseberry in English, is arguably the most important medicinal agent in the traditional Indian system of Ayurvedic medicine [1,2]. It is colloquially known as amla or amlaki in most Indian languages (Table 3.1). The plant, which belongs to the family Euphorbiaceae and was originally indigenous to India, is today also found growing in other tropical countries like Pakistan, Uzbekistan, Sri Lanka, China, and Malaysia, and other areas in Southeast Asia [3]. The fruits (also known as the berries or myrobalans) are the most important plant part, and are of dietary, culinary, and medicinal use (Figure 3.1). The fruits of amla are an important dietary agent and are used to make murabbah, burfi, ladu, fresh juice, pickle, chutneys, and curries in India [4].

Amla is a rich source of vitamin C, and reports indicate that levels of this crucial vitamin in amla are greater than in oranges, tangerines, or lemons [5]. The other important constituents of amla are gallic acid, ellagic acid, chelubic acid, chebulinic acid, chebulagic acid, emblicanin-A, emblicanin-B, punigluconin, pedunculagin, citric acid, ellagotannin, trigallayl glucose, pectin, 1-O-galloyl-β-D-glucose, 3, 6-di-O-galloyl-D-glucose, corilagin, 1, 6-di-O-galloyl beta-D-glucose, 3-ethylgallic acid (3-ethoxy-4,5-dihydroxybenzoic acid), and isostrictiniin (see Figure 3.2). Amla also contains flavonoids such as quercetin (Figure 3.2), kaempferol-3-O-α-L (6″ methyl) rhamnopyranoside and kaempferol-3-O-α-L (6″ ethyl) rhamnopyranoside [3].

3.2 TRADITIONAL AND VALIDATED USES

Amla is a very important medicinal plant in Ayurveda and in the various folk systems of medicine in Southeast Asia. Amla has been used for more than 3000 years in India and, according to Ayurvedic principles, its regular consumption is considered to be extremely useful in arresting degenerative and senescence processes, promoting longevity, enhancing digestion, reducing constipation, reducing fever, purifying blood, reducing cough, alleviating asthma, strengthening the heart, benefiting the eyes, stimulating hair growth, enlivening the body, and enhancing intellect [3].

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TABLE 3.1 Colloquial Name of Gooseberry in Different Languages [6,7]

Language	Names
Scientific name	*Phyllanthus emblica* L.; synonyms *Cicca emblica* Kurz; *Emblica officinalis* Gaertn. *Mirobalanus embilica* Burm. *Phyllanthus mairei* Lév.
Sanskrit	dhatriphala, amla, amaliki, amalakan, sriphalam, vayastha, amalaka, dhatri
Arabic	haliilaj, ihliilaj
Assamese	amlakhi, amlakhu, amlaku, amlaki
Bengali	amlaki, amla, dhatri, amloki
Chinese	an mole
English	Emblica myroblan, Indian gooseberry
French	Phyllanthe emblica
German	amla
Gujarati	amla, ambala, amala
Hindi	amla
Italian	Mirabolano emblico
Kannada	nellikkai, nellikayi, beta nelli, pottadenollikayi
Kashmiri	amli, embali
Khasi	sohmylleng
Konkani	aavalo, aavnlaa, awla, awla
Lao	mak kham bom
Malay	melaka
Malayalam	nellikka
Malaysian	popok melaka
Manipuri	heikru
Marathi	aavalaa, awla, avalkathi, aavalaa
Mizo	Sunhlu
Myanmar	zee phyu thee
Nepalese	amba, amala
Odiya	aanla
Portuguese	Mirabolano emblico
Punjabi	olay, ainla, anala, aula, amla
Sinhala	nelli
Tamil	nellikkai, nelli
Telugu	usiri, usirikaya, usiri
Thai	ma kham pom
Tibetan	skyu-ru-ra
Urdu	amla, amlaj

FIGURE 3.1 Photograph of amla fruits.

Amla is regarded as a crucial constituent of many polyherbal Ayurvedic preparations, such as *Amlakadi Gritha*, *Amlakadi Tailya*, *Amlakyadi Churna*, *Aamalaki Rasayanam*, *Asokarista*, *Avipatikara Churnam*, *Chyavananaprasa Leham*, *Dasamularishta*, *Dhatri Lauha*, *Dhatryarista*, *Kumaryasava*, *Panchatika guggulu Ghritam*, *Thriphala Lepam*, *Thriphala Guggulu*, *Thriphala Ghritam*, and *Thriphala Churnam* [3,4]. Amla is also used in Siddha, Unani, Thai, Tibetan, Sri Lankan, and Chinese systems of medicine [3]. Amla fruits are an important ingredient in the pharmaceutical industry and are used to prepare Ayurvedic/herbal healthcare products like hair oil, dye, shampoo, face creams, and tooth powder [3,4].

The fruits are indispensable in the various folk systems of medicine in Southeast Asia, and are used to treat ailments like diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, erysipelas, skin diseases, hemorrhoids, nervous debility, leprosy, inflammation, emaciation, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, jaundice, strangury, diarrhea, dysentery, hemorrhage, leucorrhea, menorrhagia, cardiac disorders, intermittent fevers, anemia, jaundice, liver complaints, hematuria, osteoporosis, weak vision, and inflammation of the eyes [3,4].

Since antiquity amla has been shown to be beneficial in preventing and reducing age-related changes, and scientific studies carried out in accordance with the modern system of medicine have validated the ethnomedicinal claims. In the subsequent sections of this chapter the beneficial effects of amla in preventing diseases that increase with aging, such as cancer, diabetes, CVD, renal failure, immune suppression, arthritis, and cataract, are addressed in detail [2–7].

3.2.1 Amla in Diabetes

The global burden of diabetes is on the rise. It is estimated that 552 million people will be affected by 2030, of which 101.2 million are contributed by India. The highest incidence is seen among the age group 40–59 years [8]. Aging has been known to affect pancreatic β cell

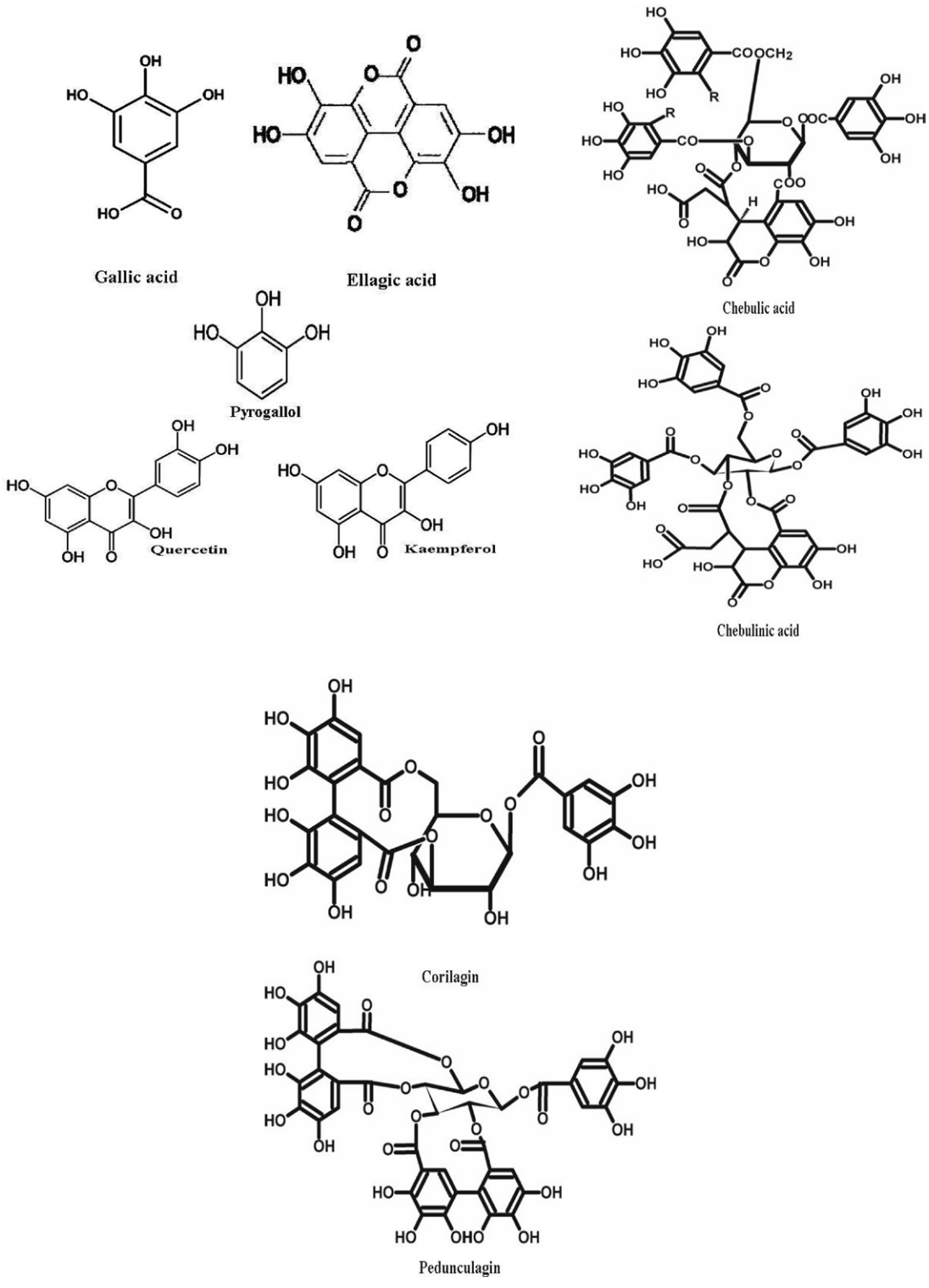

FIGURE 3.2 Some important phytochemicals of amla.

function, insulin secretion, and insulin sensitivity, thus playing a major role in the development of diabetes [9]. The gene associated with aging (*SIRT1*) has been linked with the development of diabetes, especially type 1 diabetes mellitus [10]. Oxidative stress is the key factor in the development of diabetes-related complications, and several plant polyphenols have been shown to be beneficial in diabetes. Amla (*Emblica officinalis*) contains tannins, alkaloids, vitamin C, gallic acid, and ellagic acid, which have antioxidant and immunomodulatory properties.

Several studies have demonstrated the ameliorative effects of amla in alloxan-, streptozotocin-, and high-fat diet-induced diabetic rats [11–14]. Oral administration of aqueous extract of amla (350 mg/kg) over a duration of 84 days decreased the levels of serum glucose, glycated hemoglobin, and glucose-6-phosphatase enzyme activity, while concomitantly increasing serum insulin and the glycogen stores in liver and skeletal muscle, and upregulating glucokinase in alloxan-induced type 1 diabetic rats [13]. After administering the ethanolic extract of amla (200 mg/kg) for 45 days a significant reduction in diabetes-induced atherogenesis and cardiac complications was seen among the streptozotocin-induced diabetic rats, thus endorsing its antihyperglycemic and antihyperlipidemic effects [3]. Amla extracts have also demonstrated significant free-radical scavenging activity, thus alleviating oxidative stress indices in the serum of diabetic rats. The levels of advanced glycosylation end products (AGEs) and thiobarbituric acid-reactive substances (TBARS), which have been shown to play a causative role in diabetes, decrease with amla ingestion [12].

3.2.2 Amla in Cardiovascular Disease

Globally, cardiovascular diseases (CVD) are the leading cause of death, accounting for more than 17 million deaths and contributing to approximately 30% of all deaths [15,16]. According to the most recent World Health Organization (WHO) data, more than 80% of all CVD deaths occurred in developing (low- and middle-income) countries, and estimates are that it will continue to dominate mortality trends worldwide in the future. Scientific studies by Mastan and colleagues [17] have shown that the aqueous extract of amla possesses cardiotonic activity. To further substantiate these observations, cell culture studies with cardiac myoblast H9c2 cells have also demonstrated that the ethanolic extract of amla was effective in ameliorating the doxorubicin-induced cytotoxicity secondary to its antioxidant properties [18]. Isoproterenol, a widely used experimental drug possessing β-agonistic properties, is used to produce myocardial stress and necrosis, thus inducing myocardial infarction. Oral administration of amla (100, 250, and 500 mg/kg) for 30 days was effective in preventing the isoproterenol-induced cardiotoxicity in rats. Amla increased antioxidants (SOD, CAT, GPx, and GSH), and decreased myocyte injury-specific marker enzymes (CPK-MB and LDH) and levels of LPx [19]. Among the phytochemicals, quercetin (10 mg/kg) given for 14 consecutive days was found to reduce the mitochondrial lipid peroxides and simultaneously to increase the mitochondrial antioxidants and the activity of isocitrate, succinate, malate, and α-ketoglutarate and NADH dehydrogenases and cytochrome-c-oxidase, thus ameliorating the isoproterenol-induced myocardial infarction in rats [20].

3.2.3 Amla in Renal Failure

The prevalence of chronic kidney disease (CKD) is increasing worldwide, with an annual growth rate of 8%. CKD is strongly associated with diabetes, hypertension, and chronic glomerulonephritis [21]. The loss of renal function as age progresses is a well-recognized phenomenon. A progressive fall in the glomerular filtration rate (GFR) and renal blood flow (RBF) has been documented. Eventually, a loss of renal mass, afferent arteriolar hyalinization, glomerular sclerosis, and tubulointerstitial fibrosis may occur as age advances. These changes make an aged kidney susceptible to developing acute kidney injury or chronic kidney disease [22]. The ethyl acetate extract of amla was found to reduce the levels of blood urea nitrogen and serum creatinine in aged rats. A significant reduction in the serum levels of thiobarbituric acid-reactive substances (TBARS), renal homogenate, and mitochondria in aged rats suggests that amla ameliorates oxidative stress during the aging process. Inducible nitric oxide synthase (iNOS) and cyclo-oxygenase (COX)-2 expression in the aorta of aged rats were also ameliorated by inhibition of NF-κB activation [23]. Patients with diabetic nephropathy on regular hemodialysis are found to have high levels of oxidative stress (due to neutrophil activation induced by hemoincompatibility between the hemodialyzer and blood). Though plain amla supplementation for 4 months in uremic patients led to a reduction in the plasma oxidative markers, it had no effect on diabetic and atherogenesis indices [24]. However, a mixture of green tea extract (epigallocathechin-3-gallate) and amla extract in a 1:1 ratio, given for 3 months, was found to improve significantly the diabetic and atherogenesis indices in uremic patients. Antioxidant defense was also found to improve substantially in those patients [25].

3.2.4 Amla in Cancer

The incidence of cancer increases with age. Among people above the age of 65 years, there is an 11-fold increase in the incidence of developing cancer as compared to younger individuals. The median age of cancer patients at death ranges from 71 to 77 years [26]. The radiomodulatory, chemomodulatory, and chemopreventive effects, free radical scavenging, and antioxidant, anti-inflammatory, antimutagenic, and immunomodulatory properties of amla have been demonstrated to be effective in the treatment and prevention of cancer. The phytochemicals of amla have been extensively studied in tert-butyl hydroperoxide (t-BH)-induced toxicity, as has their mechanism of hepatoprotective action in human hepatocarcinoma cells (HepG2 cell line). The hepatoprotective potential has been attributed to amla's ability to reduce lipid peroxidation and thus cellular

damage [27]. Activator protein-1 (AP-1) and human papilloma virus (HPV) transcription are implicated in the pathogenesis for tumorigenicity of cervical cancer cells. Amla fruit extract was found to inhibit AP-1 by downregulating the constituent Ap-1 proteins c-Jun, JunB, JunD, and c-Fos. Viral transcription was simultaneously suppressed, resulting in inhibition of cervical cancer cells [28]. Amla extracts have also been shown to be effective in inhibiting the cell growth of lung, liver, cervical, breast, ovarian, and colorectal cancer cell lines by producing apoptosis, thus postulating its use as a chemotherapeutic agent in future [29].

3.2.5 Amla as an Immunomodulator

Amla, being a rich source of vitamin C, improves natural killer cell activity and antibody-dependent cellular cytotoxicity. The lifespan of tumor-bearing mice was found to increase by 35% [30]. Chromium has been used as an immunosuppressive agent in various research studies. The antioxidant and immunomodulatory effects of amla helped to suppress the enhanced apoptosis and DNA fragmentation caused by chromium [31]. Similar studies in chromium-fed mice found amla offered cytoprotection against oxidative injury in macrophages. The immunosuppressive effects of chromium against lymphocyte proliferation and IL-2 and IFN-γ production were also restored by amla [32]. Arsenic-induced oxidative stress and apoptosis observed in thymocytes of arsenic-fed mice were prevented by co-administration of amla, thus emphasizing the fact that amla has immunomodulatory properties [33]. *Emblica officinalis* extract was also studied as a radioprotective agent. Radiation of Swiss albino mice resulted in reduction of glutathione and catalase concentration in the intestines. Lipid peroxidation was also observed in the jejuna cells. These changes were absent when mice were treated with amla extract prior to irradiation [34].

3.2.6 Amla in Arthritis

Rheumatoid arthritis and osteoporosis are well-known to be associated with aging. Osteoclasts (OCs), the primary cells involved in bone resorption, are involved in the pathogenesis of most age-related bone disorders. Human rheumatoid synovial lymphocytes and fibroblasts promote osteoclastogenic activity by activating the receptor activator of the NF-κB ligand (RANKL). Tumor necrosis factor (TNF-α) and IL-7 are involved in the differentiation of osteoclasts, thus representing a link between inflammation and structural damage in joints [35]. Amla extracts were demonstrated to induce apoptosis of mature human primary OCs, by inducing the expression of Fas levels – part of the apoptotic pathway. Up to 50 μg/ml of amla extracts did not demonstrate any significant cytotoxic effects on the cell populations [36]. Ganju and co-workers [37] assessed the immunomodulatory properties of amla extracts in the adjuvant-induced arthritic (AIA) rat model. The lymphocyte proliferation activity and histopathological features of synovial hyperplasia were used to evaluate the anti-inflammatory response. A marked reduction in inflammation and edema in those treated with amla extracts proved its immunomodulatory properties, and thus its use as an effective therapeutic agent in the treatment of arthritis [37].

3.2.7 Amla in Cataracts

Visual blurring and cataracts are associated with aging. A 25-fold increase in the incidence of blindness is seen as a complication of diabetes. Advanced glycosylation end products, and activation of glucosamine pathways and polyol pathways, contribute to the pathogenesis of cataracts [38]. Aldose reductase has been implicated as a major therapeutic target in diabetic complications, especially cataracts. An important component of amla, β-glucogallin has been proven to selectively inhibit sorbitol accumulation by 73% in *ex vivo* organ culture models of lenses. Therefore, amla may be used as an aldose reductase inhibitor in the prevention of diabetic cataracts [39]. The aqueous extract of amla and its tannoids was found to be effective in delaying the onset of diabetic cataracts in streptozotocin (STZ)-treated rats. Though the oral administration of amla did not prevent STZ-induced hyperglycemia, cataract progression was significantly delayed when assessed with slit-lamp microscopy. It is thus hypothesized that amla counters polyol pathway-induced oxidative stress, preventing the aggregation and insolubilization of lens proteins [40]. In animal studies, especially those performed in frogs, amla extracts hastened the process of cell proliferation and dedifferentiation of the epithelial cells of the iris, thus inducing lens regeneration [41]. This study again corroborates the use of amla as a medicinal agent for preventing and delaying the development of cataracts.

3.2.8 Amla in Dermatological Diseases

Skin aging is a multifactorial process. Hyaluronic acid, which retains moisture, is the key molecule involved in sustaining the turgor and resilience of skin [42]. Aged skin, characterized by wrinkles, is secondary to decreased collagen content. Collagen synthesis is inhibited by the pro-inflammatory cytokine (TNF-α), and collagen degradation is enhanced by increased production of matrix metalloproteinase (MMP-9) [43]. Vitamins, carotenoids, tocopherols, flavonoids, and a variety of plant extracts possessing antioxidant properties have been widely used to maintain healthy skin. Ayurvedic cosmeceuticals

dating back to the Indus Valley Civilization have utilized many traditional plant extracts, especially amla, for defying aged skin [44]. On mouse fibroblast cells, amla extracts at concentrations of 0.1 mg/ml were found to significantly promote type-1 pro-collagen levels, while simultaneously suppressing collagenase activity in a dose-dependent manner [45]. *Emblica* extracts have also been reported to possess a special property of promoting pro-collagen content while inhibiting the MMP, thus effectively inhibiting ultraviolet B (UVB)-induced photoaging in human skin fibroblasts [46]. *Emblica* has been proven to protect the skin from the damaging effects of free radicals, non-radicals, and transition metal-induced oxidative stress, and may be used as an effective sunscreen and anti-aging skin product [47].

3.2.9 Amla and Memory

Oxidative stress and reactive oxygen species (ROS) play a significant role in the brain signaling pathways. Mitochondria-generated ROS are implicated in cellular aging and, thus, cognitive impairment. A similar mechanism is implicated in the pathogenesis of Parkinson's disease [48]. *Emblica officinalis* has been traditionally used for CNS disorders in Ayurvedic medicine. Two groups of rats with kainic acid (KA)-induced seizures and cognitive deficits were studied, the first group being pretreated with hydroalcoholic extract of amla (700 mg/kg intraperitoneally).The group that received amla showed a significant suppression in cognitive decline and seizures, documented by way of decreased levels of TBARS and TNF-α, and increased levels of GSH [49]. Anwala churna, an Ayurvedic preparation of amla, was studied in different dosages in rats with diazepam-, scopolamine-, and aging-induced amnesia. A dose-dependent improvement in memory deficits was documented with amla extracts [50]. A potential therapeutic role of amla in Alzheimer's disease and other memory disorders may thus be elucidated.

3.3 CONCLUSION

Amla has been one of the most common medicinal components used in Alternative Medicine, especially Ayurveda, Unani, Siddha, and Chinese systems. The multiple and diverse medicinal effects of amla make it an inexpensive yet effective therapeutic agent devoid of adverse effects. Amla has been documented to prevent programmed cell death by reducing TBARS and suppressing iNOS and COX-2 expression in various cell lines, thus reducing the oxidative stress. The elevated expression of pro-apoptotic proteins (bax) was also significantly reduced with the oral administration of amla. The primary cause of intrinsic biologic aging is oxidative damage to macromolecules produced during the oxidative metabolism occurring in the mitochondria. Amla has been found to reduce age-related hyperlipidemia by attenuating the oxidative stress involved in lipid metabolism and protein expression during the aging process [6]. The tannoid principles emblicanin A, emblicanin B, punigluconin, and pedunculagin of amla have also been found to normalize stress-induced perturbations in oxidative free-radical scavenging activity, hence inhibiting the aging process [7]. Several diseases that have an increased incidence with aging have also responded to the therapeutic effects of *Emblica*, thus substantiating its role as an impressive and potent agent to prevent and delay aging.

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CHAPTER

4

Sarcopenia – Potential Beneficial Effects of Creatine Supplementation

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4.1 INTRODUCTION

The age-related loss of muscle mass and muscle function (i.e., sarcopenia) decreases the ability to perform activities of daily living [1], resulting in reduced quality of life. In addition, the loss of muscle mass reduces one's metabolic rate, leading to an increase in fat mass (i.e., sarcopenic obesity). Approximately one in four adults over 70 years of age will experience rapid muscle and strength loss [2]. The estimated annual healthcare costs for treating sarcopenic-related symptoms in the United States is $20 billion [3], and $300 billion worldwide [4]. With the increasing aging population, cost-effective strategies for the prevention and treatment of sarcopenia are extremely timely and warranted.

The mechanism explaining age-associated loss in muscle and strength remains to be elucidated and is thought to be multifactorial [1,5], including alterations in muscle morphology, satellite cell activity and function, oxidative stress and inflammation, and protein and hormonal kinetics [1,5]. Mechanistically, muscle- and force-loss with aging may be partly caused by a change in muscle morphology and fiber composition [6]. There is a gradual reduction in fast-twitch (i.e., type II) muscle fibers with aging, which precedes the age-related loss of total muscle fiber number [7]. Notably, the reduction in fiber number with aging is controversial in the current literature. Recently, Nilwik *et al*. [6] demonstrated that the age-related loss in muscle cross-sectional area was almost entirely explained by a reduction in size of the type II muscle fibers. Regardless, the age-related slowing of twitch properties in motor units of both fast-twitch and slow-twitch muscle fibers is thought to be caused by alterations in sarcoplasmic reticulum functionality. Larsson *et al*. [7] found that aging had a negative effect on sarcoplasmic reticulum protein function. At the cellular level, aging is associated with a decrease in myosin per unit of muscle [8]. Myosin is the primary myofibrillar protein responsible for force production in skeletal muscle, and aging has a negative effect on myosin function [7].

In addition, aging influences satellite cell activity and function, which are important for muscle fibers to undergo hypertrophy [9]. Once activated (via mechanical stimuli from resistance exercise), satellite cells produce muscle precursor cells, which undergo activation, proliferation, and differentiation to form new myofibrils. It has recently been shown that there is a substantial attenuation in satellite cell number and function in type II, but not type I, fibers of the vastus lateralis in older adults [10]. This indirectly suggests that the reduction in satellite cell activity with age is fiber-specific, which may help explain the reduction in type II muscle fibers.

Oxidative stress may also contribute to the deterioration of muscle tissue with aging (for review, see Johnston *et al*. [11]). The ratio of pro-oxidants (i.e., reactive oxygen species) to antioxidants increases with aging, leading to cellular senescence [11]. Chronic elevations of oxidative stress with aging may exhaust the antioxidant defense system, resulting in cellular disturbances. These disturbances are associated with elevations in inflammatory cytokines that are known to negatively affect aging muscle health [12].

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4.2 CREATINE AND AGING

Creatine supplementation has emerged as one of the few effective interventions attenuating age-related declines in muscle and strength [1,5,13,14]. Creatine is a nitrogen-containing compound, naturally produced endogenously or found in the diet largely from red meat and seafood [15]. Creatine is primarily produced in a two-step process beginning in the kidneys and finishing in the liver, but can also be synthesized entirely in the pancreas or liver. The majority of creatine is transported from areas of synthesis (i.e., liver, kidney, pancreas) to areas of storage and utilization (i.e., skeletal muscle [16]). The creatine content of skeletal muscle is dependent on muscle fiber composition [16]. Type II muscle fibers have high levels of free creatine (Cr) and phosphocreatine (PCr). Since there is a decline in fast-twitch muscle fibers with aging [6], there is an associated decline in total skeletal muscle creatine and PCr concentrations in older adults [17]. A reduction in high energy phosphate metabolism may augment the age-related changes in fiber type composition. Furthermore, PCr is required to maintain the ATP/ADP ratio during resistance exercise [18]. An increase in intramuscular creatine from creatine supplementation should theoretically increase PCr resynthesis during resistance exercise and have a favorable effect on aging muscle accretion [1]. To support this hypothesis, Candow *et al*. [1] conducted a meta-analysis and found that combining creatine supplementation and resistance training enhanced muscle mass and strength gains in older adults compared to resistance training alone. Mechanistically, creatine may influence muscle hypertrophy through an increase in cellular hydration status [19], myogenic transcription factors (i.e., MRF-4 and myogenin [20]), satellite cell activity and number [21], and anabolic hormone secretion (i.e., IGF-I [13]), or by reducing whole body protein catabolism [22] and oxidative stress [11].

4.3 TIMING OF CREATINE SUPPLEMENTATION

It is well known that resistance exercise alters muscle protein turnover (i.e., protein catabolism and protein synthesis [23]). While the signaling pathways for stimulating muscle protein synthesis (i.e., mTOR) are increased after exercise, it appears this anabolic response is delayed in the post-absorptive period [23]. Emerging research suggests the strategic ingestion of creatine (i.e., with proximity to training) may be an important factor for increasing muscle mass and strength (for review, see Candow and Chilibeck [24]). For example, in comparing the effects of creatine supplementation (0.1 g/kg) immediately before or immediately after resistance training for 8 months, creatine supplementation, independent of timing, resulted in greater improvements in leg-press (29–40%) and chest-press strength (34–36%) compared to placebo (leg press, 13%; chest press, 12%; $P<0.05$). Participants who consumed creatine after exercise had a greater increase in right-leg lean tissue mass (8.6%) compared to creatine before exercise (2.4%) or placebo (1.2%; $P<0.05$), with no other differences (Candow *et al*., unpublished findings). The slightly greater benefit from post-exercise creatine supplementation supports the findings of Antonio and Ciccone [25], who found a greater muscle benefit from post-exercise creatine supplementation (5 g) in young adults. Furthermore, consuming creatine immediately before (0.05 g/kg) and immediately after (0.05 g/kg) supervised resistance-exercise sessions (leg press, chest press, lat pull-down, shoulder press, leg extension, leg curl, biceps curl, triceps extension, calf press; 3 days/week, 10 weeks) resulted in greater whole-body muscle size (ultrasound; elbow and knee flexor and extensors, ankle plantar-flexors and dorsiflexors; 2.0 m) compared to placebo (0.8 cm) and resistance exercise in healthy older males (59–77 years [22]). Interestingly, the ingestion of creatine before and after resistance-exercise sessions increased lean tissue mass compared to consuming creatine in the morning and evening on training days. While the mechanistic actions explaining the possible increase in muscle mass and strength when creatine is consumed shortly before and after resistance exercise sessions are unknown, possible contributing factors include exercise-induced skeletal muscle blood flow and delivery of creatine to exercising muscles [26], an upregulation of the kinetics involved in creatine transport [27], and increase in Na^+–K^+ pump function during muscle contraction [27].

4.4 SAFETY OF CREATINE FOR OLDER ADULTS

Research examining the potential health risks associated with creatine supplementation in older adults is limited. The International Society of Sport Nutrition as well as the American College of Sports Medicine evaluated the safety and efficacy of creatine supplementation [28,29]. Adverse events associated with creatine supplementation include nausea, vomiting, diarrhea, excessive thirst, and gastrointestinal tract complications [16]. However, these symptoms are usually based on anecdotal reports, and creatine supplementation can be considered safe for young and older adults [1]. There is evidence in younger individuals that a high dose (>20 g/day) of creatine supplementation may increase formaldehyde production [30]; therefore, a low dose of creatine supplementation may be suggested for healthy older adults [22]. Candow *et al*. [22] found that a low dose

of creatine (0.1 g/kg per day) combined with protein was effective for increasing muscle mass and enhancing strength without altering formaldehyde production. Walliman *et al*. [31] recommended long-term low-dose creatine for health benefits as one ages. However, it must be noted that the long-term effects are still unknown, and it is recommended that individuals with pre-existing renal disease or those with a potential risk for renal dysfunction do not supplement with creatine [28].

4.5 SUMMARY

Age-related loss in muscle mass and strength is a global health crisis, and cost-effective strategies are warranted. Resistance exercise is a simple and effective strategy to maintain or increase aging muscle mass and strength, which may lead to greater quality of life in older adults. In addition to resistance exercise, creatine supplementation augments gains in muscle mass and strength in older adults. Recent evidence suggests that timely ingestion of creatine in proximity to resistance exercise (before and after) has a positive effect on muscle mass and strength. Future research should continue to determine the mechanistic actions of how creatine influences muscle protein kinetics, especially in aging adults.

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CHAPTER

5

Dietary Spices in the Prevention of Rheumatoid Arthritis: Past, Present, and Future

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5.1 INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory disease caused by the activation of synovial tissue lining forming a pannus in the joint, is a major ailment [1]. The prevalence and incidence increase with age, peaking at about 70 years, and a considerable population of the world is affected [1]. It usually affects the peripheral joints in symmetrical fashion and progressively causes damage to the cartilage and bone, leading to joint dysfunction [2–4]. Rheumatoid arthritis is a systemic autoimmune disease, and the chronic immune activation is regarded as a central triggering factor for joint destruction. Studies suggest that both genetic and environmental factors are responsible for the initiation of rheumatoid arthritis and the associated pathological events. The characteristic feature in rheumatoid arthritis is persistent inflammatory synovitis (pannus) in the peripheral joints in a symmetrical fashion as a result of immune cell interactions involving the T and B lymphocytes, monocytes/macrophages, and dendritic cells. The pannus formed erodes the articular cartilage and the subchondral bone. Additionally, the influx of inflammatory cells from the circulation and hyperplasic synovial cells triggers the release of inflammatory cytokines (such as IL-1, IL-6, IL-17, and TNF-α) and contributes to the damage by upregulating the cartilage-degrading enzymes (matrix metalloproteinases 1, 8, and 9) at the cartilage–pannus junction. The chemokines and prostaglandins (PGE2) also contribute to the inflammation and tissue catabolism. Excess generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) causes oxidative damage to the membrane lipids, proteins, deoxyribonucleic acid, hyaluronic acid, and cartilage. They also contribute towards the pathological process by activating the transcription factors (NF-κB) for pro-inflammatory cytokines [2–4].

Pharmacotherapy serves an important role in the treatment of rheumatoid arthritis, and the current therapies are directed primarily towards diminishing the inflammation present in joints rather than preventing or completely arresting the progression of the disease. The use of NSAIDs (non-steroidal anti-inflammatory drugs) followed by disease-modifying antirheumatic drugs (DMARDs), which prevents further progression of the disease, is the common treatment protocol. However, regular use of methotrexate, the most commonly used DMARD, is associated with hepatotoxicity, myelotoxicity, lung fibrosis, and mutagenesis. The introduction of biologicals such as the IL-1 receptor antagonist (anakinra) and anti-TNF-α agents (etanercept, infliximab, and dalimumab) although beneficial is extremely expensive, and they have unproven long-term safety and the potential for serious infection and malignancy [2–5].

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5.2 USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES IN THE TREATMENT OF ARTHRITIS

Rheumatoid arthritis is a protracted disease and requires regular use of conventional treatment that invariably causes deleterious effects, which with time may negate the beneficial effects. In this context, there is a need for safe alternative treatments that are effective and devoid of any side effects. Reports also suggest that the use of complementary and alternative medical therapies, which include traditional medicines (such as Ayurvedic, Chinese, Unani, Siddha, Arabic, Srilankan, Tibetan, etc.), acupuncture and acupressure, homeopathy, dietary restrictions or vitamin supplementation, as well as spiritual healing and prayer, has been increasing among patients with rheumatologic diseases. Reports also suggest that nearly 47% of older adults with osteoarthritis use complementary medicine [3]. Of these, the use of herbs utilized in various traditional and folk systems of medicine is the most prevalent. The main reason is that many of the plants used have been documented for their use in traditional systems since antiquity, and are cheap and easily available [3].

Spices, which are defined as aromatic vegetable substances, in the whole, broken, or ground form, and whose significant function in food is seasoning rather than nutrition, are important constituents of Indian curries [6]. In addition to their organoleptic properties, spices also are useful in prolonging the shelf-life of foods by preventing rancidity through their free radical scavenging effects, and also by imparting antimicrobial activities on the microbes [6]. Additionally, most of the spices possess myriad medicinal benefits and are extensively used either alone or in combination with other plants for treating various diseases and ailments [7]. Historical reports support the fact that ancient physicians, like Charaka, Sushrutha, Hippocrates, and Dioscorides, used spices extensively in their practices [8,9].

Numerous studies in the past three decades have conclusively shown that most of the commonly used Indian spices contain flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, and phthalides [6]. Pharmacological and biological activity studies have also shown that these molecules possess antioxidant, free radical-scavenging, anti-inflammatory, antibacterial, and antiviral effects; modulate detoxification enzymes, stimulate the immune system, and reduce inflammation; have antimutagenic and anticarcinogenic effects; protect against a wide range of cancers, heart disease, and other chronic diseases; and are devoid of any toxicity [6,10]. Additionally, studies have shown spices like fenugreek, coriander, ginger, and turmeric, and their phytochemicals 6-shogaol, curcumin, eugenol, and thymoquinone (Figure 5.1), possess beneficial effects in amelioration of RA in preclinical studies. In subsequent sections, the protective effects of these spices and some of their phytochemicals will accordingly be addressed.

5.2.1 Fenugreek

Trigonella foenum-graecum, colloquially known as fenugreek and belonging to the Fabaceae family, is a plant originally native to India and Northern Africa [11]. The seeds are the most important plant part, and have been used extensively as a spice in the preparation of various curries and dishes [12]. The leaves and seeds also possess medicinal benefits and are used in various traditional and folk systems of medicine to treat numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health [11]. Preclinical studies have shown that fenugreek possesses pleiotropic actions and is useful in the amelioration of hypertension, cataracts, inflammation, thyroid dysfunction, malaria, endothelial dysfunction, hyperlipidemia, and diabetes [11–16]. Fenugreek has also been investigated for its anti-arthritic effects, and studies with rats have shown it to be effective in ameliorating complete Freund's adjuvant (CFA)-induced arthritis [14]. The results also showed that, in addition to reducing the inflammation and arthritic index and restoring body weight, fenugreek (200 and 400 mg/kg) was also effective in reducing the differential white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and WBC content, and increasing the red blood cell (RBC) count and hemoglobin (Hb) [14]. Administering fenugreek also reduced the levels of lipid peroxidation (LPO) and concomitantly increased the levels of superoxide dismutase (SOD) and glutathion (GSH) in the cartilage [14]. Fenugreek also caused a decrease in the levels of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-2, IL-6, and TNF-α in blood [14]. Together, all these observations clearly indicate that fenugreek mediates its protective effects by increasing antioxidant levels and decreasing pro-inflammatory cytokines.

5.2.2 Coriander

Coriandrum sativum, colloquially known as coriander, originated in Italy but is today cultivated widely in The Netherlands, Central and Eastern Europe (Russia, Hungary, and Holland), the Mediterranean (Morocco, Malta, and Egypt), North Africa, China, India, and Bangladesh [17–20]. All parts of the plant are edible, but the fresh leaves and dried seeds are commonly used in cooking in India and Pakistan. In addition to its culinary use, the coriander seed possesses medicinal properties

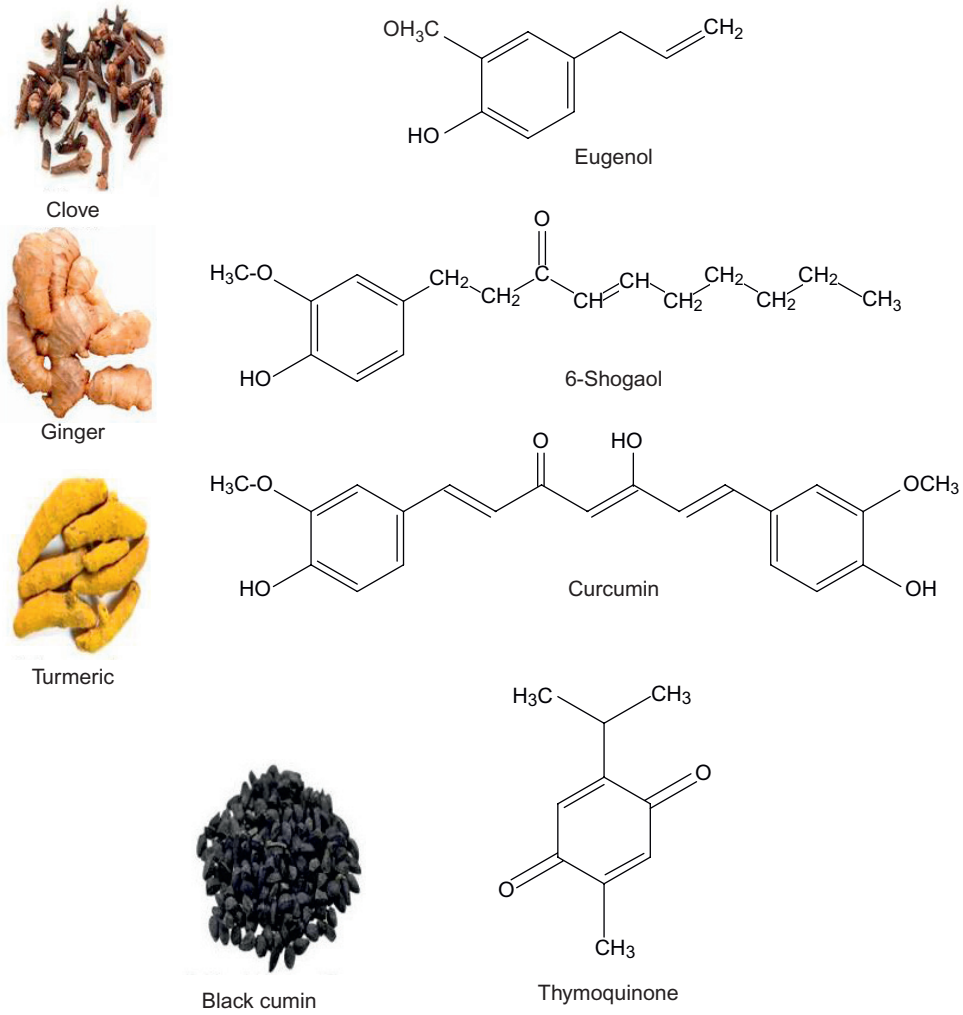

FIGURE 5.1 Spices and some of their phytochemicals that are useful in rheumatoid arthritis.

and has been used since time immemorial to treat disorders of the digestive, respiratory, and urinary systems, as it has diaphoretic, diuretic, carminative, and stimulant effects [18,19].

Phytochemical analyses have shown coriander leaves to contain linalool (coriandrol), α-pinene, β-pinene, limonene, γ-terpinene, ρ-lymene, borneol, citronellol, geraniol, pyrazine, pyridine, thiazole, furan, coriandrin, dihyrocoriandrin, coriandrones A–E, glazonoids, neochidilide, and Z-digustilide. The seeds contain coriandrol, jireniol, vebriniol, coriandrones C–E, linalool, geranyl acetate, and γ-terpinene [17]. Coriander has also been reported to exhibit several pharmacological effects, including antioxidant, antidiabetic, antimutagenic, anthelmintic, sedative-hypnotic, anticonvulsant, diuretic, cholesterol-lowering, antifungal, anticancer, anxiolytic, hepatoprotective, and anti-ulcer activities [19].

With regard to anti-arthritic effects, Nair and coworkers [19] investigated the protective effects of the hydroalcoholic extract of coriander on formaldehyde and CFA-induced arthritis in rats. The results showed that, when compared to the placebo-treated cohorts, administration of the coriander extract produced a dose-dependent inhibition of joint swelling in both formaldehyde- and CFA-induced arthritis. Mechanistic studies showed that, when compared to control, the cohorts treated with the extract had reduced levels of pro-inflammatory cytokines/cytokine receptor in the synovium [19].

5.2.3 Clove

Cloves, the aromatic dried flower buds of the tree *Syzygium aromaticum*, are an important culinary agent in various Asian foods. The clove tree, an evergreen, was originally a native to Indonesia but is today found growing in Sri Lanka and the Moluccas [21]. Cloves have been used in various folk systems of medicine to treat asthma,

and various allergic and inflammatory disorders [21]. Additionally, the essential oil of cloves has anesthetic and antimicrobial qualities, and is used to prevent halitosis and ameliorate dental pain [21]. Eugenol, chemically known as 4-allyl-2-methoxyphenol, is the principal active component of clove oil and a minor constituent of aromatic plants like nutmeg, basil, cinnamon, and bay leaves [22]. Eugenol has been very well investigated for its pharmacological effects, and studies have shown it to possess significant antioxidant, anti-inflammatory, cancer-preventive, analgesic, and local anesthetic activity [22]. With respect to the anti-arthritic effects of eugenol, Sharma *et al.* [23] observed that administration of eugenol (33 mg/kg) orally for 26 days to rats with arthritis induced by administering dead *Mycobacterium tuberculosis* bacilli caused a significant suppression of both paw and joint swelling, indicating its anti-inflammatory and antirheumatic properties. Subsequent studies have shown that eugenol was also effective in collagen-induced arthritis, reducing the periarticular erythema and edema in the hind paws of mice [24]. Mechanistic studies have shown that the cohorts receiving eugenol had reduced levels of mononuclear cell infiltration and the cytokines (TNF-α, IFN-γ, and TGF-β) within the ankle joints [24].

5.2.4 Black Cumin

Nigella sativa, a plant originally native to Southern Europe, North Africa, and Southwest Asia, is today cultivated in many countries in the world, including the Middle Eastern Mediterranean region, Southern Europe, India, Pakistan, Syria, Turkey, and Saudi Arabia [25]. The seeds are the most important plant part, and have been used since time immemorial in various traditional systems of medicine, such as Unani-Tibb, Ayurveda, and Siddha, to treat various ailments [25]. The seeds and oil have a long history of folklore usage, being widely used as an antihypertensive; as a liver tonic, diuretic, digestive, antidiarrheal, appetite stimulant, and antibacterial; for skin disorders; and as an analgesic [25]. Scientific studies carried out in the recent past have validated the ethnomedicinal uses, and reports indicate it to possess antidiabetic, anticancer, immunomodulatory, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal-protective, gastroprotective, antioxidant, and anticancer properties [25,26]. Studies have also shown that thymoquinone, which is the major bioactive component of the essential oil of the seed, possesses myriad benefits and is pleiotropic in its action [25–29].

With respect to its antiarthritic effects, studies have shown that thymoquinone was effective in reducing the inflammation and arthritis induced by incomplete Freund's adjuvant (IFA)-induced arthritis in rats, as evaluated by the clinical and radiological gradings [30]. Detailed studies showed that, when compared to placebo-treated cohorts, administering thymoquinone caused decreases in the levels of TNF-α and IL-1β [30]. Subsequent studies have shown that oral administration of thymoquinone to arthritic rats caused a decrease in arthritis scoring and bone resorption, as well as bone turnover markers such as alkaline phosphatase and tartrate-resistant acid phosphatase [31].

Additionally, cell culture studies with human RA fibroblast-like synoviocytes have also shown that thymoquinone inhibited lipopolysaccharide (LPS)-induced proliferation of the synoviocytes, generation of H_2O_2-induced 4-hydroxynonenal, and levels of IL-1β, TNF-α, metalloproteinase-13, cyclooxygenase-2, and prostaglandin E2 [31]. Thymoquinone blocked LPS-induced phosphorylation of p38, MAPK, ERK1/2, and NF-κB p65 in a time-dependent manner [31]. Additionally, studies have shown that oral administration of thymoquinone to arthritic rats reduces the serum levels of HNE, IL-1β, and TNF-α [31].

Clinical studies have also shown that consumption of 500-mg capsules of *Nigella sativa* oil twice daily significantly decreased the disease activity score (DAS-28) in people with RA when compared with before and after placebo [32]. Similarly, the number of swollen joints and the duration of morning stiffness improved. A marked improvement in disease activity was shown by both the ACR20 and EULAR response criteria in 42.5% and 30% of the patients, respectively, after intake of *Nigella* [32]. Together, these observations clearly indicate that supplementation with *Nigella sativa* during DMARD therapy in RA may be considered an affordable potential adjuvant biological therapy [32].

5.2.5 Ginger

Ginger belongs to the family Zingiberaceae, and is believed to be originally native to the north-east region of India; however, today it is also found growing in China, Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica, Nepal, Haiti, Mexico, and Hawaii [33]. Ginger is one of the world's most important culinary and medicinal agents in various alternative systems of medicines [33]. It has been documented to be of use in treating colds, headache, nausea, stomach upset, and diarrhea; to help digestion; to treat arthritis, rheumatological conditions, and muscular discomfort; and as a carminative and antiflatulent [33,34]. Scientific studies have shown that ginger has antimicrobial, antischistosomal, anti-inflammatory, antipyretic, antioxidative, hypoglycemic, hepatoprotective, diuretic, and hypocholesterolemic effects [34,35].

The pharmacological properties of ginger are attributed to the presence of volatile constituents,

including zingeberene, curcumene, farnesene, bisabolene, β-sesquiphellandrene, 1,8-cineole, linalool, borneol, neral, and geraniol; and non-volatile components such as gingerols, shogaols, paradols, and zingerone [33]. Ginger protease, capsaicin, gingediol, galanolactone, gingesulfonic acid, galactosylglycerols, gingerglycolipids, diarylheptanoids, neral, monoacyldi vitamins, and phytosterols are among other important compounds found in ginger [33–36]. Ginger has been well-documented as being very effective against symptoms of gastrointestinal problems, such as constipation, indigestion, nausea, and vomiting, in the Chinese, Ayurvedic, Arabic, Tibetan, Unani, and various other folk systems of medicines [33–35].

Ginger has been highly investigated for its antiarthritic effects, in both preclinical and clinical studies. In one of the earliest studies, Sharma and colleagues [37] observed that ginger oil was effective in reducing *Mycobacterium tuberculosis*-induced paw and joint swelling in rats. Subsequent studies have also shown that the hydroalcoholic extract of ginger was effective in reducing the collagen-induced inflammatory process and arthritis in rats, and that the extract (200 mg/kg per day) was better than the 2 mg/kg per day of indomethacin used as a positive control [38]. The cohorts administered with ginger had improved clinical scores and disease incidence, and reduced joint temperature, swelling, and cartilage destruction [38]. Additionally the ginger extract rich in gingerols and shogaols (200 mg/kg body weight) has been shown to suppress the incidence and severity of arthritis in rat adjuvant-induced arthritis, and the antiarthritic effect to be better than that of indomethacin [39].

In a study that has many implications, Funk *et al*. [40] studied the antiarthritic effects of two extracts (a well-characterized whole crude ginger extract, and a fraction containing only gingerols and their derivatives) in streptococcal cell wall-induced arthritis in rats, and observed that the cohorts receiving the crude dichloromethane extract containing the essential oils and more polar compounds had superior medicinal benefits. These observations clearly indicate a very significant cooperative protective effect of these ginger samples. The results also suggest that non-gingerol components are the most bioactive, and can enhance the antiarthritic effects of the gingerols [40]. With respect to the phytochemical studies, these have also shown that 6-shogaol (6.2 mg/kg), an important constituent of dry ginger, possesses anti-inflammatory and antiarthritic properties, and its effect was better than that provided by indomethacin (2 mg/kg per day) as observed from clinical, biochemical, and histopathological observations in CFA-induced arthritic rats [41]. In spite of all supportive preclinical observations, clinical studies with humans are scarce. The only study indicating ginger to be effective in the treatment of RA was from Srivastava and Mustafa [42], where the investigators studied the protective effects of ginger on RA in seven volunteers and observed it to be effective in ameliorating the inflammation.

Mechanistic studies indicate that ginger or its phytochemicals mediate the beneficial effects by scavenging free radicals [43–47], increasing antioxidant molecules and antioxidant enzymes [48,49], decreasing infiltration of leukocytes (including lymphocytes and monocytes/macrophages) into the synovial cavity of the knee [41], and inhibiting nitric oxide and iNOS [50–53] anti-inflammatory activity [39,45,54–57]; by suppressing prostaglandin synthesis through inhibition of cyclooxygenase-1 and -2, and leukotriene biosynthesis by inhibiting 5-lipoxygenase [42,54,58–60]; and by modulating cytokines [61–65] and decreasing activation of NF-κB [62,66–68].

5.2.6 Turmeric

Curcuma longa Linn., a perennial shrub belonging to the family Zingiberaceae, is indigenous to India but is today also cultivated in China, Sri Lanka, and other tropical countries [69]. The roots are the most important part of the plant, and are used as a religious, culinary, and medicinal agent in India [69]. Turmeric is one of the most highly investigated plants, and studies have shown it to contain curcuminoids such as curcumin, desmethoxy curcumin, bisdemethoxy curcumin, monodemethoxy curcumin, dihydrocurcumin, and cyclocurcumin. The essential oil obtained by steam distillation is shown to contain α-phellandrene, sabinene, cineol, borneol, zingiberene, and sesquiterpines [69].

Turmeric is an integral part of the various traditional and folk and tribal systems of medicine in Southeast Asia, and has been used to treat biliary disorders, jaundice, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, inflammation, sinusitis, menstrual difficulties, hematuria, and hemorrhage. Turmeric is also used as an antiseptic, analgesic, anti-inflammatory, antimalarial, and insect repellant [69]. The principal compound and the yellow bioactive component of turmeric, curcumin, is one of the most researched phytochemicals globally, and studies have clearly shown it to possess antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, anti-allergic, gastroprotective, and wound-healing properties; to increase bile secretion and prevent cataractogenesis; to reduce blood cholesterol, prevent LDL oxidation, inhibit platelet aggregation, and suppress thrombosis and myocardial infarction; and to prevent/ameliorate multiple sclerosis and Alzheimer's disease [69–72].

Turmeric is a prominent anti-inflammatory drug in various systems of medicine, and studies have validated the ethnomedicinal claims and observations of it to be effective in the treatment of RA. Chandra and Gupta

[73] investigated the protective effects of turmeric oil on chemically induced arthritis in rats, and observed it to be effective in reducing both inflammation and arthritis. Subsequent studies with turmeric extracts depleted of essential oil and extracts containing 41% of the major curcuminoids were effective in preventing streptococcal cell wall-induced rheumatoid arthritis [40], and to possess better anti-inflammatory effects than that afforded by ginger or indomethacin in the adjuvant-induced arthritis in rats [39].

In addition to turmeric, experiments with laboratory rats have shown that curcumin is also effective in preventing collagen-induced rheumatoid arthritis [74]. Co-treatment with curcumin and methotrexate was shown to possess subadditive protective effects in ameliorating the adjuvant-induced arthritis, and to also reduce the hepatic and hematological toxicity of methotrexate [75, 76]. Additionally, combining curcumin with ibuprofen is reported to decrease inflammation in adjuvant-induced chronic inflammation in rats, and to mediate these protective effects by reducing the levels of NO and TNF-α [77]. Curcumin is also shown to be effective in humans, and reports indicate that 2 weeks of supplementation with curcumin was effective in improving morning stiffness, walking time, and joint swelling, and that the beneficial effects were comparable to those afforded by phenylbutazone [78]. Additionally, recent studies in patients with active rheumatoid arthritis have shown that curcumin was more effective than diclofenac sodium in reducing the Disease Activity Score, and tenderness and swelling of joints [79]. Curcumin is a pleiotropic molecule, and mechanistic studies have shown it to possess free radical scavenging effects [80–83]; to decrease activation of signal transduction pathways [84,85] and nuclear transcription factors like NF-κB and AP-1 [86–88]; to possess anti-inflammatory effects [89,90], modulate cytokines [91–93], and induce apoptosis of synovial fibroblasts [94–96]; to decrease matrix metalloproteinases [97–99]; and to suppress production of B cell-activating factor [100–102] – all of which have a beneficial role in ameliorating arthritis.

5.3 CONCLUSIONS

Preclinical studies have shown that the commonly used dietary spices fenugreek, coriander, ginger, and turmeric, and their phytochemicals 6-shogaol, curcumin, eugenol, and thymoquinone, are effective as antiarthritic agents. Further exploration and randomized clinical studies are necessary to elucidate their pharmacological activities and clinical utility in treating arthritis. The outcomes of such studies may be useful regarding the applications of these spices in the prevention and treatment of RA, either alone or as adjuncts to DMARDs in humans, and may open up new therapeutic avenues. Due to its abundance, low cost, and safety in consumption, these spices remain dietary agents with tremendous potential to develop as non-toxic broad spectrum antiarthritic agents when the gaps in existing knowledge are bridged. In addition to their antiarthritic effects, these spices are observed to possess free radical scavenging, antioxidant, chemopreventive, and anti-inflammatory effects, and to reduce muscular discomfort. All these beneficial effects will also be of help in improving the general health of the individual without imparting any toxic effects.

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CHAPTER

6

Medicinal Benefits of Ginger in Various Gastrointestinal Ailments: Use in Geriatric Conditions

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6.1 INTRODUCTION

The gastrointestinal tract (GIT), including the oral cavity, esophagus, stomach, intestine, colon, and rectum, along with the hepatobiliary systems constitute the gastrointestinal system, which is the primary organ for digestion and absorption. The synchronous peristalsis and secretions help in physical and chemical (enzymatic) digestion [1]. The Auerbach and Meissner's plexuses, along with a host of neurotransmitters – noradrenaline, acetylcholine, the non-adrenergic and non-cholinergic transmitters, and serotonin – play an important role in the process [1]. The GIT serves as the portal for the entry of essential nutrients, and a path for the elimination of waste products and harmful substances from the body [1]. Being a site that constantly interacts with substances external to the body, several disorders, including dyspepsia, distressing nausea/vomiting, inflammations, infections, infestations, cancers, physical obstruction of the intestine, volvulus, etc., may afflict the GIT [1].

The hepatobiliary system, which comprises the liver, gall bladder, common bile duct, and pancreas, works closely with the GIT. The processes of digestion and assimilation are facilitated by the bile and digestive enzymes, which together work through the hepatic portal system. The liver clears any harmful agents that enter the GIT. The liver, GIT, stomach, and pancreas form a system that brings about efficient digestion of nutrients for the body and elimination of potential harmful agents that enter the body through the GIT. Common diseases afflicting these organs include cirrhosis, hepatic encephalopathy, Wilson's disease, cholelithiasis, pancreatitis, and adenocarcinoma [2].

In the geriatric population, the function of the gastrointestinal system is compromised and elderly individuals frequently have oropharyngeal muscle dysmotility and difficulty in swallowing food. Gastroesophageal reflux is also common, and is possibly due to decreased esophageal peristalsis and lower esophageal sphincter (LES) pressures; delayed motility and gastric emptying are also observed. There is a decrease in gastric secretions (acid, pepsin) and impairment of the mucus–bicarbonate barrier, which can lead to gastric ulcers. Exocrine pancreatic secretion is often decreased, as is the bile-salt content of bile. Additionally, the propulsive motility of the colon is decreased, and this is associated with neurological and endocrine–paracrine changes in the colonic wall. Cumulatively, these changes contribute towards

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symptomatic gastrointestinal dysfunctions in the elderly, such as dysphagia, gastroesophageal reflux disease, primary dyspepsia, irritable bowel syndrome, primary constipation, maldigestion, and reduced absorption of nutrients [3]. Considering the vital functions that the gastrointestinal system performs, the maintenance of its health is very important.

Reports suggest that the kitchen spice ginger, a rhizome obtained from the plant *Zingiber officinale* Roscoe, is extremely beneficial in ameliorating various gastrointestinal disturbances, ailments, and diseases [4,5]. This is the focus of this chapter.

6.2 GINGER IN TRADITIONAL MEDICINE

Ginger belongs to the family Zingiberaceae, and is believed originally to have been native to the northeast region of India [6]. Ginger has been cultivated for thousands of years both as a spice and for its medicinal purposes in India, and is today found growing in China, Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica, Nepal, Haiti, Mexico, and Hawaii [6]. Ginger is one of the world's most important culinary agents, and is used as a medicinal agent in various forms of alternative medicine [6]. It is documented as being of use in treating colds, headache, nausea, stomach upset, diarrhea, arthritis, rheumatological conditions, and muscular discomfort; in assisting digestion; and as a carminative and antiflatulent [4,6]. Scientific studies have shown that ginger possesses antimicrobial, antischistosomal, anti-inflammatory, antipyretic, antioxidative, hypoglycemic, hepatoprotective, diuretic, and hypocholesterolemic effects [4,5].

6.3 CHEMISTRY OF GINGER

The pharmacological properties of ginger are attributed to the presence of both volatile constituents, including zingeberene, curcumene, farnesene, bisabolene, β-sesquiphellandrene, 1,8-cineole, linalool, borneol, neral, and geraniol, and non-volatile components such as gingerols, shogaols, paradols, and zingerone [6]. Ginger protease, capsaicin, gingediol, galanolactone, gingesulfonic acid, galactosylglycerols, gingerglycolipids, diarylheptanoids, neral, and phytosterols are among other important compounds found in ginger [4,6,7]. Some of these phytochemicals are depicted in Figure 6.1. The composition of these phytochemicals in ginger varies according to temperature, availability of water, humidity, soil conditions, harvesting time, and age of the plant/rhizome [6].

6.4 GINGER IN GASTROINTESTINAL AILMENTS

Ginger has been well-documented as being very effective against symptoms of gastrointestinal conditions, such as constipation, indigestion, nausea, and vomiting, in the Chinese, Ayurvedic, Arabic, Tibetan, Unani, and various folk systems of medicines [4,6]. Ginger also acts as a stimulant and carminative, and is used frequently against dyspepsia and colic [4,5]. Preclinical studies have shown that ginger stimulates the production of saliva and promotes the release of bile from the gall bladder [4,5]. It is also gastroprotective, anti-ulcerative, anti-emetic, and preventive against epigastric discomfort, dyspepsia, stomach ache, abdominal spasm, and cancer of the gastrointestinal system [4,5]. In the following sections, the beneficial effects of ginger on the gastrointestinal system are addressed.

6.4.1 Ginger in Oral Health

Globally, dental caries and periodontal diseases are among the most common infections and at times can affect the quality of life. Park *et al*. [8] investigated the antibacterial effects of ginger and its constituents on the growth of the oral bacteria that cause periodontitis. It was observed that the ethanol and n-hexane extracts of ginger could exhibit antibacterial activity on *Porphyromonas gingivalis* ATCC 53978, *Porphyromonas endodontalis* ATCC 35406, and *Prevotella intermedia* ATCC 25611. Fractionated studies were also performed, based on the differential activities, and it was observed that 10-gingerol and 12-gingerol could effectively inhibit the growth of these oral pathogens at a minimum inhibitory concentration range of 6–30 μg/ml and a minimum bactericidal concentration (MBC) range of 4–20 μg/ml [8].

6.4.2 Ginger Prevents Epigastric Discomfort and Dyspepsia

In various systems of medicine ginger has been reported to possess carminative effects, including decreasing the pressure on the lower esophagus; reducing intestinal cramping, flatulence, and bloating; and preventing dyspepsia [4,5]. Recently, Lohsiriwat *et al*. [9] studied the effect of ginger on the esophagus and on lower esophageal sphincter function in healthy male volunteers [9]. It was observed that ginger administration enhanced relaxation of the lower esophageal sphincter and decreased esophageal contraction velocity, possibly leading to the antiflatulent effects of ginger. The consumption of ginger (1 g of dried powder) did not, however, affect the lower esophageal sphincter pressure at rest, or esophageal contractile amplitude and duration while swallowing.

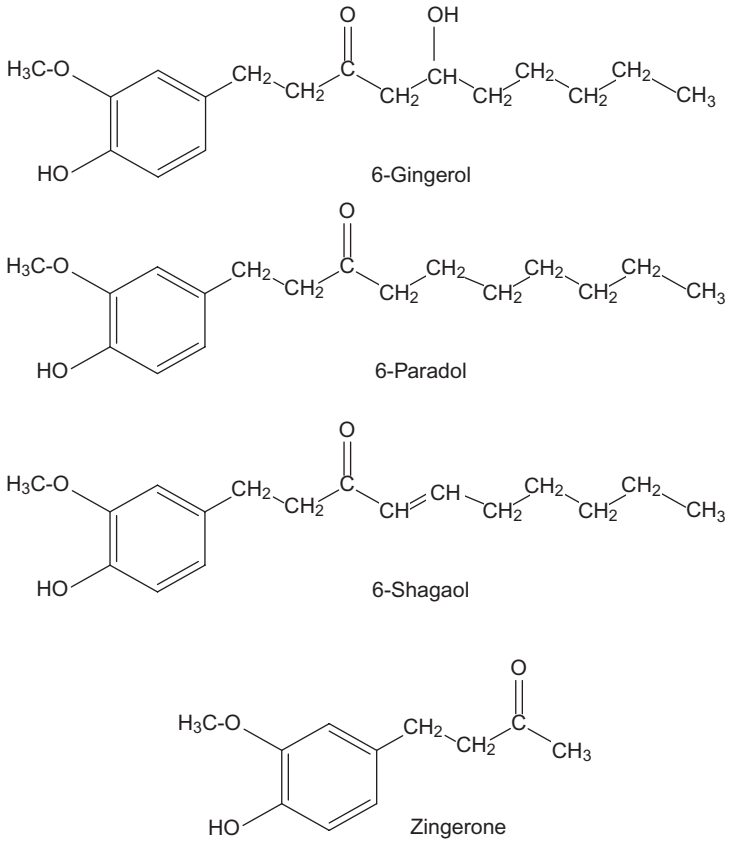

FIGURE 6.1 Structures of some phytochemicals present in the ginger rhizome.

6.4.3 Ginger is Effective Against Various Gastric Ulcerogens

Gastrointestinal ulceration, characterized by severely inflamed lesions of the gastrointestinal mucosa, is one of the most common disorders affecting humans and is associated with severe morbidity and mortality. Factors such as smoking, anti-inflammatory drugs, alcohol, stress, fatty foods, and *Helicobacter pylori* infection are known to initiate and aggravate peptic ulcers [10]; however, an exact cause is still unknown. Conventionally used drugs are often associated with serious side effects, including arrhythmia, impotence, gynecomastia, arthralgia, hypergastrinemia, and hemopoeitic changes, and may also be ineffective at preventing relapses. Hence, further studies are required to develop a drug that is efficient at preventing ulcers while having minimal or no side effects.

Whole ginger and its phytochemicals have been shown to be gastroprotective in various standard experimental models, such as ulceration induced in rats by HCl/ethanol, 80% ethanol, 0.6-M HCl, 0.2-M NaOH, 25% NaCl, indomethacin, aspirin, reserpine, hypothermic restraint, swim stress/ethanol stress, and pylorus ligation [11–13]. The decoction prepared from dry ginger is better than that prepared from roasted ginger in preventing gastric ulcers in rats, indicating that roasting may deactivate its active compounds [14]. Moreover, 500 mg/kg of orally administered ginger extract can prevent the ulcerogenic effects of indomethacin, including generation of free radicals, inhibition of prostaglandin synthesis, and increased expression of IL-1 and TNF-α [15,16]. According to a recent report, oil of ginger protects against aspirin- and pylorus ligation-induced ulcerations in rats [17]. Likewise, the phytochemical zingiberene is effective against HCl/ethanol-induced gastric ulceration in rats [11], while gingerol is effective against HCl- [18] and HCl/ethanol [16] -induced ulceration, and 6-gingesulfonic, 6-gingerol, and 6-shogaol are effective against HCl/ethanol [16] -induced gastric ulcerogenesis in rats.

Studies by Mahady *et al*. [19] have shown that the methanol extract of ginger, the extract fractions, and the isolated constituents 6-,8-,10-gingerol and 6-shogoal inhibited the growth of different strains of *H. pylori in vitro* with a minimum inhibitory concentration in the range of 6.25–50 μg/ml [19]. The fraction containing gingerols was the most active, inhibiting the growth of all *H. pylori* strains. A significant amount of activity was

reported even against the more virulent CagA$^+$ strains [19]. It has been shown that ginger possesses inhibitory effects on the growth of *H. pylori*, and that this effect is mediated at least in part by the scavenging of free radicals, inhibition of lipid peroxidation, and protection of DNA against possible damaging effects [20].

6.4.4 Ginger is an Effective Anti-emetic Agent

Nausea (from the Greek for motion sickness) and vomiting (emesis) are the two common distressing symptoms associated with derangement of the GIT or adverse effects of drugs [21]. Ginger has been shown to be efficacious in preventing nausea and vomiting during early pregnancy [22–26] without increasing the chances of any pregnancy-related complications, changing the pregnancy outcome, or the occurrence of congenital abnormalities [27]. Ginger is also reported to prevent postoperative nausea and vomiting [28–30].

With regard to motion-related sickness, the possible anti-motion sickness effects of ginger are thought to be due to its effect on the central and peripheral cholinergic, histaminergic, and serotonergic pathways [31]. Ginger has been observed to prevent motion sickness in humans [32,33], and has also been shown to be effective against sea sickness in naval cadets [34,35]. However, a study by Stewart *et al*. shows rather divergent results, observing that pretreatment with ginger was ineffective in preventing motion sickness [36].

The effectiveness of ginger against chemotherapy- and radiation-induced nausea and emesis has been proved in studies on healthy mongrel dogs. Here, the acetone extract was more valuable than ethanolic extract, but was less effective than granisetron [37]. Acetone extract, 50% ethanolic extract, and fresh ginger juice have also been reported to be effective in preventing cisplatin-induced delayed gastric emptying in rats [38]. The reversal produced by the ginger juice was better than with the 5-HT3 receptor antagonist ondansetron, while that of the acetone extract was similar to it [38]. Although ginger has been proven to be effective in preventing chemotherapy-induced nausea in animals, the observations are not consistent in other studies performed in humans. In a double-blinded crossover study by Manusirivithaya *et al*. [39] the addition of ginger to the standard anti-emetic regimen of gynecologic oncology patients receiving cisplatin was ineffective in reducing chemotherapy-induced nausea and vomiting (CINV) in an acute phase of cisplatin-induced emesis, while in the delayed phase its effect was comparable to that of metoclopramide [39]. However, in combination with ondansetron and dexamethasone, ginger proved to be effective in reducing both acute and delayed CINV in bone sarcoma patients undergoing cisplatin and doxorubicin chemotherapy [40].

Ginger also reduces the chemotherapy-induced nausea and vomiting stimulated by low-dose cyclophosphamide when used in combination with other emetogenic anticancer drugs [41]. Interestingly, these results indicate that the anti-emetic effect of ginger is equal to that of metoclopramide but lower than that of ondansetron [41]. The effects of ginger against chemotherapy-induced delayed nausea can also be enhanced by combining it with a high-protein diet in cancer patients [42]. Contradicting these observations, a study by Zick *et al*. [43] revealed that ginger is of no benefit in reducing the prevalence or severity of acute or delayed CINV when combined with 5-HT3 receptor antagonists and/or aprepitant. Furthermore, the authors observed that the participants who took both ginger and aprepitant had more severe acute nausea than did participants on aprepitant only, suggesting a possible antagonistic effect of ginger on aprepitant [43]. The anti-emetic property of ginger attributed to the active component gingerol, especially against cisplatin, is similar to that of ondansetron, used as a positive control [44]. Mechanistic studies have shown that gingerol causes dose-dependent suppression in the levels of substance P and NK1 receptors in the area postrema and ileum, suggesting its action to be similar to that of aprepitant [44].

Sharma and colleagues [45] have also observed that intraperitoneal administration of the hydroalcoholic extract of ginger 1 hour before exposure to 2 Gy of γ-irradiation was effective in blocking the saccharin avoidance response for 5 post-treatment observational days. A time- and dose-dependent protective effect was observed, and the dose of 200 mg/kg body weight was found to be the most effective in males [45] while 250 mg/kg body weight was the most effective in female rats, suggesting the existence of sex dichotomy in the effect [46].

Mechanistic studies suggest that 6-, 8-, and 10-gingerol and 6-shogaol exert their anti-emetic effect at least in part by acting on the 5-HT(3) receptor ion-channel complex, probably by binding to a modulatory site distinct from the serotonin binding site. This may include the indirect effects via receptors in the signal cascade behind the 5-HT(3) receptor channel complex, such as substance P receptors and muscarinic receptors [47]. The activity can be summarized as 5HT3 antagonist, NKI antagonist, antihistaminic, and prokinetic effects, and is devoid of any adverse effects.

6.4.5 Ginger Alters Gastrointestinal Motility

Preclinical studies have shown that ginger extract enhanced the intestinal travel of charcoal meal in mice [48]. Other *in vitro* studies have also suggested that ginger (0.01–1000 mg/ml) inhibits both prejunctional and postjunctional ileal contractility, and that

the prejunctional inhibitory effect of ginger on enteric excitatory transmission might involve a capsazepine-sensible site [49]. The phytochemicals 6-shogaol and 6-, 8-, or 10-gingerol were also observed to be effective at enhancing the transport of a charcoal meal, and their effects were similar to or slightly weaker than those of metoclopramide and domperidone [50]. Zingerone, a pungent phytochemical, was shown to inhibit the spontaneous contractile movements in the isolated colonic segments in a concentration-dependent manner. This inhibitory effect was not affected by pretreatment with capsazepine or tetrodotoxin (an antagonist of transient receptor potential vanilloid 1, and a blocker of voltage-dependent sodium channels, respectively) on neurons, suggesting that zingerone is capable of acting on smooth muscle [51]. Zingerone is also known to assuage colonic motility in rats in a reversible and reproducible manner without affecting blood pressure and heart rate [51]. Clinical studies support that ginger accelerates gastric emptying and stimulates antral contractions in healthy volunteers [52].

6.4.5.1 Ginger Affects Digestive Enzymes

In the various traditional systems of medicine, consumption of tea brewed from fresh ginger after lunch or dinner is regarded to enhance digestion. Scientific studies have substantiated this hypothesis. Preclinical studies have shown that feeding rats with a ginger (50 mg%)-incorporated diet for 8 weeks enhanced the intestinal lipase, sucrase, and maltase activities, and stimulated the synthesis of trypsin and chymotrypsin [53,54]. Apart from these digestive enzymes, ginger is also known to increase the activities of pancreatic lipase and amylase *in vitro* [55] and *in vivo* [54]. According to recent reports, ginger also stimulates the activity of the brush-bordered membrane enzymes (glycyl-glycine dipeptidase, leucine amino peptidase, and gamma-glutamyl transpeptidase) in the jejunal mucosa [56].

6.4.6 Ginger Increases Brush-Border Surface Area and Alters its Membrane Fluidity

Absorption along the 6-meter long intestine depends on the absorptive surface area in particular. Ginger has been shown to alter the membrane fluidity of the brush border favorably. Feeding ginger (0.05%) to Wistar rats for 8 weeks in their diets has been associated with an increased fluidity of the brush-border membrane in the jejunal and ileal regions by decreasing the cholesterol to phospholipid ratio. Scanning electronic microscopy of the intestinal villi of the experimental rats showed an increase in the microvilli length and perimeter, suggesting that the beneficial effects may partly be due to the increase in the absorptive surface of the small intestine [56].

6.4.7 Effect of Ginger on Intestinal Pathogens

Intestinal infections by viruses, bacteria, protozoans, nematodes, and helminths affect intestinal absorption, nutrition, and development. Preclinical research proved that ginger possesses anthelmintic effects against human *Ascaris lumbricoides* [57,58], *Anisakis* larvae [59] and *Haemonchus contortusa* – a pathogenic nematode of ruminants [60]. However, it is ineffective in preventing entry of rotavirus into the MA-104 cells and the trophozoites of *Giardia lamblia in vitro* [61].

Multiple studies have shown the antibacterial effect of ginger and some of its phytochemical components on both sensitive and drug-resistant bacteria [62–64]. The essential oil of ginger is observed to be effective on *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* [65]. However, contradictory reports also exist, and recent reports by Daswani *et al*. [61] suggest ginger to be ineffective as an antibacterial agent on some strains of the enteropathogenic *E. coli*, *V. cholerae*, and *S. flexneri.*

6.4.8 Ginger is Effective in Inflammatory Bowel Disease

Inflammatory bowel disease is an important immune-mediated disease of the gastrointestinal tract, comprising ulcerative colitis and Crohn's disease, whose management presents a constant challenge for gastroenterologists and researchers. Conventionally, surgery and medical treatment with 5-aminosalicylates, antibiotics, steroids, and immunomodulators have been used to reduce the symptoms and prevent remission of the condition. Unfortunately, long-term use of these therapeutic agents inflicts severe toxicity, which is a deterrent for users [66]. Experimental studies have shown that pretreatment with ginger extract ameliorates the acetic acid-induced edematous inflammation in the colon. Furthermore, histopathological studies confirm that ginger attenuates the extent and severity of edema, desquamation, necrosis, and inflammatory cell infiltration in the mucosa. The levels and activity of colonic myeloperoxidase (MPO), lipid peroxides, protein carbonyl content, TNF-α, and prostaglandin E2 (PGE2) were also decreased. Administration of ginger has been shown to lead to restoration of the levels of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD); reduction in the levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IFN-γ), colonic MPO, lipid peroxides, protein carbonyl content, and PGE2; and a reduction in the elevated expression of NF-κB [67]. The protective effects of the highest doses of ginger have shown to be comparable to that of the standard sulfasalazine [68].

6.4.9 Ginger Prevents Diarrhea

Globally, diarrhea, which is caused by intestinal pathogens, is a major health concern and a major cause for infant mortality in developing countries [69]. The majority of diarrheal incidences are due to the enteric bacteria, especially the enteropathogenic *E. coli*, *Vibrio cholerae*, *Campylobacter*, *Salmonella typhi*, and *Shigella flexneri.* Preclinical studies in this regard have shown that ginger blocks the binding of heat-labile enterotoxin to the cell-surface receptor GM1, resulting in the inhibition of fluid accumulation in the closed ileal loops of mice [70]. Biological activity-guided studies indicate that zingerone might be responsible for ginger's antidiarrheal efficacy [70]. Ginger reduces colonization of the epithelial cells HEp-2 by enteropathogenic *E. coli*, enteroinvasive *E. coli*, and *Shigella flexneri* [61]. *In vitro* studies have also shown that ginger does not arrest the growth of *V. cholerae* but is capable of inhibiting the production of cholera toxin, thereby implying that it selectively affects the metabolic pathway(s) responsible for toxin production [61].

Other experimental studies have also been successful at showing that ginger administration significantly inhibits 5-HT induced diarrhea [71]. Also, the active principles 6-shogoal, 6-dehydrogingerdione, and 8- and 10-gingerol have been reported to possess anticathartic action [71]. *In vitro* studies with guinea pig ileum, rat stomach fundus, and rabbit aortic strips have successfully demonstrated the inhibition of contractile responses to 5-HT by galanolactone, a diterpenoid isolated from ginger. The inhibitory effect of galanolactone on the 5-HT response in the stomach fundus and aortic strips was found to be less than that in the ileum, an effect related to antagonism of 5-HT3 receptors [71].

6.4.10 Hepatoprotective Effects of Ginger

Myriad preclinical studies have shown ginger to possess hepatoprotective action against different hepatotoxins, when administered either in the form of alcoholic extract, aqueous extract, and oleoresin or as a dietary constituent, at varying effective doses. Administration of ginger has also been reported to reverse the histological abnormalities in liver, enhance the antioxidant status, and normalize the activity of liver marker enzymes in the serum, which are altered by oxidants and xenobiotics [72–78]. Ginger increases the activities of detoxifying enzymes in the liver of experimental animals following administration of xenobiotics. In studies conducted at the National Institute of Nutrition in Hyderabad, India, feeding ginger powder at 0.5–5% to rats increased the activities of the enzymes glutathione S-transferase, UDP-glucuronyl transferase, and quinine reductase in the liver [79,80]. Administration of ginger oil could also elevate glutathione S-transferase and aryl hydrocarbon hydroxylase activities in the liver of mice [81]. Ginger extract fed to experimental rats increased the levels of cytochrome P450 and cytochrome b5 [82,83].

Ginger prevents p-hydroxybenzoic acid- [84] and paraben-induced lipid peroxidation [84]. Studies by Tao *et al*. [85] have also shown that ginger diarylheptanoids and monoterpenoid possess superoxide anion scavenging effects, thereby preventing oxidant-induced lipid peroxidation in mouse liver microsomes and protecting cultured hepatocytes against the cytotoxic effects of oxidants. Ginger has also been proven to be effective in reducing hepatotoxicity induced by paraben [74], ethanol [75,76,86], macozeb [77], bromobenzene [83], cadmium [87], ethionine [88], acetaminophen [89], paracetamol [90], the anticancer drug adriamycin [77], and carbon-tetrachloride [72]. Ginger regularizes deranged liver enzymes, lipid metabolism, and blood parameters, thus minimizing histopathological damage caused by poisoning [91]

Administration both of ginger and atorvastatin (a statin effective in reducing hypercholesterolemia) reduces atorvastatin-induced hepatic injury and lesions. Also, administering ginger extract reverses atorvastatin-induced liver injury and decreases the serum levels of serum aminotransferases, hepatic malondialdehyde, and nitric oxide. Together, these observations indicate that a combination of ginger and statins could be used in treating hypercholesterolemia, and might be helpful in decreasing statin-induced hepatotoxicity [92].

6.4.11 Ginger Rectifies Hepatic Lipid Metabolism

Hyperlipidemia contributes to cardiovascular disease, and observations that ginger possesses hypolipidemic effects have generated a lot of interest. Studies suggest that ginger mediates these effects by inducing bile acid synthesis, repressing cholesterol synthesis, inhibiting low-density lipoprotein (LDL) oxidation and aggregation, and promoting the uptake and catabolism of LDL – the "bad" cholesterol [78,93–97]. A double-blinded controlled clinical trial involving patients with hyperlipidemia showed a significant reduction in serum levels of triglycerides, cholesterol, low-density lipoprotein, and very low-density lipoprotein (VLDL) [98].

Fuhrman and colleagues [96] have also investigated the effects of standardized ginger extract on the development of atherosclerosis in apo-E deficient mice *ex vivo*, showing a significant reduction in atherosclerotic lesions in the aorta, and in plasma triglycerides, cholesterol, and low-density lipoprotein. A significant inhibition of cellular cholesterol biosynthesis in peritoneal macrophages

was also observed in the apo E-deficient mice fed on 250 μg/day ginger for 10 weeks. Reduction in the LDL basal oxidative status and inhibition of LDL aggregation were also reported in animals fed on 25 or 250 μg ginger extract [96].

Ginger supplementation is also shown to upregulate LDL receptor gene expression and downregulate the HMG-CoA reductase gene expression in the liver of rats [94]. Another mechanism responsible for the hypolipidemic action of ginger is an increase in the activity of hepatic 7-α hydroxylase, the rate-limiting enzyme of bile acid synthesis [93]. Ginger supplementation to rats supported on a cholesterol-enriched diet causes a decrease in the expression of retinoid-binding protein and fatty acid binding protein genes in liver and adipose tissue [99]. Ginger has also been reported to reduce the plasma levels of triglycerides, cholesterol, and low-density lipoprotein, with a concomitant increase in the level of high-density lipoprotein, in high-fat or high-cholesterol diet-fed rats [78,95–97].

Ginger serves as a natural supplement in the prevention of and reducing the progression of non-alcoholic fatty liver disease, by sensitizing insulin effects; downregulation of various pro-inflammatory cytokines; and activating peroxisome proliferator-activated receptor γ, which in turn induces adiponectin, thus changing the balance between adiponectin and TNF-α, promoting antioxidant effects and reducing hepatic triglyceride content which can prevent steatosis [100].

6.4.12 Ginger in the Prevention and Treatment of Gastrointestinal Cancer

Today, cancer is globally the world's second leading cause of death after CVD, and reports from the International Agency for Cancer Research indicate that approximately 12.7 million new cancer cases were diagnosed and 7.6 million cancer deaths occurred in the year 2008 [101]. Projections are that by the year 2020 the incidence of cancer will have increased three-fold and a disproportionate rise in cancer cases and deaths will occur in developing countries, which have limited resources to tackle the problem [102]. Additionally, chemotherapy and ionizing radiation, two of the most common treatment modalities, are non-specific in action and also affect healthy normal cells, causing ill effects such as nausea and vomiting, immuno- and myelosuppression, and cardio-, hepato-, and nephrotoxicity, thereby affecting the patient's quality of life [6,103].

Cancers of the gastrointestinal system, such as oral cancers and cancers of the esophagus, stomach, colon, liver, pancreas, and gall bladder, account for a large proportion of these statistics, and inflict significant morbidity and mortality [101,102]. Scientific studies carried out in the past two decades have confirmed that ginger and its phytochemicals are effective in many of the gastrointestinal cancers [6,7,104]. Cell culture studies have shown ginger extract to cause antiproliferative effects on Dalton's lymphoma ascitis tumor cells of mouse [105], and HEp-2 [106] and YYT colon cancer cells [107]. Additionally, ingestion of ginger is shown to prevent ethionine-induced hepatocarcinogenesis in Wistar rats, and to mediate these protective effects by scavenging free radicals and reducing the generation of lipid peroxidation [88]. However, with regard to the prevention of chemical (DMH)-induced colorectal carcinogenesis, the results are contradictory. While the observations of Manju and Nalini [108,109] demonstrate ginger to be effective, the results of Dias and co-workers [110] have shown ginger to be ineffective in preventing DMH-induced colon carcinogenesis in rats.

With regard to phytochemicals, experiments have shown antineoplastic effects of 6-gingerol in the human pancreatic cancer cell line HPAC [111], human liver (HepG2) cells [112], YYT colon cancer cells [107], and HCT-15 (colon cancer) cell lines [113]; of 6-shogaol in liver (Mahlavu cells) [114]; and of dehydrozingerone in HCT-15 cells [115], colon cells COLO 205 [116], and HCT-15 cells [113]. The other constituents, such as 6- and 10-paradol, are shown to induce apoptosis in the human oral squamous carcinoma cell line (KB) [117], while the 4-, 8-, and 10-gingerols and zerumbone present in the subtropical ginger *Zingiber zerumbet* cause cytotoxity and expression of NF-κB in HCT-15 (colon cancer) cell lines [113,118]. Mechanistic studies have shown that ginger mediates its protective effects by several mechanisms, the most important being free-radical scavenging; antioxidant, antimicrobial, antimutagenic and anti-inflammatory activities; an increase in the antioxidant enzymes; modulation of Phase I and II enzymes, signal transduction, transcription factors, and the cell cycle; and induction of selective apoptosis in neoplastic cells [6].

6.5 CONCLUSIONS

Ginger is used to treat several gastrointestinal diseases, and research findings in both preclinical and human studies prove most of these ethnomedicinal observations (summarized in Figure 6.2). Due to its availability, low cost, and safety in consumption, ginger is a species with tremendous potential for further investigation. The various pharmacological activities of ginger appear to be due to the presence of its many phytochemicals. Due to the contradictory results that have been seen in the use of ginger (for example, in certain pharmacological effects – antibacterial effects,

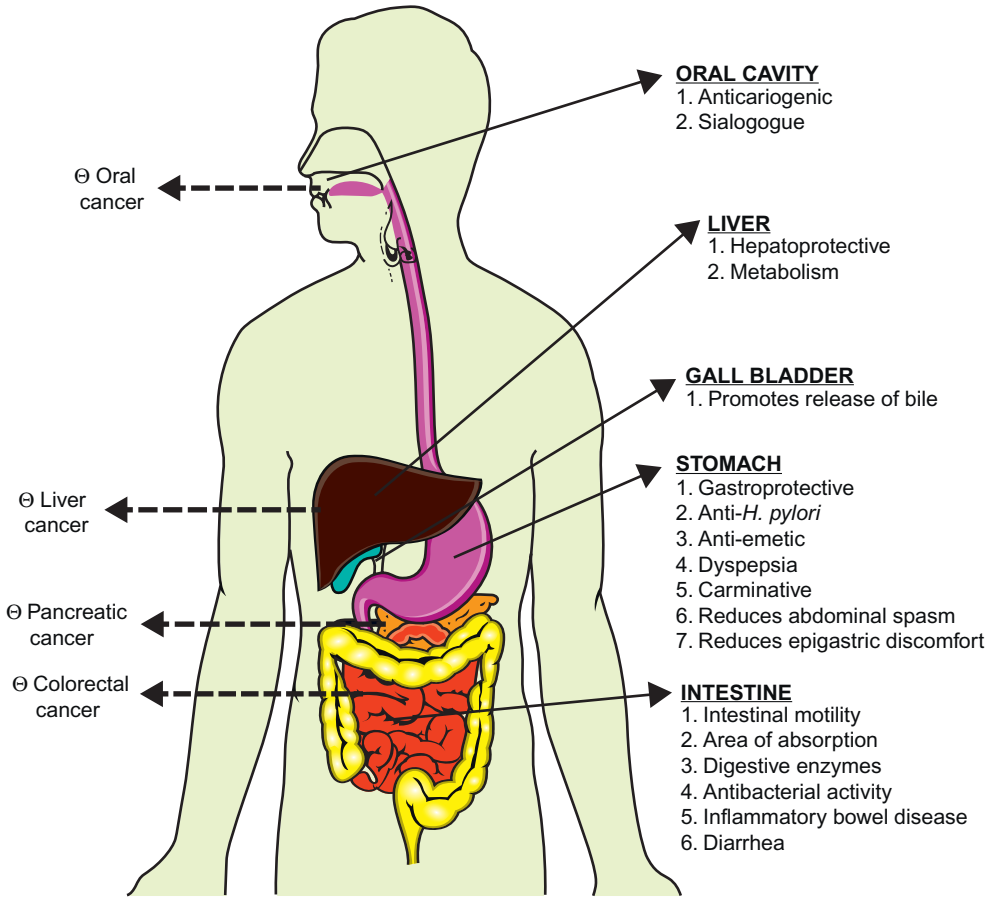

FIGURE 6.2 Effects of ginger and its phytochemicals on the various gastrointestinal functions and in inhibition of cancer (Θ = inhibition).

and anti-emetic effects against motion sickness and chemotherapy-induced nausea in humans) which could be due to several phytochemicals in the ginger used, future studies should aim at understanding which bioactive compound is responsible for the beneficial effect. This will help in understanding the possible mechanism of action responsible for the observed pharmacological effects and appreciation of ginger.

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CHAPTER

7

Foods and Dietary Supplements in the Prevention and Treatment of Neurodegenerative Diseases in Older Adults

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7.1 INTRODUCTION

The population of the world is aging. Among the most significant concerns in aging populations are mental health and nervous system diseases. A progressive neuronal atrophy may occur in older people and, consequently, neurologic functions may increasingly become reduced. Among neurogenerative diseases, Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) are of the greatest concern. Genetics is the primary cause, but some sources mention chronic inflammatory processes and oxidative stress as contributing factors. Based on these mechanisms, the proposal of the likelihood of the impact of nutrition and diet on prevention and controlling the disease arises, for instance, some pro-oxidant minerals such as iron aggregate in the brain as it is aging, especially in men, which may trigger neurodegenerative diseases like AD and PD [1]. In this chapter, the role of dietary antioxidants in the prevention and treatment of neurodegenerative diseases is highlighted.

Along with the role of poor nutritional status in the early stages of neurodegenerative diseases, at the end stages dysphagia may occur as a result of progressive neuronal and muscular dysfunction. Since it is a common problem in most neurodegenerative diseases, feeding strategies for elderly people with dysphagia are presented at the end of this chapter.

7.2 DEMENTIA AND ALZHEIMER'S DISEASE

The prevalence of dementia is increasing dramatically from 5% to 25% among adults aged 71–90 years old [2]. Alzheimer's disease (AD) and vascular dementia, respectively, are the most important types of all cases of dementia. Observational studies show that insulin resistance, adiposity, oxidative stress, inflammation, hyperlipidemia, and hypertension are among the risk factors for cognitive decline in older adults [3].

AD is a progressive neurodegenerative disease with aggregation of β-amyloid peptide in the brain. The most important risk factor for AD is age. Genetics and Apolipoprotein-E4 (Apo-E4) are the leading factors in the etiology of AD. Apo-E4 is a protein that combines with β-amyloid and takes part in cholesterol transportation. Hence, studies have suggested high serum cholesterol,

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elevated homocysteine, oxidative stress, and damage to mitochondrial components as other contributory factors. Choline may inhibit the development of β-amyloid aggregation in the brain of patients with AD [4].

Other studies discuss inflammatory and oxidative pathways as well as the pro-oxidant role of some minerals, such as lead, copper, zinc, and iron, in the pathogenesis of cognitive decline in older subjects. Because of the inflammatory and oxidative mechanisms of cognitive decline, the whole dietary pattern and some specific nutrients and supplements can affect neurodegenerative diseases. Excessive intake of pro-oxidants, saturated fatty acids, and alcohol, and deficiency of antioxidant nutrients, may be the trigger for the progressive cognitive decline. On the other hand, diets rich in vitamins, antioxidants, and unsaturated fatty acids (such as sea foods, poultry, nuts, curcumin, tomatoes, fruits, garlic, and dark and green leafy vegetables) may be supportive.

7.2.1 Antioxidants and Alzheimer's Disease

The oxidative properties of neurodegenerative diseases have led to the proposal of antioxidant use for controlling them. The antioxidant properties of dietary phytochemicals, such as theaflavins and catechins, anthocyanins, curcumin, and resveratrol, are sufficient to justify the inclusion of tea, green tea, grapes, peanuts, berries, turmeric, and other dietary sources of polyphenols in dietary planning for patients, with the intention to prevent or control AD [5].

Some clinicians consider the use of supplements such as vitamin C, lipoic acid, carotenoids, or polyphenols in AD prevention and/or treatment [6]. A number of investigators have reported that patients with AD may benefit from the intake of 800–2000 IU/day vitamin E supplementation, but others declare that the evidence is not convincing [7]. In all, better designed and larger clinical trials are needed to reveal the effects of antioxidants on AD.

7.2.2 Vitamin D and Alzheimer's Disease

Some observational studies have reported low serum vitamin D concentrations in patients with AD [8]. Vitamin D is a regulator of signal transduction and gene expression. It regulates calcium-sensing receptor expression, increases the clearance of β-amyloid peptides, and modulates inflammatory cytokine expression. Annweiler and colleagues studied 498 healthy elderly women and found that those with an initial lower intake of dietary vitamin D were more likely to develop AD in a 7-year follow-up period [9]. In all, vitamin D may play a beneficial role in AD. The metabolically active form of vitamin D3, 1,25-dihydroxyvitamin D3 (calcitriol), is the most effective supplementation form of vitamin D in treating AD [10].

7.2.3 Omega-3 Fats and Alzheimer's Disease

Omega-3 fatty acids accumulate physiologically in the brain, where they may play structural or functional roles. Observational studies have shown a reverse correlation between fish intake or serum omega-3 fatty acid concentration, and dementia. Clinical findings on the effects of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on AD have shown conflicting results. Some effects of DHA on mild dementia have been observed [11]; however, Quinn *et al*. reported no superiority of DHA supplementation over placebo in controlling the cognition and functional downturn in patients with mild to moderate AD [12]. Well-designed and in-depth clinical trials are needed to ascribe the precise effects.

7.2.4 The Role of a Good Caregiver in Nutritional Status of Patients with Alzheimer's Disease

As elderly patients with dementia decline in cognition, they may forget to eat, or may eat other people's foods, or even consume non-edible or poisonous items. Hence the role of a good caregiver is very important regarding the emotional and nutritional status of patients with AD.

Impairment in swallowing is often observed in the end stages of AD. Strategies for controlling dysphagia are discussed at the end of this chapter.

7.3 MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) in which demyelinization of nerves occurs and multiple regions of CNS undergo "sclerosis."

Adrenocorticotropic hormone (ACTH) and prednisolone are the most prescribed drugs for treating MS, particularly in patients of less than 5 years' duration. However, increasing appetite, weight gain, edema, and reduction in the serum levels of vitamin B12 and folate are common side effects of steroids. Methotrexate is another drug that may be prescribed with ACTH. Nausea and anorexia are side effects of methotrexate [4]. However, methotrexate also interacts with some dietary factors – for example, caffeine can reduce the efficacy of methotrexate medication, so caffeine-rich foods (tea, coffee, chocolate, and cola) should be reduced in those being treated with methotrexate, and a dose adjustment may be necessary for patients who consume a large quantity of caffeine in their diet. Methotrexate may have an effect on the liver, and alcohol (ethanol) intake may increase the risk. Methotrexate is also an antagonist of folic acid, and may increase calcium loss [13].

Neurogenic bladder and urinary tract infections (UTIs) are common in patients with MS. Limiting fluid intake before bed time and the compensation of liquids in the early morning hours may prevent UTIs and enhance the patient's health and quality of life. Adding cranberry or cranberry juice or supplements to the diet of patients with MS may help in decreasing the risk of UTIs [4].

Neurogenic bowel is also common in patients with MS. Addition of dietary fibers, particularly soluble fibers such as in apple, figs, plums, and prunes, may be helpful [4]. At the end stages of MS dysphagia may occur. For tips on managing dysphagia, refer to the end of this chapter.

7.3.1 Vitamins, Polyunsaturated Fatty Acids, and Multiple Sclerosis

Some epidemiologic studies are uncovering the correlation of MS with serum vitamin D levels [14], while others have associated MS with exposure to sunshine and diet, revealing the role of vitamin D3 in preventing the progression of MS [4]. Immune system enhancement is proposed for this role. However, clinical studies of the application of vitamin D supplements are conflicting [14]. Supplementation with vitamin D is safe and helpful in achieving normal levels in patients with co-morbid vitamin D deficiency and MS [4]; however, studies of other vitamin supplementation, polyunsaturated fatty acids (PUFA) (including omega-6 or omega-3) supplementation, or application of special dietary regimens such as an allergen-free diet are not conclusive for treatment of MS [15] although patients with MS have shown reduced episodes of relapse following omega-6 consumption in some studies [15].

7.4 PARKINSON'S DISEASE

Parkinson's disease (PD) is another age-related neurodegenerative disease and is characterized with tremor at rest, muscle firmness, and diminished domain or slowness of movement (bradykinesia), which occurs due to decreased dopamine transmission [4]. Oxidative stress is among the proposed pathogeneses of PD.

Exelon® and L-dopa are the most useful drugs for controlling PD symptoms. In order to tolerate the gastrointestinal side effects, it is recommended that L-dopa be taken with meals. Limiting dietary protein at breakfast and lunch and gathering protein sources at dinner may allow L-dopa to control the bradykinesia better. L-dopa also interacts with high doses of supplementation with vitamin B6 and manganese [4].

7.4.1 B Vitamins and Parkinson's Disease

Elevated plasma homocysteine levels may damage dopaminergic nerves and can be a risk factor for Parkinson's disease [4,16]. The B vitamins, especially folate and B12, play a fundamental role in the metabolism of homocysteine; hence folate or vitamin B12 deficiency and increasing homocysteine levels may cause deterioration in patients with PD. Moreover, some observational studies have shown increased risk of PD with decreased intake of vitamin B6 [16]. Thus, vitamin B6 supplementation along with a vitamin B-rich diet may decrease the risk of PD [4].

7.4.2 Therapeutic Effects of Curcumin in Parkinson's Disease

The common medications for treating PD are used to alleviate the symptoms. In traditional medicine, some therapies involving natural antioxidants and neuroprotective plant products are used. Curcumin from turmeric is a polyphenol with antioxidant and anti-inflammatory properties. It can cross the blood–brain barrier (BBB) and play an antioxidant role exactly where it is needed to protect against the oxidative processes of neurodegenerative diseases [4]. More than a few studies in different investigational settings have strongly supported the clinical application of curcumin in PD [17].

7.4.3 Vitamin D3 and Parkinson's Disease

As discussed above, associations have been made between low serum vitamin D levels and AD or MS; moreover, lower serum 25-hydroxyvitamin D3 concentration is linked with PD. Some observational studies [18] have shown lesser serum 25-hydroxyvitamin D3 concentrations in patients with PD, compared with healthy subjects.

Suzuki and colleagues [19] assigned 114 patients with PD to either vitamin D3 supplementation or placebo for 12 months, and progression of the disease was found to be significantly lower in the vitamin D3 group. In another study, by Peterson *et al*. [20], investigators reported elevated mood and better cognition and memory in PD patients with higher serum levels of vitamin D3. Well-designed randomized clinical trials (RCTs) are needed to clarify the therapeutic benefits of vitamin D3 in patients with PD.

7.4.4 Dietary Fats and Parkinson's Disease

Some investigators (see, for example, Miyake *et al*. [21]) have shown an increased risk of PD as a result of dietary cholesterol and arachidonic acid intake. Association of PUFAs, MUFAs, and omega-3 fatty acids with PD is controversial. Da Silva *et al*. [22] showed that PD patients with co-morbid depression may benefit from supplementation with omega-3 fatty acids.

With progression of PD and at the end stages of the disease dysphagia may occur. Nutritional strategies for managing dysphagia are presented below.

7.5 DYSPHAGIA IN NEURODEGENERATIVE DISEASES

Elderly patients with more severe Parkinson's disease, multiple sclerosis, or Alzheimer's disease (AD) may encounter dysphagia. Swallowing assessment by a speech-language pathologist (SLP) is often helpful in treating swallowing disorders, especially in those who are at risk of aspiration [4].

7.5.1 Eating Strategies in Dysphagia

Observation of a patient eating may help the nurse or nutritionist to assess swallowing problems, especially aspiration. Environmental interruptions and talking during mealtime increase the risk for aspiration.

With severe dysphagia or the risk of aspiration from oral intake, the patient should be scheduled nil by mouth and enteral tube feeding may be necessary.

Elderly patients with problems swallowing usually lose weight due to insufficient nutrition. Therefore, a nutritional assessment may help. An anthropometric, biochemical, clinical, and dietary assessment is necessary while treating such a patient. A palatable diet, nutritionally adequate, and of suitable texture and temperature, is helpful. Enriched or energy-dense foods with a soft or puréed consistency are often necessary. In situations of severe dysphagia, where food intake may be reduced, multivitamin mineral supplements in liquid form can be added to tolerable foods. Food texture and temperature are also important in improving the swallowing ability of patients; patients with a poor ability to swallow can better tolerate cold foods and drinks in small and frequent meals.

Swallowing of thin-consistency juices, liquids, or water is more difficult to control. Thin liquids are simply aspirated into the lungs, maintained in the pharynx (which may or may not cleared), and can create a life-threatening situation. The need for liquid in these patients should be met by thickening water with starch or powdered milk, or by providing condensed liquid foods such as dairy products, soup, fresh leafy vegetables, or fruits. Carbonated drinks and usage of sauces can improve food texture to allow easier swallowing. Gradually, swallowing ability can be improved by practice.

7.6 CONCLUSION

Inflammatory and oxidative mechanisms are involved in the etiology of neurodegenerative diseases; therefore, the dietary regimen and some dietary supplements can play an important role in preventing and controlling them. Excessive intake of pro-oxidants, saturated fatty acids, and alcohol, and deficiency of antioxidant nutrients, may be the trigger for progressive cognitive decline. On the other hand, diets rich in vitamins, antioxidants, and unsaturated fatty acids such as sea foods, poultry, nuts, curcumin, tomatoes, fruits, garlic, and dark and green leafy vegetables may be supportive.

Lower serum vitamin D concentration is seen in most types of neurodegenerative disease, including AD, MS, and PD. Therefore, paying attention to serum vitamin D status is highly recommended, and supplementation with vitamin D is advised at least for those with vitamin D deficiency.

Old patients in the more severe stages of neurodegenerative diseases may suffer from dysphagia and are at the risk of aspiration. Swallowing assessment by a speech-language pathologist (SLP) and anthropometric, biochemical, clinical, and dietary assessment by a dietitian are necessary and often helpful. With severe dysphagia or the risk of aspiration from oral intake, the patient should be scheduled as nil by mouth and enteral tube feeding may be necessary. Food texture and temperature are important in improving the swallowing ability of patients; people with a poor ability to swallow can better tolerate and ingest cold foods and drinks in small and frequent meals. Multivitamin and antioxidant supplementation is necessary if there is poor nutritional status or malnutrition.

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Further Reading

Guidelines for the diagnosis and treatment of neurodegenerative diseases are available from the National Institute of Aging.

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PART II

NUTRACEUTICALS IN CHRONIC DISEASE AND CANCER THERAPY IN SENIORS

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CHAPTER

8

Targeting Mitochondria for Healthy Brain Aging

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8.1 INTRODUCTION

Mitochondria are key to our understanding of the cell, the basic unit of life. The study of mitochondria has revealed fundamental insights that cover a broad spectrum ranging from genetics to biophysics and cell biology. Additionally, their demonstrated role in various neurodegenerative diseases, their role in aging, and the recent implications of mitochondria in cell death pathways has further advanced mitochondria to occupy a central position in the science of cell biology as well as neurobiology.

Since neuronal function is seemingly dependent on mitochondrial bioenergetics, studies concentrating on aging and age-related neurodegenerative disease like Alzheimer's disease (AD) are investigating improving mitochondrial function as a therapeutic target. Many therapeutic interventions that improve mitochondrial function in aging and disease show positive results. Interestingly, since mitochondria are necessary for normal metabolism many of these mitochondrial targeting interventions can be ingested through foods or by supplementation with, for example, vitamin E, vitamin C, and coenzyme Q10, to name but a few.

Here, we will discuss mitochondrial bioenergetics and their importance in the brain, with a focus on aging and age-related disease such as AD. A number of important findings from the literature will be discussed, regarding the use of both antioxidants and mitochondrial co-factors to improve bioenergetics in order to promote neuronal health in the brain. The findings from these studies have been promising although controversial, suggesting discrepancies due to variations in dosage, time of life when treatment was initiated, and, most importantly, the animal models used. We also provide a discussion of a study of mitochondrial co-factors in a canine model of human brain aging and AD to illustrate the importance of such animal models for the use of aging studies and age-related cognitive decline in normal and disease states.

8.2 MITOCHONDRIA BIOENERGETICS

The history of mitochondria dates back to the mid-1800s, when words such as "chondros," "Korn," and "grain" were used to describe intracellular structures. As staining techniques improved, so did the terminology. Eventually the Greek term *mitos* was adopted, which led to "mitochondria" being coined in 1898 (see review by Chowdry 1918, quoted in Lehninger [1] and Scheffler [2]). Remarkably, in the 1890s Altman termed these intracellular granules "bioplasts" and proposed they were autonomous, forming bacterial-like colonies within their host cells [2]. Based on our current knowledge, this was indeed a very astute observation.

In order to understand the importance of mitochondrial dysfunction in aging and disease states, we must first discuss normal mitochondrial function (Figure 8.1). In cells of higher animals, 95% of the common energy currency, adenosine triphosphate (ATP) is produced by oxidative phosphorylation within mitochondria. As discussed above, mitochondria are intracellular organelles with a dual (inner and outer) membrane system, each being responsible for specific functions. The outer membrane (OM) contains multiple transporter proteins tasked with the import and export of many ions and proteins necessary for mitochondrial function [3]. The inner

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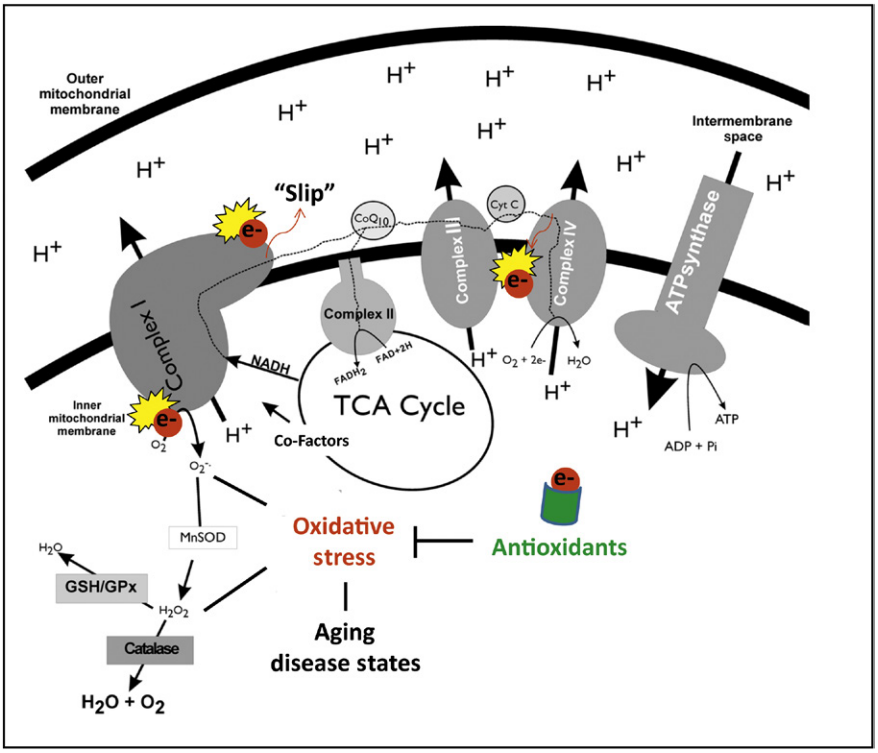

FIGURE 8.1 Mitochondrial function is crucial for the healthy aging of neurons. Within the electron transport chain, metabolic-mediated oxidative stress is generated as a result of electrons being released and reacting with molecular oxygen.

membrane (IM) has many folds, termed cristae, which increase the surface area available for mitochondrial respiration and are the site of electron transport and oxidative phosphorylation (OXPHOS). The space enclosed by the IM is termed the matrix, and contains enzymes involved in cellular metabolism and calcium regulation.

Within the cristae of the IM there are five protein complexes that comprise the electron transport chain (ETC). In terms of thermodynamics and electrochemical properties, each step in the ETC is bound to free-energy changes as each component cycles through oxidation and reduction reactions. The components of the ETC include Complexes I, II, III, IV, as well as the mobile carriers, coenzyme Q10, and cytochrome C. Complex I (NADH–ubiquinone oxidoreductase), which is embedded within the IM, converts NADH to NAD^+ by accepting an electron into the Fe–S center of the protein. As a byproduct of this electron donation, a proton is translocated from the matrix into the intermembrane space [4], which is located between the inner and outer membranes. Complex II (succinate dehydrogenase), in addition to its function as an ETC protein, is also a key component of the Krebs cycle, which converts the glycolytic product pyruvate into substrates for the ETC. Complex II utilizes the conversion of succinate to accept electrons from FADH2 into the ETC with no translocation of protons from the matrix to the IMS. Complexes I and II transfer their electrons to ubiquinone (coenzyme Q10) located within the IM. These electrons are then passed to Complex III (ubiquinone–Cytochrome-C oxidoreductase) via the Q cycle, resulting in protons being translocated into the IMS. Cytochrome c then accepts the electron and transports it to Complex IV (cytochrome-C oxidase); again translocating a proton into the IMS. It is at Complex IV that oxygen plays its vital role as the final electron acceptor for the ETC, where it is reduced by these electrons to form H_2O. The protons that have been translocated into the IMS generate a chemiosmotic gradient/membrane potential ($\Delta\Psi$) which is utilized by Complex V (ATP synthase) to facilitate the phosphorylation of ADP into ATP for use as an energy source for cellular processes.

Although OXPHOS is credited for its ability to keep ATP:ADP (adenosine diphosphate) ratios high so cells are never depleted of their desired energy currency, ATP, it is also the source of various reactive oxide species (ROS) and reactive nitrogen species (RNS). As mentioned previously, during OXPHOS Complex IV will release an electron to molecular oxygen (O_2), allowing for the formation of H_2O, although, within Complexes I, II, or III, an electron can "slip" from the chain and bind to O_2, resulting in the formation of superoxide ($O_2^{\bullet -}$). This highly reactive compound can bind to proteins, nucleic acids, and fatty acids within the membrane, leading to damage, which is often referred to as metabolic-mediated

oxidative damage. Mitochondrial manganese superoxide dismutase (MnSOD, SOD2), a mitochondrial located metalloenyzme that is crucial for the dismutation of this highly reactive $O_2^{\bullet-}$ into H_2O_2, will neutralize the $O_2^{\bullet-}$. However, H_2O_2 is also a strong oxidizing agent. Therefore, glutathione peroxidase is located within the mitochondria and can convert H_2O_2 into H_2O [5,6].

Although it is unclear why, cells within the brain seem to be particularly vulnerable to this metabolic-mediated oxidative damage. This has been attributed to the fact that the brain consumes approximately 20% of the body's total oxygen and has lower levels of endogenous antioxidant activity relative to other tissue [7–9]. Additionally, neurons have high energy demands compared to other cell types. The Na^+/K^+ ATPase pumps located within their cellular membrane function to maintain the membrane potential necessary for action potentials to be generated. It is estimated that one-third of the entire body's ATP production in 1 day is used by these pumps [10]. Due to the fact that neurons primarily undergo aerobic respiration, the ATP used is generated primarily by OXPHOS. However, OXPHOS also leads to increased oxidative stress, which can be detrimental to the cell over time.

The damage within mitochondria due to "electron slippage" seems to be self-perpetuating. As oxidative stress builds up, damage to proteins within the ETC will occur, leading to more "electron slippage." The ETC will reach a point where it can no longer maintain the necessary $\Delta\Psi$ needed to allow Complex V to function properly. As the mitochondria remain in a dysfunctional state with a decreased $\Delta\Psi$ hovering around 100–120 mV, which is a drastic decrease from the normal membrane potential ranging around 180 mV, the electron transport systems will begin to slow down or even halt. Reactive superoxide molecules will continue to be released within the mitochondria [11]. As this perpetuates, crucial mitochondrial proteins like adenine nucleotide translocator (ANT) undergo oxidation modifications, specifically a thiol oxidation, resulting in a conformational change that promotes cyclophilin D (cypD) binding with ANT. Once ANT, an inner membrane mitochondrial protein, is bound to cypD, an appropriate conformational change occurs to promote ANT, cypD, and many other proteins within the complex to bind with the voltage-dependent anion channel, VDAC [12–14]. VDAC is an outer membrane mitochondrial protein that, once bound to this inner mitochondrial membrane protein complex (cypD, ANT, and many other proteins), forms the mitochondrial permeability transition pore (MPTP). The MPTP formation exposes the inner mitochondrial matrix to the cytosol, allowing for the release of apoptotic initiating factors like cytochrome C, SMAC/diablo, and AIF, which all promote cell death. Neuroprotective agents that target mitochondrial dynamics and the membrane permeability pore formation can improve neuronal function in many disease states, and therefore it is important to consider when studying age-related neurodegenerative diseases, like AD, which have a hallmark of increased oxidative stress.

8.3 CHANGES THAT OCCUR IN THE BRAIN WITH AGE

The brain is constantly undergoing structural changes. Imaging studies in humans have shown that aged brains have decreased gray and white matter, and enlarged ventricles [15–17]. However, these age-related changes in brain volume do not appear to be due to a loss of neurons and are seemingly the result of shrinking neurons, reduced synaptic spines, decreases in synapses, and changes in myelination [17–26]. As these structural changes in the brain occur, functional changes ensue. With age-related structural changes, the functional changes observed are generally deficits of some kind. For instance, reduced cognitive abilities, such as processing speed, attention, executive functions, and episodic memory, are all commonly seen in healthy aging [27–32]. In a large population, these age-related functional deficits can progress into neurodegenerative disease states like AD.

With age-related structural and functional changes, increasing evidence provides strong support that specific cellular changes occur in brain as well [33–36]. One of the most common cellular changes observed with age is the oxidative damage to lipids, proteins, RNA, and DNA. In the 1950s, a researcher at the University of Berkley, Denham Harman, proposed a compelling mechanistic link between the functional and cellular changes observed with aging. At the time Dr Harman had conducted years of radiation experiments, radiation being known to induce free radicals and thereby cause cellular damage. During these studies he also observed metabolic changes that were similar to those observed in aging. With these findings, he hypothesized that as an organism ages, free radical damage accumulates in the cell, similar to that seen with radiation treatment. Years of free radical production, generated by metabolic-mediated oxidative stress, eventually result in oxidative damage to intracellular components, leading to cellular dysfunction and changes in metabolism. This theory became the foundation for the Free-Radical Theory of Aging (FRTA) [37], and provides a mechanistic link between oxidative damage, cellular dysfunction, changes in metabolism, and age. To further support this theory, studies of human autopsy tissue show higher levels of oxidative damage to nucleic acids [38–40], proteins [39,41–44], and lipids [40,44,45] in aged brain as compared to young brain, and changes in brain metabolism have been linked to various age-related diseases [46–48].

At this booming time in science, mechanistic studies into the sources of oxidative stress progressed. It was

then that Chance and colleagues showed that the mitochondrial electron transport chain was a source for the production of reactive H_2O_2, which is a strong oxidizing agent, as mentioned earlier [49]. Further expanding upon these findings, his studies showed that the H_2O_2 produced was a product of MnSOD converting $O_2^{\bullet -}$ to H_2O_2 [50–53]. As stated previously, MnSOD, also known as SOD2, is a mitochondrial located metalloenyzme that is crucial for the dismutation of highly reactive $O_2^{\bullet -}$ into H_2O_2 and O_2 [54]. Chance's finding was important for two reasons: (1) it proved mitochondria were an important site for ROS production; and (2) it proved, for the first time, that oxidative stress was linked directly to metabolism. As mentioned previously, in normal metabolic processes ROS and RNS are produced [55–58]. In a healthy system, after production these reactive species are immediately neutralized, avoiding deleterious consequences. However, if not neutralized they can diffuse away from the site of production and damage proteins, lipids, DNA, or RNA [55].

With the production of ROS and RNS being understood as a byproduct of OXPHOS, Harman expanded upon FRTA to acknowledge mitochondria as the major source of oxidative stress within a cell. He modified FRTA to the mitochondrial free-radical theory of aging (MFRTA) [37,59]. The MFRTA was the first theory of its kind to acknowledge a possible link between mitochondrial oxidative phosphorylation, ROS production, cellular damage, and functional deficits related to aging [60]. In this theory, it is proposed that mitochondrial-produced ROS will diffuse away from the site of production to damage proteins and mtDNA. This leads to increased damage to proteins within the ETC, resulting in continually increased ROS production as electron slippage occurs by the damaged proteins [61,62]. Therefore, mitochondrial dysfunction and the production of ROS, combined with lower endogenous antioxidant activity, may lead to increasing oxidative damage to molecules critically important to neuronal function. The combination of mitochondrial dysfunction and production of ROS may be a key contributor to the deleterious effects of aging on the brain [63–71]. This mitochondrial dysfunction can lead to increased oxidative stress, changes in protein processing, and changes in metabolism.

Linking age-related functional deficits to the function of an organelle became an important turning point in the study of aging. As the powerhouse of the cell, mitochondria are extremely important organelles for normal cellular homeostasis [72]. Interestingly, within anaerobic cells and tissues 90% of the oxygen consumed is used by mitochondria. Mitochondria primarily use this oxygen for the production of ATP during oxidative phosphorylation within the electron transport chain. In most aerobic healthy cells, mitochondria are the main source of the ROS produced [59,73–75]. However, due to the high production of ROS, mitochondria are extremely vulnerable to oxidative damage [76]. Well aligned with the MFRTA is the understanding that one of the primary hallmarks of aging is mitochondrial dysfunction [73,77]. The literature suggests that mitochondrial function is compromised with age in the human brain [57,58,67,78]. In normal aging, mitochondrial respiratory chain activity declines [79], mitochondrial metabolism-associated enzymatic activity such as aconitase decreases [80], and the rate of somatic mitochondrial DNA (mtDNA) mutations increases [70,81].

Although evidence is now arising that contradicts Dr Harman's MFRTA, there are still many supporters [60]. Therefore, this will be the mechanistic foundation used in this chapter to explain the deficits seen related to aging, and the therapeutic rational for antioxidants and mitochondrial co-factors to improve aging and decrease the onset of cognitive disorders.

8.4 AGE-RELATED CELLULAR DYSFUNCTION AND ASSOCIATION WITH ALZHEIMER'S DISEASE

Many disease states are thought to be precipitated by increased oxidative stress within the cell causing damage and, consequently, dysfunction. This is congruent with the MFRTA discussed earlier. Further elaboration on this theory helps to explain the pathology seen in age-associated neurodegeneration [82], since oxidative damage has specifically been linked to age-associated neurodegenerative diseases, including AD [83–85]. AD is a progressive neurodegenerative disease that causes dementia in the elderly, affecting an estimated 5.5 million people in the United States [86]. It is characterized by the accumulation of beta-amyloid (Aβ) in extracellular senile plaques, and intracellular hyperphosphorylated tau protein in neurofibrillary tangles [87]. Consequently, extensive neuron loss is observed in the AD brain in the cortex and particularly within the hippocampus, a region of the brain involved with memory. In an AD-affected brain, further increases in oxidative damage to protein [39,42,43,88–95], lipid [40,45,96–99], DNA [100–102], and RNA [65,103,104] are seen relative to elderly controls. In addition, endogenous antioxidant activity in the AD brain is reduced relative to age-matched controls [39,44,105]. Proteins particularly vulnerable to oxidative damage have been identified by proteomics, with a subset of these proteins putatively involved directly or indirectly in the production and accumulation of AD neuropathology [106].

Mitochondrial dysfunction also occurs in AD, with decreased respiratory chain activity [68,107,108] and increased mitochondrial DNA mutations [109] observed at higher rates when compared to age-matched controls.

Further, decreased cytochrome oxidase activity in the posterior cingulate cortex of AD patients is correlated with hypometabolism seen by positron emission studies [47]. A gene array study in the cingulate cortex has also shown that energy metabolism-related genes decrease in AD; specifically, a 65% reduction in expression of mitochondrial electron transport chain genes has been reported [46].

The observed mitochondrial dysfunction in AD may also account for the "Ca^{2+} hypothesis of AD" [110,111]. As discussed earlier, mitochondria are bioenergetically active due to the maintenance of a membrane potential that is primarily generated by the electron transport chain. As a result of this membrane potential, mitochondria are an integral part of one of the cell's best Ca^{2+} buffering mechanisms. As Ca^{2+} enters the cell, mitochondria are able quickly to uptake the Ca^{2+} in an electrogenic fashion due to the $\Delta\Psi$ generated by the ETC. In the Ca^{2+} hypothesis of AD it is believed that mitochondria are inherently dysfunctional, thus hindering their ability to generate the membrane potential necessary to buffer intracellular calcium properly. As cytosolic Ca^{2+} levels remain high, signaling cascades that affect synaptic stability and function are initiated, such as the activation of calcineurins and calpains, leading to detrimental neuronal health [112]. Based on the MFRTA, the initial mitochondrial dysfunction that inhibits proper Ca^{2+} buffering is thought to be the result of mitochondrial metabolic-mediated oxidative stress within the electron transport chain leading to diminished membrane potential. Without the generation of an appropriate membrane potential, mitochondria are unable to buffer calcium, leading to eventual initiation of mPTP and resulting in neuronal cell death.

Based on these mechanistic theories that eloquently link mitochondrial function, oxidative stress, and metabolism to aging and AD, diet seems to be an important factor in healthy aging. It is thought that a healthy diet may slow or prevent the onset of many age-related neurodegenerative diseases, such as AD. For instance, antioxidants are a critical component of the proper cellular homeostasis. These compounds are necessary for keeping levels of oxidative stress low and therefore decreasing damage to lipid, proteins, DNA, and RNA [113]. Based on correlative human neuropathology studies, antioxidants are predictive of healthy aging, may reduce the risk of developing AD, and may improve cognitive function in AD patients. However, studies in humans have shown either a positive effect of antioxidant use on cognition and risk reduction for developing AD [114–116] or no significant effects [117–120]. This suggests the need for more systematic and controlled clinical trials to evaluate the effects of antioxidants on cognition in aged individuals or patients with AD.

8.5 DIETARY SUPPLEMENTATION TARGETING MITOCHONDRIAL FUNCTION TO IMPROVE AGE-RELATED COGNITIVE DEFICITS

As mentioned previously, mitochondrial dysfunction, oxidative stress, and changes in metabolism are important factors in natural aging. Based on the MFRTA, it is thought that appropriate dietary supplementation can help prevent many of the age-related deficits that arise, such as impaired cognition. Two major dietary factors/supplementations that have shown some promise are the use of antioxidants and of mitochondrial co-factors.

One specific antioxidant that has been well studied in aging and various age-related neurodegenerative states is vitamin E. Vitamin E is an important lipid-soluble antioxidant that is obtained through the diet [121]. Commonly referred to as tocopherol, the vitamin E isomers are found in high concentrations in almonds, roasted sunflower seeds, and various plant oils such as olive oil, and can also be taken as a supplement. It is thought that proper supplementation with vitamin E can protect cells against the deteriorative effects of oxidative damage, and the progression of degenerative diseases and aging. However, findings in the literature have shown mixed results [113]. As a compound, vitamin E is an important antioxidant shown to protect against lipid peroxidation in cell culture models [122]. However, in single-cell organisms, rotifers, *Caenorhabditis elegans*, *Drosophila melanogaster*, and rodent models, vitamin E has shown the full gamut of results, ranging from increased lifespan with supplementation, to no observed changes in lifespan, to detrimental effects with supplementation [123–135]. Similar to these previous findings, human clinical trials with vitamin E have also produced mixed results. In one study, intake of vitamin E delayed institutionalization of AD patients [136], suggesting some beneficial effects. However, vitamin E alone did not improve cognition in patients with mild cognitive impairment, which is thought to precede AD [137]. Further, in non-demented elderly women, vitamin E treatment was associated with little improvement in cognition [119]. Because of these mixed findings, it becomes apparent that more research is needed in this area for conclusive findings to be reported and understood.

Vitamin C is another antioxidant that has been studied for years and has shown mixed results in the treatment of various age-related diseases. Since vitamin C is a water-soluble vitamin, it is not stored in the body and therefore needs to be consumed regularly through the diet. Not only has it shown to be important in the maintenance of connective tissues; it can also assist in the conversion of the amino acid tryptophan, to the neurotransmitter serotonin, and is an antioxidant that protects the body

from free radical damage [138]. Foods rich in vitamin C include guava, kale, and kiwi, and vitamin C can also be taken in supplement form [139]. To date, research using vitamin C as an antioxidant has led to mixed results, although results seemingly are improved when vitamin C is combined with other antioxidants, such as vitamin E [140]. Research has found that in low concentrations vitamin C has antioxidant properties; however, at high concentrations it is a potent pro-oxidant, with intra- and extracellular mechanisms that generate hydrogen peroxide [141]. Treatment with aspirin plus ascorbic acid/vitamin C in aged rats was shown to enhance cognitive performance and increase the expression of several receptors related to the learning and memory process [142]. Recently, a rodent student also observed that high supplementation of vitamin C was able to decrease the amyloid plaques found in the cortex and hippocampus of an AD transgenic mouse model that normally shows increased amyloid plaques in the brain compared to wild-type mice [143]. However, even with the positive results found in animal testing, the results pertaining to human clinical trials have not been as positive [144]. Similar to vitamin E, cognitive studies in aged humans using vitamin C have also shown the full gamut of results pertaining to cognition [145,146].

Coenzyme Q10 (CoQ10) is another significant, naturally occurring compound that is hypothesized to be important in various neurodegenerative diseases and cognitive disorders related to age [147]. As an important co-factor needed in the mitochondrial electron transport chain, CoQ10 is necessary for proper aerobic cellular respiration and ATP production, both of which have been found to decline with age. It is thought that when CoQ10 is deficient, mitochondrial function decreases, ROS production increases, and inflammation ensues due to increased electron slippage [86,148]. CoQ10-H2, the reduced form of CoQ10, is a fat-soluble antioxidant that is found in cell membranes. This important lipid soluble antioxidant is the only antioxidant that animal cells synthesize *de novo* [149]. Although it has an integral role in proper cellular function related to both metabolism and oxidative stress, its importance in aging and proper supplementation in many diseased states has been highly debated. In laboratory studies where CoQ10 was supplemented in cell-culture and slice-culture studies, it was found to be neuroprotective through the reduction of oxidative stress leading to decreased cell death [150]. Rodent studies have also shown improvements in cognition with later-life supplementation with CoQ10 [151]. Additionally, a study measuring cognition in aged beagles following statin usage found lower levels of serum CoQ10 in the parietal cortex that correlated with decreased cognition, suggesting the need for a proper CoQ10 level in order to retain proper cognition [86]. Decreases in plasma CoQ10 levels seen in a canine model of human brain aging and AD are consistent with reports in humans. A correlative study recently completed in humans found that plasma levels of CoQ10 are significantly reduced in older patients, suggesting a decreased antioxidant capacity [152]. Since both cognition and CoQ10 levels tend to decline with age, it is hypothesized that decreases in CoQ10 may lead to the mitochondrial dysfunction seen with AD [153]. However, another interpretation of the data could suggest that age-related impaired mitochondrial function makes aged cells more vulnerable to naturally occurring decreased levels of CoQ10 [154].

Taken together, studies of dietary or supplemental antioxidant intake in humans reveal variable results and appear far less robustly associated with positive functional outcomes than those reported in the rodent aging literature [71,76,155–162]. However, the variability in the outcomes of human antioxidant clinical trial outcomes may reflect inconsistencies in the amount of supplementation provided, its form and source (e.g., lower AD brain neuropathology is associated with cerebrospinal fluid levels of alpha-tocopherol and not gamma-tocopherol [163]), its duration and regularity of use, and the extent of antioxidants and mitochondrial co-factors used before the study [164]. However, it is also possible that the results comparing combinations of antioxidants may be superior to those with single compound supplementation [165], and dietary intake of antioxidants superior to supplements in human studies on cognition and risk of developing AD [166,167]. Positive results related to combination antioxidant treatments have been observed. A recent study found that supplementation with a combination of vitamins E and C in elderly women can lead to improved memory [168]. Therefore, antioxidants may prove to be more efficacious if administered in combination with other antioxidants (e.g., vitamin C, which helps to recycle vitamin E) and through diet, rather than as a supplement.

In addition to investigating the effects of cellular antioxidants on cognition and risk of AD, several studies have examined the effects of targeted co-factors that improve mitochondrial function, including acetylcarnitine (ALCAR) and lipoic acid (LA). It is thought that ALCAR and LA may improve mitochondrial function and reduce the production of ROS, and thus reduce oxidative damage to proteins, lipids, and DNA/RNA [169]. In studies where ALCAR was administered to patients with moderate to severe AD, either improved cognition and/or slower deterioration was observed [170–173]. In early-onset AD patients (less than 65 years of age) only small cognitive improvements were noted [174], although younger patients with AD (less than 61 years) may also have experienced slowed disease progression [175,176]. When the results of all these studies were combined in a meta-analysis, ALCAR administration in patients with AD was clearly beneficial, particularly

with respect to slowing cognitive decline [177]. Further, combining ALCAR with acetylcholinesterase therapy in AD may provide additional benefits [178]. Similar evidence of maintenance of function was observed in an open label study of nine patients with AD or related dementias receiving 600 mg/day of LA for an average of 337 days [179]. In a larger follow-up study of 48 patients for a 48-month treatment period, maintenance of function was also observed [180].

However, even with some positive studies, the definitive answer as to whether antioxidants and co-factors improve aging is still a controversial subject. A panel of experts for the Duke Evidence-based Practice Center for the US Department of Health and Human Services recently reviewed the literature and reported no consistent or robust evidence to suggest that single or dual antioxidant use is protective against AD [181]. In terms of preventing cognitive decline with aging, vegetable intake was only weakly associated with decreased risk of developing AD. Thus, the role of either dietary or supplemental antioxidants and the level of protection against cognitive decline or AD have yet to be clearly established.

Additional reasons for the small or negative effects of antioxidants on cognition in the elderly and for treatment of AD [155,182] include the limitations of animal models (primarily rodent) in terms of ability to predict human response. Therefore, it is useful to consider other animal models of human aging and AD, and also to test the potential for combinations of antioxidants/mitochondrial co-factors to improve cognition and reduce Aβ. Specifically, dogs are frequently used to evaluate the safety of drugs and in food metabolism studies, given their substantial similarities to humans. Therefore, preclinical studies of mitochondrial function and interventions should be completed in an aging canine model of human brain aging and AD in order to provide results that can better be translated to humans.

8.6 THE IMPORTANCE OF CANINE STUDIES IN AGE-RELATED COGNITIVE DEFICITS

Dogs may be particularly useful in studying human brain aging because they naturally develop cognitive decline with age and accumulate oxidative damage and Aβ protein [183]. In dog brain, oxidative damage to proteins increases with age [184,185] and is associated with reduced endogenous antioxidant enzyme activity or protein levels [184,186–188]. In several studies, a relationship between age and increased oxidative damage has been inferred by measuring the amount of end products of lipid peroxidation to predict oxidative damage to lipids. These end products included 4-hydroxynonenal [188–191] and malondialdehyde [184]. Additionally, reported evidence suggests increased oxidative damage to DNA or RNA (8OHdG) in aged dog brain [183,191].

Oxidative damage may also be associated with behavioral decline in dogs. In a study completed by Rofina and collaborators examining oxidative end products (lipofuscin-like pigment and protein carbonyls) in aged companion dog brain, a correlation was found between increased oxidative end products and severity of behavior changes due to cognitive dysfunction [185,190,191]. Similarly, work completed by Head and colleagues in aging beagles found that higher protein oxidative damage (3-nitrotyrosine) and lower endogenous antioxidant capacity (superoxide dismutase and glutathione-S-transferase) are associated with poorer prefrontal-dependent and spatial learning [187]. These correlative studies imply a link between cognition and progressive oxidative damage in the dog, suggesting a utility in testing antioxidant treatment strategies.

To test the hypothesis that reduced oxidative stress leads to cognitive benefits, longitudinal studies in aged dogs have also been completed. In one study, a combination of antioxidants and mitochondrial co-factors was provided in food [192–196]. Here, 48 aged beagles (between ~8 and 12 years of age) were divided into four groups that were balanced with respect to baseline cognitive ability, sex, and age: (1) no behavioral enrichment/control diet group; (2) behavioral enrichment/control diet; (3) no behavioral enrichment/antioxidant diet; and (4) combined behavioral enrichment and antioxidant diet. In a subset of experiments, an additional 17 young beagles (<5 years of age) were included for comparison to aged dogs. Young dogs were all placed in the behavioral enrichment condition, with half provided with the antioxidant diet (i.e., similar to groups 2 and 4).

Three unique features of the experiment included: (1) a combination of antioxidants and mitochondrial co-factors; (2) incorporation of all antioxidants and mitochondrial co-factors into food; and (3) evaluation of dietary treatments in combination with behavioral enrichment. An antioxidant-enriched dog diet was formulated to include a broad spectrum of antioxidants and two mitochondrial co-factors [195]. Based on an average weight of 10 kg per animal, the daily doses for each compound were 800 IU or 210 mg/day (21 mg/kg per day) of vitamin E, 16 mg/day (1.6 mg/kg per day) of vitamin C, 52 mg/day (5.2 mg/kg per day) of carnitine, and 26 mg/day (2.6 mg/kg per day) of lipoic acid. Fruits and vegetables were also incorporated at a one-to-one exchange ratio for corn, resulting in 1% inclusions (dehydrated) of each of the following: spinach flakes, tomato pomace, grape pomace, carrot granules, and citrus pulp. This was equivalent to raising fruit and vegetable intake from three servings per day to five to six servings per day, based upon ORAC values [197]. Additionally, vitamin E was increased by ~75% in dogs

treated with the antioxidant diet [198]. The behavioral enrichment condition consisted of additional cognitive experience (20–30 min/day, 5 days/week), an enriched sensory environment (housing with a kennelmate, weekly rotation of play toys in kennel), and physical exercise (two 20-minute outdoor walks/week) [195].

Dogs were evaluated over a 2.8-year period to determine short-term and chronic treatment effects. Treatment with the antioxidant diet led to cognitive improvements in learning within 2 weeks, with aged animals showing significant improvements in spatial attention (landmark task) [198]. Subsequent testing of animals with a more difficult complex learning task (oddity discrimination) also revealed benefits of the diet [192]. With antioxidant treatment, visual discrimination improved and reversal (frontal function) learning ability was maintained over time, while untreated animals showed a progressive decline [195]. This was despite the fact that, for each time point where discrimination learning was re-administered, the task was made more difficult (harder to distinguish objects) to prevent a practice effect. Thus, the progressive increase in error scores over time in untreated dogs reflects both increased task difficulty and, possibly, longitudinal aging effects. Interestingly, the dogs fed an antioxidant diet benefited from behavioral enrichment, in that cognitive scores of aged dogs receiving both treatments were superior to either treatment alone [194,195]. For example, in singly-treated animals spatial memory showed a trend toward improvement, reaching statistical significance only after long-term treatment (>2 years), with a combination of both the antioxidant diet and behavioral enrichment [196]. The antioxidant diet selectively repaired an aging deficit, in that cognitive scores from young dogs treated with the antioxidant diet did not differ from those of young dogs fed control diet [199]. Neurobiological studies showed reduced oxidative damage and increased endogenous antioxidant activity in antioxidant-fed dogs, particularly among animals receiving the combination of antioxidants and behavioral enrichment [187]. Age-associated mitochondrial dysfunction was significantly improved in the antioxidant-fed dogs and not in behaviorally enriched dogs [154]. Interestingly, behavioral enrichment but not the antioxidant diet protected against neuron loss in the hilus of the dog hippocampus [200]. Further, brain-derived neurotrophic factor mRNA increased in aged dogs provided with the combination treatment [201]. These results suggest that cognitive benefits of antioxidants can be further enhanced with the addition of behavioral enrichment, perhaps due to different yet synergistic mechanisms of action in the brain, including reduced oxidative damage and maintenance of neuron health. In addition to brain, however, peripheral benefits were also seen, including less cellular degeneration in the inner ear [202].

In another recent study of aged dogs, the formulation of the diet was modified to compare only the mitochondrial co-factors used in this previous study and effects on cognition [203]. Aged dogs were treated with lipoic acid, ALCAR, or a combination of the two, and tested with spatial learning and discrimination/reversal tasks. When these compounds were included with a broad spectrum of antioxidants, no cognitive benefits were observed when evaluated singly or in combination. Additionally, protein carbonyl accumulation in the plasma of treated dogs was increased. This increased oxidative damage may reflect either higher doses of the mitochondrial co-factors used in this study, or increased oxidative stress resulting from not counterbalancing mitochondrial co-factors with cellular antioxidants. Further study is needed to address this.

8.7 SUMMARY

Mitochondrial dysfunction is consistently observed in normal aging brain, and is exacerbated in AD. Mitochondrial-targeted interventions show somewhat limited benefits in clinical populations, but have shown significant benefits in cell culture and animal models. In future studies it will be important to consider mitochondrial co-factors and/or antioxidant diet interventions using a prevention approach, when providing neurons with the ability to reduce mitochondrial dysfunction and oxidative damage can lead to improved neuronal repair and function.

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CHAPTER

9

The Progression of Non-alcoholic Fatty Liver Disease and Lifestyle Intervention in Older Adults

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9.1 INTRODUCTION

The term "non-alcoholic fatty liver disease" (NAFLD) describes a spectrum of liver histology characterized by excess fat within the liver in individuals who drink little or no alcohol. It ranges from simple fatty liver (steatosis), through fat accompanied by signs of hepatocyte injury, mixed inflammatory cell infiltrate, and variable hepatic fibrosis (non-alcoholic steatohepatitis, NASH), to cirrhosis and hepatocellular carcinoma (HCC) [1,2]. The histological characteristics of NAFLD are indistinguishable from alcoholic liver disease; however, excluding patients with a history of excessive alcohol use is critical in defining who has NAFLD [1,3]. NAFLD has become a common reason for liver transplant. It also has been identified as an important risk factor for the development of primary liver cancer, mostly due to NAFLD-associated cirrhosis [1–3].

Understanding the burden of NASH in the NAFLD population is important because whereas hepatic steatosis alone is considered to be relatively benign from a liver perspective, with a 0–3% liver-related mortality rate over 10–20 years, NASH presence has been associated with a 17.5% risk of liver-related mortality over approximately 20 years of follow-up in a series of 131 subjects [4,5]. A significant minority of people with simple steatosis will also develop NASH over time, as illustrated by one comprehensive follow-up series using serial liver biopsy, where 23% of patients with simple steatosis were found to progress to NASH in a period of 3 years [6].

9.2 PREVALENCE OF NAFLD AND NASH

NAFLD is rapidly becoming a global public health problem. It is the most common liver disease in the United States, and indeed worldwide. Current estimates are that about 19.0% of the general population of the United States had NAFLD detected by hepatic ultrasonography [7,8], and that around 11.8% of NAFLD patients developed NASH [8]. The prevalence of NAFLD among adults in the general population in China was found to be approximately 15.0% [9]. NAFLD was found in over one-quarter of the general adult Chinese population in Hong Kong, but the proportion of patients with advanced fibrosis was low (3.7%) [10]. NAFLD was highly prevalent (29.7%) in the general population in Japan in 2009–2010, and the estimated prevalence of NASH was less than 10.0% in subjects with NAFLD [11]. Additionally, the prospective definition of the prevalence of NAFLD and NASH was higher than estimated previously: NAFLD and NASH affected as many as 46.0% and 12.2% of United States middle-aged adults, respectively;

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moreover, Hispanics had a higher prevalence of NAFLD and NASH compared with Caucasians [12].

Previous studies have reported that the prevalence of NAFLD increased to 60–70% in obese patients [13]. However, non-obese individuals could still have NAFLD and NASH [8,14–16]. NAFLD was present in 7.39% of the lean individuals in the United States, but was significantly less common than in overweight or obese individuals (27.75%) [8]. Likewise, the prevalence of NAFLD in non-obese subjects in China was 7.27% in a cross-sectional study [15]. Interestingly, a high percentage (15–21%) of Asia-Pacific NAFLD subjects have been found to be non-obese [16]. NAFLD prevalence is increased in people with type 2 diabetes and has been estimated at around 70% using ultrasound techniques [17]. Additionally, NAFLD and NASH were found to be more common in men compared with women [7,12]. In total, NAFLD is the most common liver disorder in Western industrialized countries. With the introduction of a Westernized lifestyle and the increasing frequency of obesity and diabetes in Asia, the prevalence of NAFLD/NASH will likely continue to rise. This disorder will therefore contribute substantially to the burden of chronic liver disease in coming decades.

9.3 RISK FACTORS ASSOCIATED WITH NAFLD AND NASH

9.3.1 Metabolic Syndrome

The etiology of NAFLD and its progression are complex and remain incompletely understood. It is clearly multifactorial. Most cases of NAFLD/NASH occur in overweight or obese individuals, and there are particularly strong links to central obesity, insulin resistance (IR), type 2 diabetes, dyslipidemia, and hypertension, all of which are elements of metabolic syndrome (MetS). NAFLD can now be regarded as the hepatic manifestation of MetS [7,8,12]. Obesity, as classified by the body mass index (BMI), has become a worldwide concern reaching epidemic proportions. While obesity in the United States is defined by BMI >30 kg/m^2, this cutoff is not universal and different classifications exist based on racial phenotypic characterizations. Obesity represents a state of chronic low-grade inflammation that exists in peripheral fat depots. This observation is based on the increased presence of circulatory pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP), C-reactive protein, and the decrease in anti-inflammatory adipokines such as adiponectin and IL-10 [18]. This phenomenon is thought to be causal in the development of IR and MetS, and is without doubt a major risk factor for NAFLD. The prevalence of NAFLD in morbidly obese subjects was found to be 93%; of those with NAFLD, 26% had NASH, and 9% had advanced fibrosis [19]. It is important to be aware that factors other than BMI, such as body fat distribution, lifestyle, and genetics, have a large role in obesity-induced NAFLD [18]. Although less prevalent than in the obese, both NAFLD and NASH are observed in non-obese individuals with a BMI in the normal range. Differential distribution of visceral adipose tissue; waist circumference; levels of triglyceride, high-density lipoprotein cholesterol, serum uric acid, hemoglobin, and platelet count; recent increase in body weight; intake of a high cholesterol diet; and most MetS components and genetic background are likely associated with the development of NAFLD in these non-obese subjects [8,15,16].

The liver, together with skeletal muscle and adipose tissue, displays exquisite sensitivity to insulin, with classical insulin signaling occurring mainly through specific cell surface receptors and a series of post-receptor signaling pathways. By insulin-dependent processes, the liver dynamically regulates glucose flux and metabolism and, consequently, glycemia [20,21]. Hepatic IR is a common feature that predisposes one to compensatory hyperinsulinemia, often followed by pancreatic β-cell dysfunction and development of type 2 diabetes [22,23]. NAFLD prevalence is increased in people with type 2 diabetes. Although studies of patients with type 2 diabetes that examine the spectrum of pathology within NAFLD utilizing liver biopsy are limited to only a few, they collectively estimate the prevalence of NASH at 63–87% and moderate–severe fibrosis at 22–60% [24]. In a recent study of patients with type 2 diabetes and an average BMI of 36 kg/m^2, over 60% of patients who underwent weight reduction surgery had moderate to severe NAFLD upon liver biopsy [25]. Presence of diabetes is particularly associated with fibrosis in NASH. When diabetes and obesity coexisted, 66% of patients with NAFLD had advanced fibrosis [26]. This figure is much higher than that estimated for patients without diabetes or obesity [17]. Furthermore, in a study of 92 patients with type 2 diabetes, three patients were found to have histological evidence of cirrhosis secondary to NAFLD without clinical evidence of liver disease [24]. Diabetes also has been shown to be a marker of progression for fibrosis in longitudinal series. In a clinical cohort of 103 patients with the average age of 45 ± 11 years who underwent serial liver biopsy at an average interval of 3.2 ± 3.0 years (range, 0.3–21.3 years), and in whom 42% had diabetes, pre-existing diabetes was found to be a predictor of fibrosis progression on multivariate analysis with a regression coefficient of 0.35 ($P = 0.007$) [27]. As previously discussed, the increased rates of NASH and fibrosis and the apparent increases in fibrosis progression observed in populations with diabetes are of great importance, because NASH, particularly with increasing

fibrosis, appears to be far more significant than simple steatosis in leading to liver-related adverse morbidity and mortality outcomes [17,28]. Models of NAFLD that include diabetes mellitus implied that dyslipidemia, IR, and possibly hyperglycemia were involved in the effects of type 2 diabetes on NAFLD and its progression, whereas describing the pathogenesis of NAFLD and NASH in a human with diabetes is complex and challenged [29]. Factors causing NAFLD progression in diabetes are not well defined, but they are likely to involve an interplay of dysfunctional lipid metabolism and disordered glucose regulation related to IR with hyperinsulinemia and a relative insulin deficiency, increased oxidative stress, and local and systemic inflammation. Both genetic and environmental conditions are likely to interact to cause NAFLD and NASH, and also influence their strong relationship with type 2 diabetes [29].

Hyperinsulinemia and IR play a role in the pathogenesis of NAFLD. Studies have shown that patients with NASH are more insulin-resistant than patients with fatty liver alone [30]. Chitturi and colleagues [31] tested the hypothesis that IR is an essential requirement for the development of NASH, and that a high association between IR and liver disease is relatively specific for NASH. Sixty-six patients with NASH were studied. IR was found in virtually all patients (98%), both lean and overweight. A subset of 36 patients with less severe NASH was compared to 36 age- and sex-matched patients with chronic hepatitis C. The prevalence of IR was significantly higher in those with NASH than in their counterparts (75% vs 8.3%) [31]. Issues regarding the nature of hyperinsulinemia in NASH have been raised. It has been questioned whether hyperinsulinemia and IR occur as part of the MetS, or whether liver damage itself leads to chronic hyperinsulinemia and IR from impaired insulin degradation, as is seen in cirrhosis [32]. An aforementioned study [31] conducted a comparison between NASH patients having mild or absent fibrosis and age- and sex-matched patients with chronic hepatitis C found that NASH patients showed more attributes of IR than the controls. They had much higher levels of IR, serum insulin, and C-peptide. However, the serum C-peptide/insulin ratio was similar in both groups. Pagano *et al*. [33] addressed the same issue, comparing 19 patients with histologically mild NASH, who had functionally competent livers, with 19 normal subjects. Patients with NASH showed marked hyperinsulinemia and IR as compared with controls; however, the hepatic insulin extraction was similar in both groups. These two studies showed that insulin hypersecretion, and not just impaired insulin degradation, was the basis for hyperinsulinemia in NASH. Dixon *et al*. [34] studied 105 severely obese individuals undergoing bariatric surgery, and showed that hyperinsulinemia and increased IR were associated with adverse histologic findings. The study found that C-peptide was the best predictor of advanced fibrosis (stage 3–4), and that patients with advanced fibrosis had significantly higher C-peptide levels. The IR index and systemic hypertension were independently associated with advanced NAFLD. IR was found to be the best predictor of zone 3-centric steatosis, inflammation, and fibrosis. In addition, metabolic risk factors such as hypertension and dyslipidemia were highly prevalent among patients with NAFLD [35,36]. Finally, a recent investigation of NAFLD and MetS showed that the presence of MetS carried a high risk for NASH among NAFLD patients, and was also associated with a high risk of severe fibrosis [30]. Thus, features of MetS, like obesity, IR, and hypertriglyceridemia, are not only predisposing factors for NASH but also risk factors for more severe fibrosis and advanced disease.

9.3.2 Age and Gender

In studies, NAFLD and NASH exhibited differences in both prevalence and severity in relation to age and gender. These age and gender differences are caused by differences in the prevalence of obesity, hypertension (influenced by older age of women), and lifestyle-related diseases [35,37]. Of 492 biopsy-proven NASH patients, NASH was more common in men (two- to three-fold) in the younger group (<55 years old); however, the number of NASH cases in women was higher than in men in those over 50 years of age (probably post-menopause) [38]. According to annual health-check findings in Japan, the prevalence of NAFLD in men was around 27% for all ages above 30 years, while in women it gradually increased from 7% of those in their 30s to 23% of those above 60 years of age [39]. A cross-sectional study recruiting 193 biopsy-proven NASH patients compared younger (<55 years old) with older (≥55 years old) groups, and showed that older patients had much more advanced fibrosis than the younger ones (23.8% in younger group vs 54.3% in older group, $P < 0.001$). Women were predominant in the older group (23.8% in younger group vs 67.4% in older group, $P < 0.001$). Age was an independent predictor of advanced fibrosis in the younger group by multivariate analysis ($P = 0.007$) [37]. Likewise, an animal model study of NASH also indicated that aging promotes the progression to steatohepatitis, manifested by increased hepatocellular injury and inflammation [40]. Lee *et al*. [41] evaluated the prevalence and risk factors of biopsy-proven NAFLD in potential living liver donors, finding that NAFLD was highly prevalent in these participants, and that an age of over 30 years was an independent risk factor for significant steatosis (>30%) by multivariate analysis (odds ratio [OR] = 2.223, $P = 0.014$). Furthermore, Valantinas *et al*. [35] observed that NAFLD-related metabolic risk factors and lifestyle were more common in older patients.

Those with liver complications are more often in their sixth through eighth decades of life [42,43]. This could be related to the increasing rate of fibrotic progression with age [37], or to mitochondrial dysfunction (which causes steatosis and hepatic insulin resistance) developing in the elderly [44]. Concerning cirrhotic NASH patients, the prevalence in women was higher than that in men (57% in women and 43% in men) [45]. In contrast, the prevalence of hepatocellular carcinoma was higher in men (38% in women vs 62% in men) [46]. This gender difference may be attributable to differences in exposure to risk factors for HCC, such as cigarette smoking. However, it was recently reported that estrogen-mediated inhibition of IL-6 production in mice models explains the gender disparity in the development of HCC [47]. Estrogen may play a role in the pathogenesis of HCC. Further studies are needed to clarify the pathogenesis of gender differences.

9.3.3 Genetics

A number of studies over the years have revealed a genetic predisposition to NAFLD. As data have accumulated, it has become clear that ethnic differences play a role in susceptibility to NAFLD, especially progressive NAFLD, which cannot be explained simply on the basis of diet or socioeconomic differences. Recent reports of the Third National Health and Nutrition Examination Survey [7], conducted in the United States from 1988 to 1994, found a more common incidence of NAFLD in Mexican Americans (24.1%) compared with non-Hispanic whites (17.8%) and non-Hispanic blacks (13.5%) ($P = 0.001$). A prospective study newly reported that Hispanics had the highest prevalence of NAFLD (58.3%), followed by Caucasian (44.4%) and African Americans (35.1%), in a middle-aged United States population [12]. Because of the mixed racial heritage of such populations in the United States, it would be useful in the future to identify them more precisely using accepted racial-origin genetic markers. This is of particular importance when accumulating data that may ultimately be used to set public policy regarding population screening and/or public health intervention strategies.

Several case studies of familial clustering of NAFLD and NASH have been reported [48], further suggesting that genetic factors may play a role in the pathogenesis of NAFLD. Recently, samples from populations with well-defined NAFLD have begun to be used for genome scans to pinpoint gene variants that are more common in NAFLD patients than in control populations. Romeo *et al*. [49] reported that genetic variation in PNPLA3 confers susceptibility to NAFLD. Other groups have examined single nucleotide polymorphism variants in candidate genes chosen for their known implication in the regulation of lipid metabolism, or their relationship with risk factors for NAFLD [50–52]. In addition, gene/environment interactions are increasingly being explored, and studies have begun to look for chromosomal regions harboring gene variants that affect the onset of NAFLD and its progression [2].

9.3.4 Lifestyle

Non-alcoholic steatohepatitis has been referred to as a disease of the West, in which altered socioeconomic circumstances and related changes in food intake, food composition, and physical activity (together referred to as "lifestyle") may each play a role [1]. Poor dietary habits have been implicated in the development of NAFLD; however, little is known about the role of specific dietary patterns in its development. Oddy *et al*. [53] examined prospective associations between dietary patterns and NAFLD in a population-based cohort of adolescents, and found that NAFLD was present in 15.2% of them. A higher Western dietary pattern score at 14 years was associated with a greater risk of NAFLD at 17 years (OR 1.59, $P < 0.005$), although these associations were no longer significant after adjusting for BMI at 14 years. However, a healthy dietary pattern at 14 years appeared to be protective against NAFLD at 17 years in centrally obese adolescents (OR 0.63, $P = 0.033$), whereas a Western dietary pattern was associated with an increased risk of NAFLD [53]. Nutritional assessments showed that NAFLD patients consumed low-nutrient food, more high-sodium food, more high-fat sources of meat/protein, and few calories from fruits [54].

A cross-sectional study investigated dietary patterns associated with primary NAFLD, and found that the NAFLD group consumed almost twice the amount of soft drinks ($P = 0.030$) and 27% more meat ($P < 0.001$) [55]. In contrast, the NAFLD group consumed somewhat less fish rich in omega-3 ($P = 0.056$). Moreover, a higher intake of soft drinks and meat was significantly associated with an increased risk for NAFLD, independent of age, gender, BMI, and total calories [55]. It has been noted that soft drinks are the leading source of artificially added sugar in the world, and have been linked to obesity in children and adolescents [56]. Recent evidence suggests an association between the intake of sugar-sweetened soft drinks and the risk of obesity and diabetes, because the drinks contain large amounts of high-fructose corn syrup, which raises triglycerides and blood glucose similarly to sucrose [56,57]. Individuals consuming more than one soft drink daily showed a higher prevalence of MetS than those consuming less than one soft drink per day [57]. It has been reported that soft drink consumption is a strong predictor of NAFLD independent of a MetS diagnosis [58]. In that study, 80% of patients with NAFLD had excessive intake of soft drinks (>500 cm^3/day) compared to 17% of healthy controls ($P < 0.001$). The NAFLD

group consumed five times more carbohydrates from soft drinks compared to healthy controls (40% vs 8%, $P < 0.001$). Of these patients, 7% consumed one soft drink per day, 55% consumed two or three soft drinks per day, and 38% consumed more than four soft drinks per day on most days, and for the whole study period [58]. Indeed, dietary fructose consumption by NAFLD patients was excessive, nearly two- to three-fold higher than in controls [59]. Furthermore, hepatic metabolism of fructose favors *de novo* lipogenesis and adenosine triphosphate depletion, which contribute to the development of NAFLD [59]. An additional study found a significant association between higher carbohydrate intake in NAFLD patients and liver inflammation [60].

A study of dietary habits revealed that dietary intake of NASH patients was high in saturated fat and cholesterol, but low in polyunsaturated fat, fiber, and antioxidant vitamins C and E, in comparison with healthy controls. Saturated fat intake correlated with the insulin sensitivity index, the different features of MetS, and the postprandial rise of triglycerides [61]. Here, it is of importance to understand that dietary cholesterol is an important risk factor for the progression of steatosis, and for the inflammatory recruitment and fibrosis in NASH patients with or without obesity and insulin resistance [61–63], as well as in a wide variety of animal models [64–67]. Taken together, unhealthy dietary habits may promote steatohepatitis directly by modulating hepatic triglyceride accumulation and antioxidant activity, and indirectly by affecting insulin sensitivity and postprandial triglyceride metabolism.

Another reported risk factor is cigarette smoke, which contains more than 4000 toxic chemicals, including tar, nicotine, and carbon monoxide [68–70]. An association of cigarette smoking with MetS, such as IR, diabetes, and dyslipidemia, has been reported [71–73]. As we know, MetS is a major risk factor for the development of NAFLD, and the question of whether cigarette smoking impacts the development of NAFLD has been raised. Azzalini *et al*. [74] reported that cigarette smoking caused significant oxidative stress and hepatocellular apoptosis, and worsened the severity of NAFLD, in obese Zucker rats. Their results indeed provided important data for improving our understanding of the relationship between cigarette smoking and NAFLD. However, whether this association holds true in humans remains unclear; moreover, it is not clear whether cigarette smoking independently increases the risk for NAFLD. In a large-scale retrospective study, Hamabe *et al*. [75] found that cigarette smoking was a risk factor for NAFLD development independent of MetS risk factors.

In addition, lack of exercise is a major cause of chronic diseases, including NAFLD [76]. Therefore, when unhealthy dietary patterns and habits are coupled with a sedentary Western lifestyle, caloric imbalance can occur, resulting in increased weight gain in most individuals. The increasing prevalence of overweight/obesity is associated with the epidemic of NAFLD.

9.4 PATHOGENESIS OF NAFLD AND NASH

The pathogenesis of NAFLD and its progression to NASH has not been fully described. An older concept of NASH pathogenesis, the so-called "two-hit" hypothesis of Day and James [77], proposed that hepatocyte triglyceride accumulation resulting from MetS (obesity, IR, and diabetes) is what leads to steatosis (the "first hit"), and that the lipid-laden liver is then vulnerable to injurious processes ("second hit" insults) such as cytokines and oxidative stress. Damaged and dying hepatocytes and/or recruited and activated inflammatory cells, such as Kupffer cells, generate other signals (cytokines, growth factors, and oxidative stress) which activate hepatic stellate cells, with resultant development of liver fibrosis and cirrhosis [78,79].

9.4.1 Mechanism of Steatosis

Steatosis is the excessive accumulation of triglycerides (>5% of liver weight) in the liver [80]. Accumulation of fat in the liver represents an imbalance in hepatic lipid turnover. The liver plays a pivotal role in lipid metabolism. It absorbs circulating free fatty acids and other lipids that arise from intestinal uptake/dietary sources, from lipolysis of peripheral storage sites (adipose tissue), and from *de novo* synthesis (lipogenesis). The liver then exports the lipids for storage in adipose stores as triglyceride-rich very low-density lipoproteins (VLDLs) [81]. Steatosis occurs when fatty acid supplies to the liver (from dietary intake, peripheral lipolysis, and *de novo* lipogenesis) exceed hepatic fatty acid elimination (via oxidation, re-esterification, and excretion as VLDLs) [80,81].

Kinetic studies have indicated that approximately 75% of hepatic lipids in obese patients with NAFLD come from peripheral sites (60% from non-esterified free fatty acids by lipolysis and 15% from diet), with approximately 25% arising from *de novo* lipogenesis [82]. The process of *de novo* lipogenesis is governed by several nuclear transcription factors activated by insulin (in the case of sterol regulatory element binding proteins [SREBPs], 1 and 2) and glucose (in the case of carbohydrate-responsive sterol regulatory element binding protein [ChREBP], 1) [80,83]. Both SREBP1 and ChREBP activate fatty acid synthase, the rate-limiting step in the biosynthesis of long-chain fatty acids which are ultimately esterified to form triglycerides, while SREBP2 regulates cholesterol biosynthesis. These pathways provide a partial explanation of why IR and premetabolic syndrome (which is

hyperinsulinemia and glucose intolerance) are strongly associated with steatosis. Several studies have suggested that there is increased hepatic lipogenesis in hepatic steatosis [84]. Increased lipogenesis may have a dual effect: increased triglyceride synthesis and decreased fatty acid oxidation through production of malonyl-CoA [84], both leading to increased triglyceride content in fatty liver. Sanyal *et al*. reported that β-oxidation of fatty acids in the liver was increased in patients with NASH [85]. However, this increase might not sufficiently overcome the elevated rates of hepatic fatty acid synthesis.

Triglyceride accumulation in hepatocytes was considered to be the major pathogenic trigger in the development of NAFLD. Diacylglycerol acyltransferase 2 (DGAT2) catalyzes the final step in hepatocyte triglyceride biosynthesis. Suppression of DGAT2 reverses diet-induced hepatic steatosis and IR [86], and attenuates hyperlipidemia [87]. However, recent findings suggest that triglyceride synthesis may not be harmful to hepatocytes. Rather, it provides a useful mechanism for buffering free fatty acid accumulation [88]. Yamaguchi *et al*. [88] showed that inhibiting triglyceride synthesis by suppressing DGAT2 did improve hepatic steatosis, yet it exacerbated liver damage and fibrosis in obese mice with non-alcoholic steatohepatitis. Lipotoxicity arises when hepatic triglyceride synthesis is unable to accommodate increased free fatty acid accumulation.

Additionally, IR raises serum insulin and increases serum-free fatty acid levels. In the presence of a steatotic liver, the hyperinsulinemic state fails to suppress adipose-free fatty acid flux, resulting in these free fatty acids being taken up by the liver, driving triglyceride production, and ultimately perpetuating more hepatic steatosis and inflammation when the mechanisms for lipid storage in adipocytes become overwhelmed [89]. Several studies have clearly indicated that the development of NAFLD and MetS is more closely linked to the pattern of fat distribution than to total body fat. In particular, central (or visceral) adiposity is strongly implicated in the development of both hepatic steatosis and MetS [78,90]. Taken together, the following four mechanisms are possible causes of lipid accumulation within the liver: (1) increased delivery and uptake into hepatocytes of long-chain fatty acids due to excess dietary intake or release from adipose tissue; (2) increased *de novo* hepatic fatty acid and triglyceride synthesis; (3) failure of VLDL synthesis and triglyceride export; and (4) failure of fatty acid elimination due to impaired hepatic β-oxidation.

9.4.2 What Promotes Steatosis to Steatohepatitis?

In the setting of stressed and hypertrophic adipocytes caused by overnutrition and obesity, increased visceral adipose tissue was found to induce the recruitment of inflammatory cells, particularly macrophages, resulting in dysregulation of adipocytokines (TNF-α, leptin, resistin, and, most notably, adiponectin) [91]. Visceral adipose tissue secretes more pro-inflammatory cytokines (TNF-α, IL-6, and MCP1), and this, coupled with direct drainage to the liver via the portal circulation, emphasizes the ability of visceral adipose tissue to directly impair hepatic insulin signaling and promote inflammation [81,91]. TNF-α can activate both nuclear factor-kappa B (NF-κB) and c-jun *N*-terminal kinase, promoting serine phosphorylation of an insulin receptor substrate which directly impairs insulin signaling. Additionally, MCP1 can activate inflammatory pathways and promote hepatocyte triglyceride accumulation directly. The NF-κB signaling pathway, in particular, plays a role in the pathogenesis of a wide variety of liver conditions, such as steatohepatitis [92]. NF-κB, commonly referred to as the p65/p50 protein heterodimer, accumulates in the initiation phase of inflammation. It has been reported that the NF-κB activation pathway is involved in the pathogenesis of inflammation in the non-obese and non-diabetic NASH model [80].

Significant hepatocyte death (apoptosis or necrosis) is a feature of NASH [93,94], which triggers regenerative mechanisms to replace dead hepatocytes. However, aberrant repair in some individuals eventually leads to activation of hepatic stellate cells and their transformation to myofibroblasts, and to hepatic recruitment of immune cells that produce pro-inflammatory and profibrogenic cytokines [79].

9.5 ANIMAL MODELS

Studies of NAFLD/NASH using human materials have limitations, because the occurrence and progression of NAFLD/NASH requires a period of several decades, and ethical limitations exist regarding administering drugs to patients or collecting their liver tissue. Animal models of NAFLD/NASH give crucial information, not only for elucidating the pathogenesis of NAFLD/NASH but also for examining therapeutic effects of various agents [95]. An ideal animal model of NAFLD/NASH should reflect the hepatic histopathology and pathophysiology of human NAFLD/NASH. Accordingly, the liver of the NASH animal model should show steatosis, intralobular inflammation, hepatocellular ballooning, perisinusoidal fibrosis in zone 3, and susceptibility to liver tumors. Furthermore, the animal should show metabolic abnormalities such as obesity, IR, fasting hyperglycemia, dyslipidemia, and an altered adipokine profile [95]. Established animal models of NAFLD/NASH are classified into genetic models, nutritional models, and models with a combination of genetic and nutritional factors. Here, we introduce several representative and popular animal models.

9.5.1 Genetic Models

SREBP-1c is a transcription factor involved in adipocyte differentiation. Transgenic mice overexpressing nuclear SREBP-1c in adipose tissues, an inherited lipodystrophic model with severe IR, spontaneously developed steatohepatitis [96]. The animals had a marked fatty liver accompanied by hyperglycemia, hypoleptinemia, and hypoadiponectinemia. Liver histology similar to NASH (i.e., mononuclear cell infiltration, pericellular fibrosis, ballooning degeneration, and Mallory hyaline body formation) was seen in the livers from transgenic mice at 20 weeks of age or older.

The leptin-deficient ob/ob mouse is a commonly studied model for obesity and hepatic steatosis, in which reduced hepatic mitochondrial content and function and an upregulation in *de novo* lipogenesis contribute to obesity-associated NAFLD [97]. Secondary insults such as a methionine- and choline-deficient (MCD) diet or a high fat (HF) diet are needed to trigger steatohepatitis in ob/ob mice [98].

The db/db mouse is hyperleptinemic and develops obesity and severe type 2 diabetes partly due to a functional defect in the long-form leptin receptor, which plays a significant role in the regulation of food intake and control of body weight [99]. These mice have only modestly increased liver triglyceride content, and do not spontaneously develop steatohepatitis or liver fibrosis. They develop NASH when a second hit, such as an MCD diet, is added [99]. The advantage of ob/ob and db/db mice is that the phenotype of these mice simulates the human condition of MetS in many aspects. However, these mice have a disadvantage in that they do not spontaneously develop steatohepatitis or liver fibrosis.

Phosphatase and tensin homolog (PTEN) is a multifunctional phosphatase whose substrate is phosphatidylinositol-3,4,5-triphosphate, and it is also a ubiquitously expressed tumor suppressor gene [100]. Liver-specific PTEN knockout mice showed massive hepatomegaly and steatohepatitis with triglyceride accumulation, a phenotype similar to human NASH [101]. Importantly, the loss of PTEN function in the liver led to tumorigenesis [100–102]. The advantage of this model is that the histological phenotype resembles that of human NASH, while its disadvantage is that it is hypersensitive to insulin [101].

9.5.2 Dietary Model

Feeding mice an MCD diet is a frequently used nutritional model of NASH that induces aminotransferase elevation and hepatic histological changes characterized by steatosis, focal inflammation, hepatocyte necrosis, and fibrosis [88,103,104]. These histological changes occur rapidly, and are morphologically similar to those observed in human NASH.

Alternatively, long-term HF diet feeding with adequate methionine and choline was envisaged as having the potential to induce NASH [105]. C57BL/6J mice fed HF diet for 20 weeks developed histopathological features of human NASH, including hepatic steatosis, ballooning, inflammation, and fibrosis.

Our group established a rat model (stroke-prone spontaneously hypertensive rat, SHRSP5Dmcr) by feeding a high fat-cholesterol (HFC) diet for up to 14 weeks, which exhibited histological changes (especially severe fibrosis) similar to those of human NASH, without obesity or diabetes, but with hypertension [67,80,94,106]. Dietary cholesterol played a more important role in the pathogenesis of liver lesions than dietary fat did via dysregulating bile acid homeostasis in this model (Figure 9.1) [67]. A recent mouse model fed an HFC diet induced hepatic features of NASH, as well as obesity [107].

NAFLD/NASH is regarded as a hepatic manifestation of MetS. Experimental animals fed a fructose-enriched diet are recognized as good models of MetS [108]. Furthermore, mice fed high fructose for 16 weeks had increased hepatic reactive oxygen species and a NASH-like phenotype with significant fibrosis, in addition to developing obesity [109].

9.5.3 Combined Model of Genetic Modification and Dietary Challenges

Many animal models combine naturally occurring genetic mutations or targeted gene modifications with dietary or chemical challenges so that the histopathology and pathophysiology of the models more closely resemble those of human NAFLD/NASH. For example, ob/ob mice fed an HF diet or an MCD diet for 4 weeks developed moderate diffuse macrosteatosis, hepatocellular ballooning, and a diffuse inflammatory infiltrate [98]. Sahai *et al*. [99] fed an MCD diet to ob/ob and db/db mice and observed that db/db mice had significantly higher serum alanine aminotransferase (ALT) levels and more severe hepatic inflammation and fibrosis than in ob/ob and wild-type mice.

9.6 NAFLD AND NASH IN THE ELDERLY

NAFLD is principally a disease of middle-aged and elderly people, with a mean age of presentation between 44 and 50 years [110,111]. The prevalence of NAFLD in the general population increases with age, from 1–3% in children, 5% in teenagers, 18% in those between 20 and 40 years, and 39% in those aged 40–50 years, to over 40% in those greater than 70 years [81]. In general, NAFLD is more predominant in males than females up to the age of 60 years. Beyond menopause, the prevalence of NAFLD rises sharply in women and exceeds that observed in

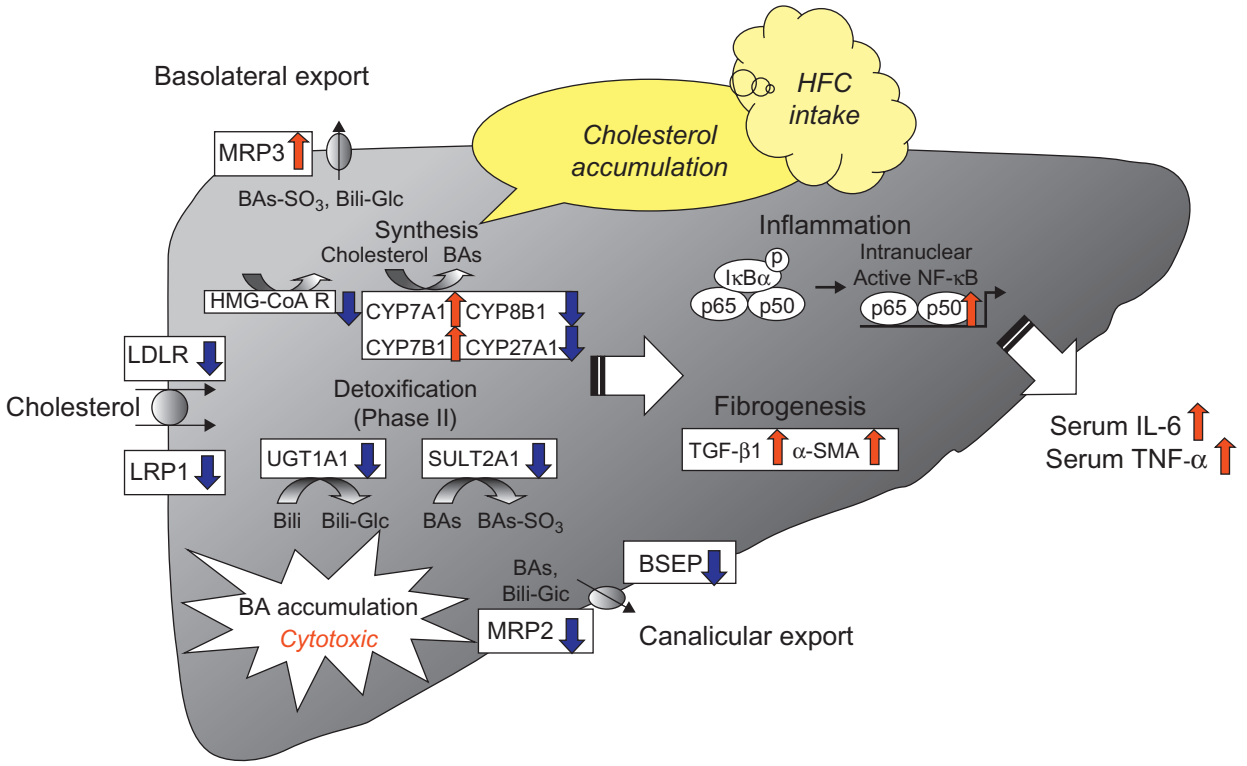

FIGURE 9.1 **A possible mechanism of HFC diet-induced fibrotic steatohepatitis in SHRSP5/Dmcr rats without obesity and diabetes, but with hypertension [67,80,94,106].** A HFC diet suppressed cholesterol uptake (LDLR and LRP1) and synthesis (HMG-CoAR) in response to cholesterol accumulation in liver. A HFC diet accumulated bile acids (BAs) by upregulating CYP7A1 and CYP7B1,while downregulating BSEP as well as UGT1A1 and SULT2A1, which are detoxification enzymes of BAs. Cytotoxic BA accumulations induced hepatitis by increasing inflammatory cytokines such as TNF-α and IL-6, and upregulating the NF-κB subunit p50/p65. All these processes led to fibrosis by upregulating TGF-β and α-SMA. BA, bile acid; BA-SO_3, sulfated BA; Bili, bilirubin; Bili-Glc, glucuronidated bilirubin; BSEP, bile salt export pump; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; HFC, high fat-cholesterol; HMG-CoAR, hydroxymethylglutaryl-CoA reductase, HMG-CoA reductase; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, LDLR, low density lipoprotein receptor; LRP1, lipoprotein receptor related protein-1; MRP3, multidrug resistance-associated protein-3; NF-κB, nuclear factor kappa B; α-SMA, alpha smooth muscle actin; SULT2A1, sulfotransferase 2A1; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor-alpha; UGT1A1, UDP-glucuronosyltransferase 1A1.

their male counterparts [81]. The prevalence of NAFLD in women increases with age, but does not alter with age in men. Furthermore, the prevalence of NAFLD in premenopausal women was found to be lower than that in men and in postmenopausal women. Aging is a risk factor for NAFLD in premenopausal women, independent of weight gain or influence of metabolic syndrome [112]. History of miscarriage and induced abortion were associated with prevalent NAFLD in middle-aged and elderly women [113].

There have been few epidemiological studies reporting on NAFLD in older people. Kagansky *et al*. [114] reported NAFLD to be benign in old age, using a clinical examination which had very low sensitivity as a diagnostic modality. However, more recent studies suggest that there is an increased mortality in those >60 years old [115]. A unique study, which examined risk factors, laboratory data, and histological severity between different age groups (an older group, ≥60 years; a middle-aged group, ≥50 to <60 years; and a younger group, <50 years) in a large cohort with biopsy-confirmed NAFLD, provided some valuable clinical information for a physician when faced with an older person with liver disease [115]. Older patients had significantly more risk factors (hypertension, obesity, diabetes, hyperlipidemia). Albumin, ALT, ALT/aspartate aminotransferase ratio, and platelets significantly reduce with advancing age. Mean cell volume and alkaline phosphatase significantly increase with increasing age. Older patients had significantly greater fibrosis on biopsy with a lower percentage of fat, with cirrhotic patients being significantly older than non-cirrhotics. However, IR was similar among younger and older groups. Another previous study of an Asian population with histologically confirmed NAFLD demonstrated that an age of ≥60 years was significantly associated with severe fibrosis [116]. It has been suggested that NAFLD can cause insults to the liver through an increased load of pro-inflammatory cytokines,

generated through visceral obesity [117]. These insults, alongside an age-related decline in Kupffer cell deactivation of endotoxins, and a slower, reduced ability to regenerate hepatocytes, may contribute to the increased fibrosis [118]. Taken together, age is recognized as an independent risk factor for NAFLD development. The aging process results in increased prevalence of MetS for NAFLD development, changed fat distribution, a reduced liver blood flow, and a reduced ability of the liver to adapt to injury [81]. Such changes could contribute to the poorer liver histology of NAFLD in older people.

9.7 MANAGEMENT OF NAFLD/NASH

Non-alcoholic fatty liver disease has been recognized as a major health burden. It has been independently associated with atherosclerosis [119], arterial stiffness [120], and cardiovascular disease [121], especially in the elderly population [119,121]. In patients with NAFLD, metabolically abnormal individuals are at a higher risk for mortality [122]. Therefore, intervention and treatment for NAFLD/NASH is urgent and important. The cornerstone of NAFLD management is to correct the disturbed metabolic milieu by encouraging an active lifestyle that counteracts increases in body weight and improves insulin sensitivity. Furthermore, treatment of coexisting metabolic disorders such as hypertension, dyslipidemia, and glucose intolerance/diabetes is important in the overall management plan [81]. However, the efficacy and safety profile of pharmacotherapy in the treatment of NAFLD remains uncertain. This section focuses on recent advances in lifestyle intervention for patients with NAFLD or NASH.

The usual management of NAFLD includes gradual weight reduction and increased physical activity, which lead to an improvement in serum enzymes showing liver function, reduced hepatic fatty infiltration, and, in some cases, a reduced degree of hepatic inflammation and fibrosis [123]. Based on available data, patients should optimally achieve a 5–10% weight reduction. Setting realistic goals is essential for a successful long-term lifestyle modification, and more effort must be devoted to informing NAFLD patients of the health benefits of even a modest weight reduction. Furthermore, all NAFLD patients, whether obese or of normal weight, should be informed that a healthy diet has benefits beyond weight reduction. They should be advised to reduce saturated/trans fats and increase polyunsaturated fats, with special emphasis on omega-3 fatty acids. They should reduce added sugar to a minimum, try to avoid soft drinks containing sugar (including fruit juices that contain a large amount of fructose), and increase their fiber intake. For heavy meat-eaters, especially those who consume red and processed meats, less meat and increased fish intake should be recommended. Minimizing fast food intake will also help in maintaining a healthy diet. Physical activity should be integrated into behavioral therapy in NAFLD, as even small gains in physical activity and fitness may have significant health benefits. Potentially therapeutic dietary supplements are vitamins E and D; potentially, good dietary habits are reduced caffeine consumption, modest wine intake, and modest alcohol consumption, although these warrant further research [123–126]. In individuals aged over 65 years, the beneficial effect of diet and exercise on physical fitness, muscle strength, and metabolic fitness has been confirmed, as shown by reductions in hepatic steatosis, serum cholesterol, and high blood pressure, and improved IR [127].

9.8 CONCLUSIONS

The prevalence, severity, and progression of NAFLD/NASH are significantly associated with the presence of components of MetS, especially obesity and diabetes, and are also governed by the interactive effects of age, sex, genetic susceptibility, and lifestyle (diet and exercise). Advanced age is associated with disease severity and fibrosis progression. As an efficacious and safe pharmacotherapy for the treatment of NAFLD has not been established, a combination of dietary modification and physical activity should be recommended as a potential option for patients with NAFLD.

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CHAPTER

10

Use of Tea (*Camellia sinensis* [L.] Kuntze) as a Hepatoprotective Agent in Geriatric Conditions

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10.1 INTRODUCTION

The world's elderly population is growing rapidly, and reports from the Western world, where data are available, indicate that the number of individuals over 65 years of age is expected to rise from 35.1 million in 2000 to 86.7 million in 2050 (US Census Bureau). Aging increases the incidences of various ailments and conditions, such as neurological disorders, diabetes, and degenerative arthritis, and cancer increases almost exponentially, consequentially increasing morbidity and mortality in the afflicted individuals [1]. In addition to the above, the prevalence of chronic liver diseases also increases in the elderly population, but is largely unreported due to non-specific presentation [2].

The liver plays a cardinal role in most metabolic processes, digestion (bile synthesis), and excretion of waste metabolites, and a pathological liver will invariably affect the health and life of the individual [3]. The liver, as the chemical factory of the body, has a remarkable ability to regenerate and maintain function. However, in aging the ensuing biochemical and histological changes reduce the overall physiological functioning of the liver, and this at times compromises the health of the individual [2,4,5].

The aged liver takes on a dark, macroscopic appearance known as "brown atrophy," resulting from the accumulation of intracellular debris, lipofuscin that may arise from defective protein synthesis, and degradation secondary to cumulative oxidative stress [2,6]. Additionally, the activities of the phase I detoxification enzymes, especially the hepatic cytochrome P450 and the antioxidant enzyme superoxide dismutase, are also reduced with age, and both these occurrences contribute to increased sensitivity of hepatocytes to xenobiotic agents [2,4,5]. Thus, several age-related morphological variations in the liver secondary to (1) decreased liver volume, (2) increase in the lipofuscin content, (3) decreased phase I metabolism of certain drugs, (4) shifts in the expression of proteins, and (5) decline in the function of the liver contribute to the reduced hepatic regenerative capacity in the elderly [7].

Diet has a substantial influence on liver functions, health, and aging [8], and numerous studies have shown that *Camellia sinensis* (L.) Kuntze, commonly known as tea, possesses beneficial effects in various geriatric conditions and also acts as a hepatoprotective agent against various hepatotoxins [3]. Globally, tea is the second most widely consumed beverage after water, and it has been cultivated and consumed by humans for thousands of years [9]. Historical evidence suggests that the tea plant was originally native to China, Burma, Thailand, Laos, and Vietnam, but today tea is also cultivated in Sri Lanka, India, and Japan [9].

Depending on the way the leaves are harvested and processed, the tea is categorized as green, black, or oolong

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tea powder. Green tea (unfermented), oolong tea (partially fermented), and black tea (fully fermented) are manufactured from the same tea plant, *Camellia sinensis* [9–11]. Of the total commercial tea production worldwide, about 80% is consumed in the form of black tea, 18% in the form of green tea, and 2% as oolong tea. Black tea is consumed principally in Europe, North America, and North Africa; green tea throughout Asia; and oolong tea in China and Taiwan [9–11].

10.2 PHYTOCHEMISTRY OF TEA

Tea is one of the most investigated plants, and detailed information on the phytochemical constituents is available (Figure 10.1). The active compounds of green tea are the catechins, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG), and proanthocyanidins, flavonols (kaempferol, quercetin, and myricitin in the form of glycosides), gallic acids, and theanine. Those of black tea are thearubigins and theaflavins. The relatively less commonly used oolong tea is reported to contain monomeric catechins, theaflavins, and thearubigins [9]. Tea leaves also contain about 2–5% of the alkaloid caffeine, and small quantities of theobromine and theophylline. Other related compounds in this class include isotheaflavins, neotheaflavins, theaflavic and epitheaflavic acids, theafulvins, and theacitrins [9].

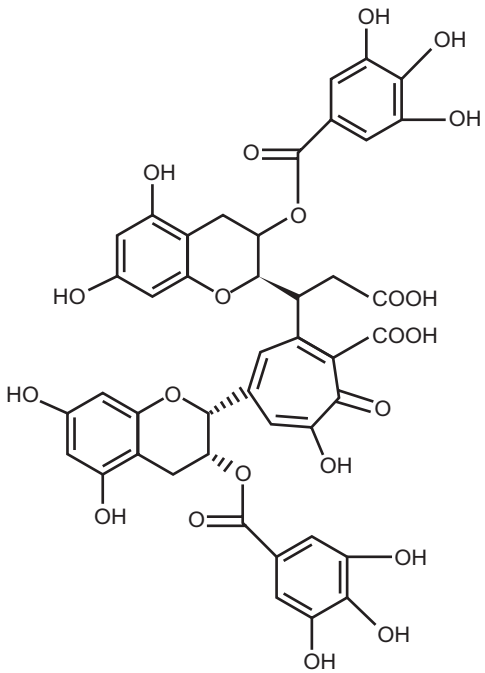

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FIGURE 10.1 Polyphenols present in black tea.

10.2.1 Validated Uses

In traditional systems of medicine, black tea is used for improving mental alertness and cognitive performance. It is also used for headache, hypotension, atherosclerosis, and myocardial infarction; for preventing Parkinson's disease; and for reducing the risk of gastrointestinal cancer, lung cancer, ovarian cancer, and breast cancer [12,13]. Studies have also shown that tea is useful in various conditions such as body weight control and energy metabolism, impaired glucose tolerance and diabetes, cardiovascular disease, bone mineral density, cognitive function and neurodegenerative disease, and cancers of the stomach, esophagus, ovary, and colon [12–17]. Additionally, multiple studies have shown that tea possesses protective effects against various hepatotoxic agents. The following section addresses these observations.

10.3 TEA PROTECTS AGAINST ALCOHOL-INDUCED HEPATOTOXICITY

Alcohol toxicity is one of the world's major health problems, and chronic consumption of high doses of ethanol has been proved to cause liver cirrhosis and cancer [3]. Preclinical studies have shown that tea protects against alcohol-induced hepatotoxicity by ameliorating ethanol-induced oxidative stress and preventing subsequent oxidation of lipids and proteins. Luczaj *et al*. [18] studied the hepatoprotective effects of black tea in rats, and observed that administering black tea decreased ethanol-induced (chronically) increased lipid and protein oxidation products and increased the levels of sulfhydryl groups in liver tissue. Studies have also shown that epigallocatechin-3-gallate (EGCG), the most abundant catechin polyphenol in green tea, shows protection against alcohol-induced cytochrome P450-dependent liver damage, and formation of fatty liver. Dietary supplementation with EGCG (3 g/l with liquid diet for 7 weeks) prevented increases in serum ALT and AST, and ameliorated the reduced hepatic phospho-acetyl CoA carboxylase (p-ACC) and carnitine palmitoyltransferase 1 (CPT-1) levels [19].

In vitro studies have also shown that theanine, a unique amino acid found in green tea, protects against ethanol-induced hepatocytotoxicity, as indicated by amelioration of decreased viability and increased release of LDH and AST. L-theanine inhibited ethanol-induced L02-cell apoptosis and loss of mitochondrial membrane potential, and prevented cytochrome c release from mitochondria in ethanol-treated L02 cells. L-theanine also prevented ethanol-triggered reactive oxygen species (ROS) and malondialdehyde (MDA) generation in L02 cells [20]. In mice chronically intoxicated with

ethanol, L-theonine prevented depletion of antioxidants in hepatocytes and release of hepatic enzymes LDH, AST, and ALT into blood [20].

10.4 TEA PROTECTS AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY

Carbon tetrachloride (CCl_4) is a well-known toxin frequently used in preclinical experiments for xenobiotic-induced hepatotoxicity [21,22]. Green tea extract has been shown to be effective in decreasing the elevated serum levels of ALT and AST, and ameliorating reduced liver glutathione (GSH) and elevated liver lipid peroxide levels, in rats subjected to carbon tetrachloride toxicity [21]. Supplementation of the diet with black or green tea has been shown to prevent increases in plasma levels of amino transferases (ALT and AST) and decrease in plasma total antioxidant capacity in rats intoxicated with carbon tetrachloride [22]. Green or black tea in the diet was also shown to ameliorate oxidative stress in liver, as indicated by preventing decrease in GSH and increase in MDA in the liver [22].

L-theanine, the amino acid constituent of black tea, was found to prevent hepatotoxicity from carbon tetrachloride. In mice pretreated orally with L-theanine (50, 100, or 200 mg/kg, once daily for 7 days) before dosing with carbon tetrachloride, there was prevention of increases in serum ALT and AST and bilirubin level, as well as carbon tetrachloride-induced liver histopathological changes [23]. L-theanine significantly prevented CCl_4-induced production of lipid peroxidation, and decreased hepatic GSH content and antioxidant enzyme activities. L-theonine was shown to downregulate CYP 2E1 expression, inhibit increase of TNF-α and interleukin-1β in serum, and suppress expression of inducible nitric oxide synthase and cyclooxygenase-2 in liver. It also prevented CCl_4-induced activation of apoptotic related proteins, including caspase-3 and PARP, in mouse livers [23]. These studies indicate mitigation of carbon tetrachloride-induced oxidative stress, inflammatory responses, and apoptosis of liver cells by theanine.

10.5 TEA IS EFFECTIVE IN VIRAL HEPATITIS

Globally, hepatitis caused by hepatotropic viruses is the most common cause of various liver diseases and cancer. Of these viruses, hepatitis B and C are responsible for most of the liver diseases. Hepatitis C virus (HCV) is a major cause of liver cirrhosis and hepatocellular carcinoma [24,25]. Green tea catechins, such as EGCG and its derivatives, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), have been found to exert antiviral and antioncogenic properties. EGCG potently inhibited cell culture-derived HCV (HCVcc) entry into hepatoma cell lines as well as primary human hepatocytes. Treatment with EGCG directly during inoculation strongly inhibited HCV infectivity [24]. A study performed by Li and colleagues [25] demonstrated the effect of catechins against viral hepatitis in Beijing ducklings. It was observed that the catechins were effective in reducing the levels of DHBsAg and DHBV-DNA, and also reversed the histopathological changes in the liver [25].

10.6 EFFECT OF TEA ON ISCHEMIA REPERFUSION INJURY

Ischemia–reperfusion injury induced by free radicals is one of the major complications of liver transplantation. Efforts have been made worldwide to prevent the hepatic damage due to reperfusion injury [26]. As the polyphenolic constituents of *Camellia sinensis* have been shown to be potent scavengers of reactive oxygen species, it can be developed as a potential agent to prevent this injury. An animal study conducted with fasted Sprague-Dawley rats showed that a single dose of green tea extract was highly effective in reducing ischemia–reperfusion injury [26]. Green tea extract acts by improving the sinusoidal circulation and also decreasing cellular activation [26].

10.7 EFFECT OF TEA PHYTOCHEMICALS ON HEPATOTOXICITY OF LEAD

Exposure to lead through occupational and environmental settings is of major concern globally. Various studies on the potential hepatotoxicity of lead in experimental animal systems and in humans exposed environmentally have reported alterations in hepatic xenobiotic metabolism, cholesterol metabolism, liver cell proliferation, and DNA synthesis indicative of lead-induced hepatic hyperplasia [27]. An animal study conducted on Sprague-Dawley rats to determine the hepatoprotective effects of green tea extract on lead-induced liver toxicity showed that administration of green tea extract produced significant reduction in the levels of ALT, AST, and ALP, thus proving its protective effects on the hepatic cells [27].

10.7.1 Effect of Tea Phytochemicals on Phenobarbitol-Induced Liver Damage

Hepatotoxicity is an infrequent but fatal adverse effect of phenobarbitol toxicity. Phenobarbitol has been shown to induce cytochrome P-450 (CYP), oxidative stress and, consequently, liver damage [28]. An animal study conducted on diethylnitrosamine-initiated male Wistar rats

showed that administration of epicatechin complex extracted from green tea along with the administration of phenobarbitol during hepatocarcinogenesis produced significant inhibition of the promotive effects of phenobarbitol [29]. Further studies with green tea extract must be conducted to develop it as a potential agent against hepatocarcinogenesis promoted by phenobarbitol.

10.7.2 Effect of Tea Phytochemicals on Hepatotoxicity of Microcystin

Microcystins are cyclic non-ribosomal peptides produced by cyanobacteria, and microcystin, the most common heptapeptide of this group, is known to cause severe hepatic damage principally by inhibiting protein phosphatases [30]. Pretreatment with green tea (12 g/l, 18 days) prior to administration of microcystin decreased the serum levels of ALT, AST, and MDA, and increased SOD and GSH [30]. Studies have shown that the phytochemical quercetin protected mice against the MC-LR-induced hepatotoxicity, and decreased the levels of serum transaminases and hepatic activity of protein phosphatase in mice [31].

10.7.3 Effect of Tea Phytochemicals on Hepatotoxicity of Azathioprine

Azathioprine (AZA) is a purine analog used as an immunosuppressive drug in organ transplantation and autoimmune diseases. The pharmacological action of this compound is mediated through its metabolite 6-thioguanine nucleotide, which is believed to induce apoptosis of activated T lymphocytes, hence leading to suppression of the overactive immune defense mechanisms [32]. AZA-induced hepatotoxicity is considered to be a rare adverse event manifested as nodular regenerative hyperplasia, veno-occlusive disease, peliosis hepatis, fibrosis, and sinusoidal dilatation [32]. The polyphenols present in green tea were shown to mitigate the hepatotoxicity of AZA [32]. Green tea prevented the elevation of enzymes such as ALT, AST, and ALP, and increased the levels of GSH, GPx, CAT, and GSSG, contributing to its anti-inflammatory activity. It also decreased the levels of TNF-α and caspase-3, thus reducing apoptosis [32].

10.7.4 Effect of Tea Phytochemicals on Galactosamine and Lipopolysaccharide-induced Liver Damage

D-galactosamine (GalN) is an important experimental hepatotoxin, and the pathogenesis it causes is akin to that in acute hepatitis. GalN causes insufficiency of UDP-glucose and UDP-galactose and alters the intracellular calcium homeostasis, consequently affecting the cell membrane, cell organelles, and energy metabolism, and the synthesis of proteins and nucleic acids [33]. Studies performed on rats have demonstrated the protective effects of tea against D-galactosamine-induced liver damage, confirming that the extract from tea attenuated the levels of plasma ALT and AST [33]. Lipopolysaccharide (LPS) causes hepatoxicity by induction of oxidative stress and consequent oxidative damage to biomolecules [34]. Pretreatment with tea polyphenols attenuated LPS-induced liver injury, and blunted the rises of serum TNF-α levels, lipid peroxidation, and the induction of expression of TNF-α and inducible nitric oxide synthase (iNOS) in the liver of rats [35].

10.7.5 Effect of Tea on Hepatotoxicity of Insecticides

Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. Induction of the CYP system and oxidative stress have been implicated as the key mechanisms in the hepatotoxic effects of these insecticides. Preclinical studies have observed protective effects of black tea extract against hepatotoxicity of chlorpyrifos and cypermethrin [36]. Administering aqueous black tea extract at a dose of 200 mg/ml to mice before the combination dose of chlorpyriphos and cypermethrin (20 mg/kg each) on alternate days over a 15-day period prevented and mitigated elevation in serum levels of enzymes ALP, AST, and ALT; decrease in hepatic antioxidant enzymes SOD, GPx, GR, GST, and CAT in liver; and increase in lipid peroxidation in liver, when compared to insecticide-alone treated cohorts [36].

10.7.6 Effect of Tea on Hepatocarcinogenesis

N-nitrosodiethylamine (DEN) is a potent hepatocarcinogenic dialkylnitrosamine extensively found in varieties of products, such as milk products, meat products, soft drinks, alcoholic beverages, and tobacco smoke. *N*-nitrosodiethylamine is a commonly used xenobiotic agent in experimental animal model systems [37]. Various preclinical studies have observed protective effects of tea against hepatic carcinogenesis induced/initiated by nitrosodiethylamine. Purified epicatechin complex (87% concentration) isolated from green tea inhibited DEN-initiated, phenobarbitol-promoted proliferation of precancerous liver cells [29]. Green tea (2.5%) administered to rats before and following DEN treatment effectively inhibited hepatocarcinogenesis [37].

Catechin components of green tea have been shown to possess anticarcinogenic properties, possibly related to their antioxidant activity. In studies by Klaunig [38], a catechin-containing green tea extract was examined for its effect on three previously defined properties of liver

tumor promoters: induction of cytolethality, inhibition of gap junctional intercellular communication, and induction of cell proliferation. Green tea extract prevented the induction of hepatocyte cytolethality by glucose oxidase, xanthine oxidase, and paraquat (all oxygen free radical inducers) in a dose-responsive manner. Green tea extract prevented the inhibition of gap junctional-mediated intercellular communication by phenobarbital, lindane, and paraquat in a dose-dependent manner. The effect of green tea extract on hepatocyte DNA synthesis was examined in male mice with preneoplastic liver lesions induced by diethylnitrosamine. Green tea extract significantly decreased the labeling index in hepatic preneoplastic foci [38]. Mice that were administered green or black tea extracts (1.25%, total 40 weeks' treatment) prior to, during, and after DEN treatment showed a decrease in the number hepatic tumor cells when compared to DEN-alone treated cohorts [39].

Tea catechins, black tea extract, and oolong tea extract (0.05% or 0.1%) were shown to significantly decrease the number and area of preneoplastic glutathione S-transferase placental form-positive foci in the liver in rats with DEN-induced hepatocarcinogenesis [40]. Green tea extract was shown to possess an anticarcinogenic effect in DEN-initiated hepatocarcinogenesis without chronic hepatocyte damage, but was not effective in the inhibiting lesion development in hepatic carcinoma with liver cirrhosis [41]. Tea polyphenols and tea pigments have been observed to effectively reduce the GST-Pi expression at both transcription and translation levels, and thus inhibition of carcinogen-induced expression of GST-Pi has been suggested as one of the mechanisms in the anticarcinogenic effect of phytochemicals in tea [42]. Tea polyphenols and pigments have been shown to modulate the phase detoxifying enzymes by inhibiting the overexpression of GST-Pi and promoting the expression of GST-α and GST-μ, thus inhibiting the occurrence and development of the precancerous lesions of rat liver [43]. Green tea was effective in inhibiting hepatocarcinogenesis in mice with DEN-induced, pentachlorophenol-promoted hepatic carcinoma [44].

Obesity associated with insulin resistance, type 2 diabetes, and pro-inflammatory status of the body is a potential risk factor for hepatic cancer. Abnormal activation of the insulin-like growth factor (IGF)/IGF-1 receptor (IGF-1R) axis is also involved in obesity-related liver tumorigenesis. Tea phytochemical EGCG (0.1%, 34 weeks) administered to obese mice (db/db model of obese type 2 diabetic mice) with DEN treatment inhibited the phosphorylation of IGF-IR, ERK (extracellular signal-regulated kinase), GSK-3β (glycogen synthase kinase-3β), and c-Jun NH-terminal kinase in the liver. EGCG also decreased the serum levels of insulin, IGF-1, IGF-2, free fatty acid, and TNF-α, and downregulated the hepatic expression of mRNAs of TNF-α and interleukins. The antitumorigenic actions of EGCG have been suggested to be brought out by improving hyperinsulinemia and attenuating chronic inflammation [45].

10.8 CONCLUSIONS

Observations from the scientific studies carried out in the recent past have clearly shown that tea possesses hepatoprotective action against diverse xenobiotic agents and heptotoxic agents. Several mechanisms are likely to account for the observed pharmacological effects, the most important being free radical scavenging, antioxidant and anti-inflammatory activity, increase in antioxidant enzymes, modulation of phase I and II enzymes, and possibly antiviral effects. Although considerable work has been done to exploit the hepatoprotective effects, countless possibilities for investigation still remain. Further in-depth mechanistic *in vitro* studies, relevant animal studies (especially with old mice), and rationally designed clinical trials are also required. The outcomes of such studies may be useful for identifying further clinical applications of tea in humans, and also open up a new therapeutic avenue, especially in the geriatric population.

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CHAPTER

11

Fruits in the Prevention of Cataractogenesis by Targeting the Aldose Reductase: Promise from Preclinical Observations

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11.1 INTRODUCTION

Recent reports indicate that, at a global level, the incidence of elder adults with visual problems is increasing, and that this is a major challenge to both the healthcare system and the individuals themselves [1]. Predictions are that advances in disease diagnosis and in healthcare facilities will increase, and that this will consequentially result in an increased population of the older adults [1]. Estimations are that by 2050 nearly 2 billion people globally will be aged above 60, and that of these nearly 39 million will be fully blind and 246 million will suffer from limited vision [1]. Additionally, facts indicate that nearly 90% of the world's visually impaired live in developing countries, where the medical facilities are less than optimal when compared with those in developed countries [1].

Of all eye ailments, the leading causes of visual loss in the elderly are cataract, glaucoma, age-related macular degeneration, and diabetic retinopathy [1]. Of these, cataract, defined as any opacity in the crystalline lens or change in its refractive index, remains the single leading cause of blindness in middle- and low-income countries [1]. To emphasize its extent and impact, the 2002 report of the World Health Organization reported that, at a global level, cataracts caused reversible blindness in more than 17 million (47.8%) of the 37 million blind individuals, and that this number is projected to reach 40 million by 2020, having a severe impact on the progress and economy of individuals, their families, the country, and the world at large [1].

11.1.1 Cataracts

The process of cataract formation is multifactorial, and is not completely understood. The prevailing concepts are suggestive of the fact that as the lens ages it increases in weight and thickness, and consequentially decreases in accommodative power [1]. Additionally, as new layers of cortical fibers are formed concentrically, the lens nucleus undergoes compression and hardening, and the underlying chemical modification and proteolytic cleavage of crystalline (lens proteins) result in the formation of high molecular weight protein aggregates. These at times become large enough to cause abrupt fluctuations in the local refractive index of the lens, thereby resulting in scattering of light and reduction in transparency [1]. The chemical modification of lens nuclear proteins also increases pigmentation, such that the lens increasingly takes on a yellow or brownish hue with advancing age. Other age-related changes include a decrease in concentrations of glutathione and potassium,

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and an increase in concentrations of sodium and calcium, in the lens cell cytoplasm [2].

In addition to the normal age-related process of cataractogenesis, certain illnesses such as diabetes, atopic dermatitis, renal and gastrointestinal diseases, and long-term steroid intake also enhance the process and aggravate the clinical condition. Of all the illnesses influencing the process and development of cataract, diabetes is the most important, and the fact that it afflicts nearly 6% of the global population [1] indicates the magnitude of the problem. Studies have shown that diabetes mellitus (DM) affects the clarity of the lens, as well as its refractive index and accommodative amplitude. Cataract formation in diabetes is mediated by both hyperglycemia-induced glycation of lens proteins and oxidative stress [1].

Hyperglycemia increases the level of glucose content in the aqueous humor, which then proportionately will lead to greater levels of diffusion into the lens, thus increasing the process of cataractogenesis [1]. Some of the glucose present in the lens is acted upon by the enzyme aldose reductase (ALR), an enzyme belonging to the aldo-ketoreductases (AKR) super-family, to give sorbitol through the polyol pathway (the sugar alcohol of glucose) (Figure 11.1). ALR is the first and rate-limiting enzyme in the polyol pathway, and reduces glucose to sorbitol utilizing NADPH as a co-factor. The sorbitol formed is then metabolized to fructose by sorbitol dehydrogenase [3] (Figure 11.1).

Evidence for the involvement of ALR2 in diabetic cataractogenesis and other complications has emerged from several independent studies [3–7]. Mice, which are devoid of lens ALR2, do not develop sugar cataract in hyperglycemic conditions [7]. In addition, transgenic mice which overexpress ALR2 develop cataract in hyperglycemic conditions [8]. Genetic deletion of ALR2 is shown to prevent all early effects of diabetes on neural, glial, and vascular cells of the retina in mice, thereby validating its role in cataractogenesis [9].

In normal situations the polyol pathway represents a minor route of glucose utilization, accounting for <3% of glucose consumption. However, in the presence of high glucose the activity of this pathway is substantially increased, and could represent up to 30% of total glucose consumption [4]. The abnormal activation of the polyol pathway during diabetes leads to accumulation of osmotically active sorbitol, leading to osmotic as well as oxidative stress and culminating in tissue injury [4]. The increase in sorbitol levels enhances the osmotic pressure, thus resulting in influx of water and swelling of the lens fibers (Figure 11.1). The increased risk of earlier onset of age-related cataracts in diabetic patients may be a result of the accumulation of sorbitol within the lens and accompanying changes in hydration, increased non-enzymatic glycosylation of lens proteins, or greater oxidative stress from alterations in lens metabolism [10].

Although cataracts are often considered to be an unavoidable consequence of aging, and surgery to be the mainstay of treatment, recent studies of the risk factors associated with human cataract have identified interventions that may prevent or slow the process. Efforts have been directed towards delaying the onset and slowing down the progression of cataract by using various agents, and aldose reductase is considered to be an important target molecule to prevent the process of cataractogenesis. Accordingly, efforts have been ongoing to develop

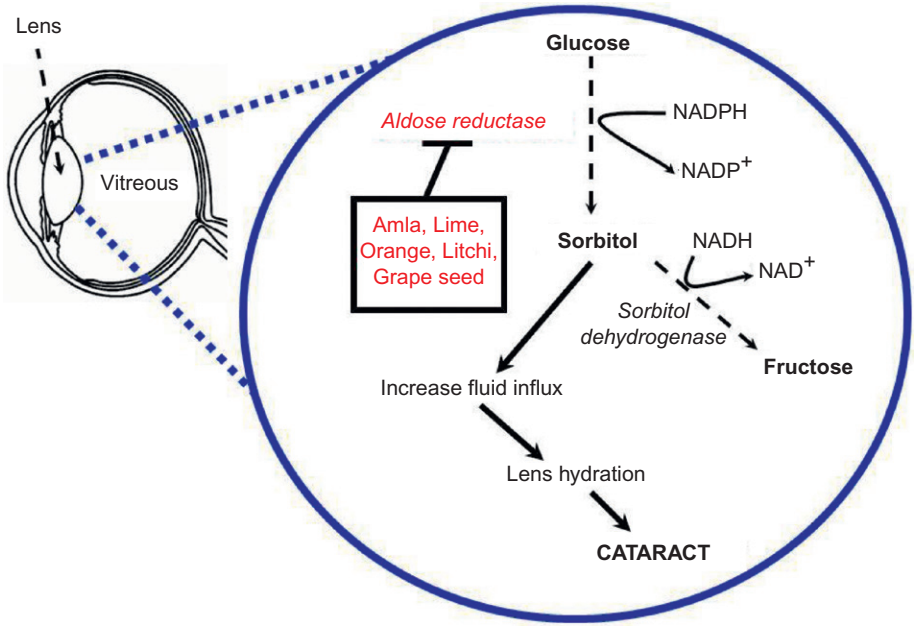

FIGURE 11.1 Process of cataractogenesis and its inhibition by fruits.

a pharmacological agent that is specific and effective in preventing its activity. However, the search for optimal agents remains a long-term objective as historical evidence suggests that numerous synthetic AR inhibitors (ARI) that have been tested are shown to inhibit the enzyme efficiently only in experimental preclinical sets [11,12]. This has necessitated the need for prospective agents preferably from dietary sources, and studies have shown that some dietary agents could be beneficial. In subsequent sections of this chapter, the beneficial effects of some of the dietary agents (the Indian gooseberry, lemon, orange, grapes, mangostene, and litchi) are addressed in detail.

11.2 BENEFICIAL EFFECTS OF DIETARY AGENTS

11.2.1 Amla

Amla, known commonly as the Indian gooseberry and scientifically as *Phyllanthus emblica* syn. *Emblica officinalis* (family Euphorbiaceae), is arguably the most important medicinal agent in the traditional Indian system of medicine, the Ayurveda [13–15]. The fruits, also known as the berries or myrobalans, are the most important part of the plant, being of dietary, culinary, and medicinal use [13–15]. Amla fruit is an important dietary agent, and is used to make murabbah, burfi, ladu, fresh juice, pickle, chutneys, and curries in India [13–15].

The fruits are indispensable in the various folk systems of medicine in Southeast Asia, and are used to treat ailments including diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, erysipelas, skin diseases, hemorrhoids, nervine debility, leprosy, inflammation, emaciation, dyspepsia, colic, flatulence, hyper-acidity, peptic ulcer, jaundice, strangury, diarrhea, dysentery, hemorrhage, leukorrhea, menorrhagia, cardiac disorders, intermittent fever, anemia, jaundice, liver complaints, hematuria, osteoporosis, weak vision, and inflammation of the eyes [13–16].

With respect to amla's role in the prevention of cataractogenesis, scientific studies have shown that 1-O-galloyl-β-D-glucose (β-glucogallin), a major component of the fruit of the gooseberry, was effective in selectively inhibiting (IC_{50} = 17 μM) AKR1B1 *in vitro* [17]. Molecular modeling studies showed that β-glucogallin was able to bind the enzyme at the active site and thereby mediate the inhibitory effects. Subsequent studies have shown that β-glucogallin effectively inhibits sorbitol accumulation by 73% at 30 μM under hyperglycemic conditions in an *ex vivo* organ culture model of lenses excised from transgenic mice overexpressing human ALR2 in the lens [17,18]. Together, these observations clearly indicate the usefulness of β-glucogallin as a novel therapeutic agent to prevent the activity of AKR1B1 and the subsequent process of cataractogenesis [17–20].

11.2.2 Lemon

The fruit of *Citrus limettoides* (family Rutaceae), colloquially known as lemon, is an important dietary and culinary agent in India, Vietnam, Egypt, and other countries around the coasts of the Mediterranean sea. The juice is supposed to possess cooling effects, and is useful in reducing nausea and vomiting, fever, and jaundice [21]. Studies by Saraswat and co-workers have reported that the aqueous extracts from lemon have considerable inhibitory action on the polyol pathway, and inhibit the activity of ALR2 isolated from rat lens [19].

11.2.3 Orange

Sweet orange, scientifically known as *Citrus sinensis* (family Rutaceae), is an important fruit globally. It is supposed to have originated in Southeast Asia, but is today also found growing in other parts of the world [22]. The pulp and the juice prepared from it are important dietary sources of vitamin C, liminoids, synephrine, hesperidin, polyphenols, pectins, calcium, potassium, thiamine, niacin, and magnesium [22]. These biologically active compounds have preventive action against arteriosclerosis, cancer, kidney stones, and stomach ulcers, and have been shown to reduce cholesterol levels [22]. The aqueous extract of orange pulp is reported to be effective in inhibiting the polyol pathway and the activity of ALR2 isolated from rat lens. Together, both these observations indicate the usefulness of orange pulp in preventing cataractogenesis [19].

11.2.4 Grapes

Grapes, known scientifically as *Vitis vinifera* (family Vitaceae), are among the most important fruits in the world, and have dietary, medicinal, and industrial uses [23,24]. Studies carried out over the past two decades have shown that the seeds of grapes (waste products of the winery and grape juice industry) possess myriad biological and pharmacological effects [23,24]. Grape seeds are rich in polyphenols, and are primarily composed of dimers, trimers, and oligomers of monomeric catechins or epicatechins commonly known as procyanidins or proanthocyanidins [23,24]. Commercial preparations of grapeseed polyphenols are marketed in the United States as GSE3, with 95% standardized procyanidins, as dietary a supplement due to its health benefits [25]. Seminal studies by Yamakoshi and co-workers [26] have shown that the grape seed procyanidins and their antioxidative metabolites are effective in preventing progression of cataract formation, through their antioxidative action. Together, all these observations clearly suggest the usefulness of grape seed procyanidins in preventing cataractogenesis, and suggest the requirement for detailed studies along these lines [26].

11.2.5 Mangosteen

The fruit of *Garcinia mangostana* (family Guttiferae), commonly known as a mangosteen, is an important fruit in the various traditional and folk systems of medicine in Southeast Asia, being used to treat dysentery, urinary disorders, cystitis, gonorrhea, inflammatory skin disorders, and wounds [27,28]. The fruits are rich in secondary metabolites known as xanthones, such as α-mangostin, β-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, garcinones A, B, C, D and E, mangostinone, 9-hydroxycalabaxanthone, and isomangostin [28]. With respect to its effect in preventing the activities of ALR, Soda and co-workers [29] have observed that the pericarps of mangosteen possess inhibitory activities against aldose reductase, and may be useful as an anticancer agent.

11.2.6 Litchi

Litchi, known scientifically as *Litchi chinensis* (family Sapindaceae) is a native tropical fruit tree of Southern China, but is today also cultivated in semitropical areas around the world [30]. Phytochemical studies have shown that litchis contain flavanols like procyanidin B4, procyanidin B2, and epicatechin; and anthocyanins like cyanindin-3-rutinside, cyanidin-3-glucoside, quercetin-3-rutinosde, and quercetin-3-glucoside. These phytochemicals possess good antioxidant activity and antineoplastic activity by upregulating and downregulating multiple genes [30]. With respect to its use in the prevention of cataractogenesis, experimental studies with rat lenses have shown that extracts of the fruit possess inhibitory effects. Of the extracts and organic fractions tested, the methanolic extract and an ethyl acetate fraction were the most effective and resulted in a low IC_{50} levels of 3.6 and 0.3 μg/l, respectively. Detailed biochemical studies with the isolated compounds showed that delphinidin 3-O-β-galactopyranoside-39-O-β-glucopyranoside was the most effective, resulting in an IC_{50} of 0.23 μg/ml. Together all these observations indicate the usefulness of litchi in preventing the activity of ALR, suggesting the need for detailed studies.

11.3 CONCLUSIONS

Preclinical studies have demonstrated the inhibitory effects of Indian gooseberry, lemon, sweet orange, grapes, mangosteen, and litchi on aldose reductase (Figure 11.1), indicating that these could be possible agents to prevent or reduce the process of cataractogenesis. These observations are preliminary, and detailed studies are required to validate their usefulness and to make their use acceptable in modern medicine. As these agents have a dietary function and wide acceptability, results from the proposed study would be highly beneficial.

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CHAPTER

12

Ginger (*Zingiber officinale* Roscoe) in the Treatment of Osteoarthritis: Clinical Observations and Mechanistic Insights

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12.1 INTRODUCTION

Osteoarthritis (OA) is a chronic, highly prevalent and disabling disease that is expected to increase in prevalence secondary to longer life expectancy and a disproportionately aging population [1]. Reports from the United States of America indicate that OA affects 33.6% of adults older than 65 years of age, and the prevalence rate increases significantly with age [2,3]. The development of OA is normally a lengthy process, and comprises two phases: (1) a degradative phase, where erosion of the cartilage is accelerated by the production of matrix digesting enzymes and simultaneous decrease in the synthesis of matrix; and (2) a biosynthetic phase, during which the chondrocytes try to repair the damaged extracellular matrix [4]. When compared with the normal chondrocytes, the synthesis–degradation equilibrium of the matrix is altered in osteoarthritic chondrocytes principally due to:

1. Increase in the inflammatory cytokines (IL-1, IL-17, and IL-18)
2. Increase in matrix degrading enzymes the metalloproteinases (MMPs 1, 2, 3, 7, 8, 13, and 14), serine and cysteine proteinases, aggrecanase 1 and aggrecanase 2
3. Generation of free radicals (ROS and RNS)
4. Induction of apoptosis
5. Decrease in the synthesis of MMP enzyme inhibitors (TIMPs)
6. Decrease in the production of anabolic factors (such as cytokines, growth factors, and bone morphogenetic proteins) [4–6].

Studies also suggest that the low-grade inflammatory response observed in OA is due to the increase in prostaglandin E2 (PGE2), which in turn contributes towards inflammatory and degradative processes by interfering in the synthesis of collagen [7].

Pharmacotherapy serves an important role in OA, and acetaminophen, traditional non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, and opioid analgesics are shown to be palliative and to reduce pain in OA. However, protracted use of aspirin is shown to increase the risk of cardiovascular dysfunction; cyclooxygenase-2 inhibitors to increase the risk of myocardial infarction and stroke; NSAIDs to cause dyspepsia, nausea, bloating, gastrointestinal bleeding, ulcer disease, edema, interstitial nephritis, renal insufficiency, and aggravation of hypertension due to their renal toxicity; and opioid analgesics to cause sedation, nausea, constipation, dryness of mouth, giddiness, etc. [1–5].

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12.2 USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES IN THE TREATMENT OF ARTHRITIS

OA is a highly protracted disease and necessitates regular use of treatment that leads to side effects and negates the beneficial effects on prolonged use. This has necessitated the requirement for safe and effective alternative treatments that are devoid of any side effects. Reports also suggest that the use of complementary and alternative medical therapies, including traditional medicines (Ayurvedic, Chinese, Unani, Siddha, Arabic, Srilankan, Tibetan, etc.), acupuncture and acupressure, homeopathy, and dietary restrictions or vitamin supplementation, as well as spiritual healing and prayer, has been increasing among patients with rheumatologic diseases. Reports also suggest that nearly 47% of older adults with osteoarthritis use complementary medicine [8].

Among these, the use of herbs as in various traditional and folk systems of medicine is the most prevalent. The main reason is that many of the plants have documented details of their use in traditional systems since antiquity, and are cheap and easily available [8]. *Zingiber officinale* Roscoe (family Zingiberaceae) is a perennial herbaceous plant that grows to a height of about 1 m. The leaves develop from the branched rhizome, and the flowers, which resemble orchids, are inconspicuous and occur in a dense spike consisting of several overlapping scales on an elongated stalk. Each flower has three yellowish-orange petals with an additional purplish, lip-like structure. It has been cultivated for thousands of years for medicinal purposes and as a spice [9].

Although probably native to North-Eastern India, *Zingiber officinale* has become naturalized in many countries, and is now widely distributed throughout tropical and subtropical parts of the world [10,11]. The ginger rhizome is frequently utilized as a condiment for various foods and beverages. It can be used fresh, dried, or in extracts (mostly decoctions), and demand for ginger is increasing globally. The US Food and Drug Administration has categorized ginger as a food additive [12].

12.3 PHYTOCHEMISTRY OF GINGER

Phytochemical studies have shown that the ginger rhizome contains a wide variety of biologically active compounds. Quantitative studies indicate that the rhizome contains fatty oil, protein, carbohydrates, crude fiber, ash, vitamins, minerals, water, and volatile oil [10,11,13]. The volatile oil and the non-volatile pungent compounds are supposed to be responsible for the characteristic organoleptic properties of ginger [10–14]. The composition of the essential oil varies, and is dependent on the growing conditions (temperature, water, humidity, soil conditions, manure levels, etc.), the time of harvesting, and the age of the plant/rhizome [10,11]. The important constituents present in the volatile oil are the mono- and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol, and α-farmesene [10–13].

The non-volatile pungent phytochemicals of ginger consists of gingerols, shogaols, paradols, and zingerone [14,15] (Figure 12.1). These compounds are responsible for the warm, pungent sensation in the mouth, and are also reported to account for many of its pharmacological effects [10,11]. The gingerols, a series of chemical homologs differentiated by the length of their unbranched alkyl chains (3–6-, 8-, 10-, and 12-gingerols, with a side-chain with 7–10, 12, 14, or 16 carbon atoms, respectively) are the major active components in fresh ginger [10,11,14]. Of all the gingerols, 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant [10–16].

Due to the presence of a -hydroxy keto group, gingerols are highly thermolabile and readily undergo dehydration to form the corresponding shogaols. The extent of this conversion is likely to have a significant impact on the medicinal benefits of ginger, as the two classes of compounds vary in their bioavailability, pharmacokinetics, and pharmacological properties [13]. Shogaols may be further converted to paradols by hydrogenations which are similar to gingerol [10,11]. The other constituents include ginger protease, capsaicin, gingediol, galanolactone, gingesulfonic acid, galactosylglycerols, gingerglycolipids, diarylheptanoids, neral, monoacyldi vitamins, and phytosterols [9,13,15,17–19].

12.4 TRADITIONAL USES OF GINGER

Ginger has been used since ancient times in Ayurvedic and traditional Chinese medicine to treat a wide range of ailments, including common cold, fever, sore throat, pain, rheumatism, and bronchitis; as a carminative, appetite stimulant, and antipyretic; and for digestive problems, gastrointestinal disorders, abdominal spasm, and nausea and vomiting associated with motion sickness and pregnancy [13,15]. It is also useful in the treatment of stomach ache, diarrhea, toothache, gingivitis, bronchitis, hypertension, dementia, fever, helminthiasis, constipation, and asthmatic respiratory disorders [13,15,20].

As a home remedy, ginger is widely used for dyspepsia, flatulence, abdominal discomfort, and nausea. It has been recommended by herbalists for use as a carminative, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, and astringent. Therefore, and deservingly, ginger has been used as medicine since the Vedic

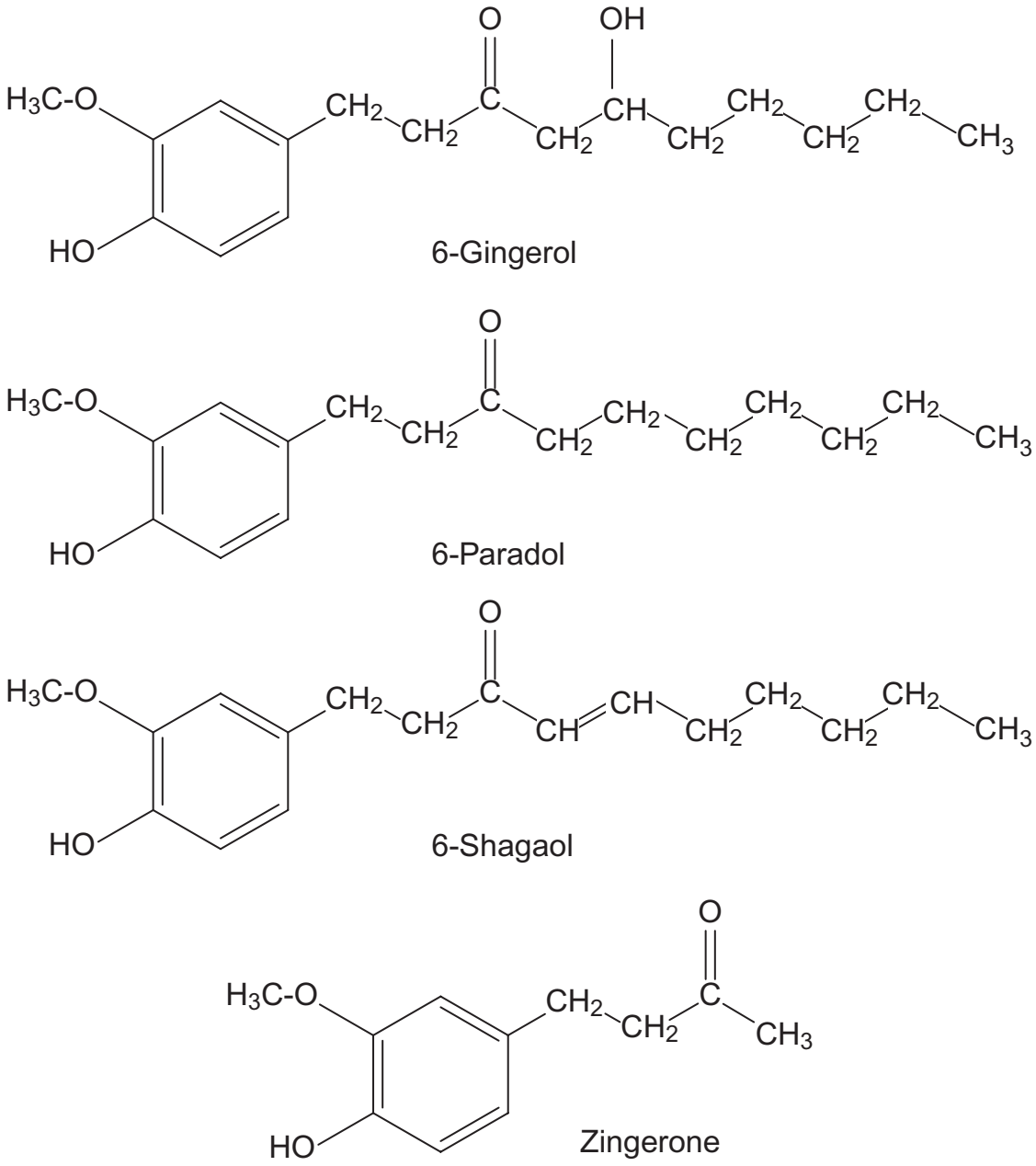

FIGURE 12.1 Structures of some phytochemicals present in the ginger rhizome.

period and is called *maha aushadhi*, meaning "the great medicine" [13,21]. Ginger is also of medicinal use in the various folk systems and the traditional systems of medicines in Asia and Africa [15].

Scientific studies have shown that ginger possesses a wide array of pharmacological and biological properties that are of immense use to humans. The various extracts of ginger have been reported to possess antibacterial, analgesic, anti-inflammatory, antiangiogenetic, and antitumoral ogenic effects [13,15,19]. Ginger is also preventive against gastrointestinal disorders and gastric ulcerogenesis. Recently, clinical studies have shown that daily consumption of raw and heat-treated ginger resulted in moderate-to-large reductions in muscle pain following exercise-induced muscle injury [22].

Ginger also has a long tradition of medicinal use, and has been used as an anti-inflammatory agent for musculoskeletal diseases, including rheumatism, in Ayurvedic and Chinese medicine for more than 2500 years [23]. Of all the actions of ginger, it is the anti-inflammatory and circulatory stimulant effects of the plant that are most important in arthritis [10,11,15]. Preclinical studies with experimental animals suggest that ginger and some of its compounds are effective in preventing

chemically-induced arthritis [23]. In the following sections, the observations from both preclinical and clinical studies will accordingly be discussed.

12.5 SCIENTIFIC STUDIES VALIDATING THE ANTIARTHRITIC EFFECTS OF GINGER

Antiarthritic studies with ginger in humans have shown mixed and contradictory results. Bliddal *et al*. [24] performed a randomized, placebo-controlled crossover study of ginger (170 mg EV ext-33 ginger extract) and ibuprofen (400 mg) in osteoarthritis for 3 weeks each. Ibuprofen performed better than ginger extract, and both treatments were better than the placebo treatment in reducing the pain. In the crossover study, no significant difference between placebo and ginger extract could be seen, suggesting no benefit [24]. Subsequently, Altman and Marcussen [25] evaluated the efficacy of ginger in reducing the pain in a randomized, double-blind, placebo-controlled, multicenter, parallel-group, 6-week study in patients with OA. At the end of the study, when compared to the placebo cohorts, administration of a ginger capsule (255 mg of extract) twice daily, morning and evening, reduced the pain on standing and after walking 50 feet [25]. In another randomized, double-blind, placebo-controlled crossover study of 6 months' duration, Wigler *et al*. [26] observed that administration of ginger extract (250 mg per capsule, four times daily) was as effective as placebo during the first 3 months of the study. However, at the end of 6 months, 3 months after crossover, ginger showed significant superiority over the placebo group in reducing the pain and discomfort associated with OA [26].

From all these studies it can be inferred that while ginger at low concentrations and for a short period of time is ineffective, with prolonged administration the therapeutic benefits are significant. Ginger also has a good safety profile, barring mild adverse effects associated with gastrointestinal functioning [25]. Future studies should involve investigation of the medical benefits of ginger when administered for prolonged periods of time (>6 months consecutively) and with a range of doses. This would clearly help in understanding ginger's efficacy in ameliorating OA-associated pain and discomfort.

12.6 MECHANISTIC STUDIES

12.6.1 Scavenging of Reactive Oxygen Species

Innumerable studies performed in the past have proved conclusively that the reactive oxygen species (ROS) have an important role in the pathogenesis of arthritis [27]. Studies have shown that the extract of ginger and its phytochemicals are free radical scavengers in different cell-free assay systems. The extract was observed to scavenge superoxide, hydroxyl, and $ABTS^{\bullet+}$ radicals in a dose-dependent manner *in vitro* [28,29]. Krishnakantha and Lokesh [30] observed that zingerone scavenged superoxide anions *in vitro*. 6-Gingerol and zingerone are reported to be good scavengers of peroxyl radicals generated by pulse radiolysis, and were observed not to accelerate DNA damage in the bleomycin–Fe (III) system [31].

Glucosides of 6-gingerdiol, 5-O-beta-D-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decane are also a good scavenger of free radicals [32]. Masuda *et al*. [33] studied the antioxidant effects of gingerol-related compounds substituted with an alkyl group bearing a 10-, 12-, or 14-carbon chain length in various *in vitro* assays, and observed that the substitution on the alkyl chain contributes to both radical scavenging effect and inhibitory effect of autoxidation of oils. Additionally, Dugasani *et al*. [34] compared the antioxidant and anti-inflammatory activities of gingerols and their natural analogs. The authors observed that in the antioxidant activity assay *in vitro*, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol exhibited substantial scavenging activities against DPPH superoxide radical hydroxyl radical scavenging assays. Further, at a concentration of 6 μM these compounds also significantly inhibited the f-MLP-stimulated oxidative burst in polymorphonuclear cells (PMNs). A concentration-dependent inhibitory activity on the production of inflammatory mediators of NO and PGE2 was also observed. Of all the compounds, 6-shogaol was observed to possess the most potent antioxidant and anti-inflammatory properties, and this could be attributed to the presence of an α,β-unsaturated ketone moiety [34].

12.6.2 Effect on Antioxidant Molecules and the Antioxidant Enzymes

To prevent or nullify the effect of free radical-induced damage, the eukaryotic cells are equipped with natural antioxidant molecules and antioxidant enzymes [35–37]. Oral feeding of ginger as well as its oil increased the levels of acid-soluble sulfhydryl levels in mice and rats [38–40]. Administration of ginger and its compound zerumbone prevented the depletion of glutathione by the carcinogens [40,41]. Feeding ginger to rats also increased the levels of activities of the antioxidant enzymes SOD, catalase, and GPx [39].

12.6.3 Inhibition of Nitric Oxide and iNOS

Reactive nitrogen species (RNS), such as nitric oxide (NO) and its derivatives (for example, peroxynitrite, $ONOO^-$), have been implicated in the exacerbation of arthritis. Inducible nitric oxide synthase (iNOS), a

pro-inflammatory enzyme responsible for the generation of nitric oxide (NO), has been implicated in the pathogenesis of inflammatory diseases, including arthritis. Pro-inflammatory cytokines such as IL-1 and TNF cause activation of the iNOS pathway in bone cells, and NO derived from this pathway potentiates cytokine and inflammation-induced bone loss. These actions of NO are relevant to the pathogenesis of bone resorption, and may act to suppress bone turnover in severe inflammation. Therefore, inhibition of iNOS represents a novel target for therapeutic intervention in the prevention and treatment of bone diseases [42].

Studies have reported that 6-gingerol inhibits NO production, reduces the levels of LPS-stimulated J774.1 cells, suppresses the peroxynitrite-induced oxidation of dichlorodihydrofluorescein, and inhibits single strand breaks in the supercoiled pTZ 18U plasmid and formation of 3-nitrotyrosine in both bovine serum albumin and J774.1 cells [43]. Other studies have shown that gingerol metabolite and a synthetic analog, capsarol, suppress the NO production in murine macrophages [44]. This effect was mediated partially by inhibiting iNOS enzymatic activity and reducing the iNOS protein production that resulted in attenuation of the NF-κB mediated iNOS gene expression [44]. 6-Shogaol is also observed to block both protein and mRNA expression of iNOS in murine RAW 264.7 cells activated with LPS [45]. Additionally, feeding of zingerone suppresses the increased levels of pro-inflammatory enzymes COX-2 and iNOS in aged rat [46].

12.6.4 Anti-inflammatory Activity

Inflammation is a complex process involving both cellular and molecular components, and, when triggered, leads to widespread changes in the physiological systems. Inflammation is characterized by pain, heat, redness, swelling, and loss of function [47,48]. Depending on the onset time and duration, inflammation is denoted as acute or chronic, and can be either beneficial or detrimental. Acute inflammation is short in duration, rapid in onset, and critical to ward off infection, while chronic inflammation is more prolonged in duration and is mostly detrimental. Synovitis results in proliferation of articular mucosa, destroys cartilage, penetrates bone, tears ligaments and tendons, and results in joint destruction [47,48].

The anti-inflammatory properties of ginger have been known and valued for centuries, and studies in the past two decades have validated this long-held belief [20]. Experimental studies have shown that intraperitoneal administration of ethanolic extract of dried ginger inhibited carrageenan-induced [49] inflammation, and fresh egg albumin provoked inflammation [50]. Recently, a well-characterized crude ginger extract and a fraction containing only gingerols and their derivatives have both been reported to possess anti-inflammatory effects on the streptococcal cell wall-induced animal model of rheumatoid arthritis [12]. Administration of the essential oil of ginger [51] and the phytochemicals 6-gingerol [52] and 6-shogaol [53] is also reported to lead to anti-inflammatory effects in rats.

Mechanistic studies have shown that ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase-1, cyclooxygenase-2, and leukotriene biosynthesis by inhibiting 5-lipoxygenase [20]. Srivastava [54] reported that the ginger extract inhibited cyclooxygenase. Later studies have shown that the ginger constituents 8-paradol and 8-shogaol possess strong inhibitory effects on COX-2 enzyme activity *in vitro* [55]. The COX-1 inhibitory activity of 8-paradol was more potent than in the gingerol analogs [55]. Studies have also shown that 8-paradol, a natural constituent of ginger, was an effective COX-1 inhibitor, while a diarylheptanoid with catechol group was the most active against 5-lipoxygenase [56].

12.6.5 Ginger Decreases the Metalloproteinase Levels

The extracellular matrix, which was formerly considered to be an environment for cell–cell interaction and a structural support, is important in controlling bone remodeling. Matrix synthesis and degradation is tightly regulated, and under normal conditions homeostasis exists [57,58]. However, in OA there is a shift towards degradation, and this sequentially leads to the observed pathological changes [58]. The zinc-dependent endopeptidases commonly known as metalloproteinases (MMPs) are recognized to be important enzymes capable of degrading practically all components of the extracellular matrix [58]. Accordingly, MMPs are considered to be ideal pharmacological targets, and several inhibitors have been investigated. However, none of the molecules, including Ro32-3555 (Trocade™), a collagenase selective inhibitor, have been able to prevent arthritis [59]. With regard to ginger and its phytochemicals, studies have shown that they possess anti-MMP activities and prevent cancer metastasis [19,60]. However, studies on their effect in arthritis are absent. It is quite possible that the mechanism that operates in antimetastatic activity extends to arthritis, and this needs to be validated.

12.6.6 Decrease in NF-κB Activation

NF-κB, a family of inducible transcription factors found virtually in all cells, serves as an important regulator of the host immune and inflammatory response [61]. Its role in inflammation is undisputed, and inhibition of the pathway is widely believed to have great potential as a therapeutic target in many diseases, including arthritis. Several studies have shown conclusively that

ginger and its phytochemicals are effective in inhibiting the activation and transactivation of NF-κB in disease models such as cancer in both *in vitro* and *in vivo* systems of study [61,62]. *In vitro* studies with synoviocytes have shown that pretreatment with ginger extract (100 μg/ml) 1 hour before activation with 1 ng/ml TNF-α and 10 ng/ml interleukin-1β significantly inhibited the activation of TNF-α and COX-2 expression, and was also accompanied by suppression of NF-κB and IκBα induction [63].

12.7 CONCLUSIONS

Ginger has been used as an antiarthritic agent for centuries in the various traditional and folk systems of medicine. However, to date, clinical data are insufficient to draw firm conclusions. The observed opposing results may be due to variations in the bioactive compounds, as these studies were performed in different countries. Studies have shown that the pharmacological activity of ginger is due to gingerol, paradols, and shogaol. The final ratio of these compounds in ginger is determined by a number of factors, including geographic origin, the maturity of rhizomes at the time of harvest, and the method by which extracts are prepared. The gingerols are thermally labile and readily undergo dehydration to form the corresponding shogaols. The extent of this conversion is likely to have a significant impact on the medicinal benefits of ginger, as the two compounds vary in their bioavailability, pharmacokinetics, and pharmacological properties. In the setting of these observations it is imperative that a quality control be established for the presence of active phytochemicals in the required levels. Additionally, preclinical studies should be performed to understand the efficacy of important ginger phytochemicals, such as gingerols, shogaols, paradols, zingerone, dehydrodzingerone, terpinolene, β-pinene, α-phellandrene, β-sesquiphelandrene, α-pinene, β-lemene, etc., as antiarthritic agents. Due to its abundance, low cost, and safety in consumption, ginger remains a species with tremendous potential and countless possibilities for further investigation as an antiarthritic agent.

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CHAPTER

13

Natural Polyphenols Target the Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) Signaling Pathway for Cancer Chemoprevention

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13.1 INTRODUCTION

Natural compounds are extensively researched for their health-promoting potential. Polyphenols, as an essential part of the human diet, play an important role in cancer chemoprevention. They occur in vegetables, fruits, seeds, grains, herbs, spices, propolis, tea, beer, and wine [1]. The epidemiologic findings and laboratory studies strongly suggest that the rates of malignant diseases are influenced by environmental factors, including diets rich in polyphenols. These compounds protect against cancers through multiple mechanisms of action [1–4].

Chemoprevention is a means of cancer control in which carcinogenesis is inhibited or reversed by nutritional or pharmacological intervention with natural or synthetic agents [5]. When Dr Michael Sporn, for the first time, introduced the term "chemoprevention," referring to the activity of natural forms of vitamin A and its synthetic analogs in preventing the development and progression of epithelial cancer, he originated a novel field in cancer research [6]. Consequently, a systematic dissection of the chemopreventive potential of polyphenolic compounds in recent years has clearly supported their health benefits, including anticancer effects.

Polyphenols exert anticancer and chemopreventive properties by multiple mechanisms of action affecting apoptotic pathways in cancer cells [7,8]. Targeting the TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) -induced apoptotic pathway in cancer cells by polyphenolic compounds could be one of the mechanisms responsible for their chemopreventive activity. Polyphenols have been shown to sensitize cancer cells to TRAIL-induced apoptosis [9–11]. The induction of cancer cell-specific apoptosis via the activation of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) signaling has become an important focus of cancer research [12]. However, as more tumor cells are reported to be resistant to TRAIL-mediated death, it is important to develop new strategies to overcome this resistance [13,14]. This chapter provides an overview of the molecular targets underlying reversal of TRAIL resistance in cancer cells by polyphenols.

13.2 CHARACTERISTIC OF DEATH LIGAND TRAIL AND TRAIL-MEDIATED APOPTOTIC PATHWAY

Apoptosis is a ubiquitous and high regulated mechanism by which cells undergo programmed death. Resistance to apoptosis is a hallmark of cancer, with loss of pro-apoptotic stimuli and the gain of anti-apoptotic

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mechanisms contributing to tumorigenesis. Apoptosis is a complex process that involves the participation of affected cells in a self-destructive cascade and is defined by a set of characteristic morphological changes, including membrane blebbing, shrinkage of cell and nuclear volume, chromatin condensation, and nuclear DNA fragmentation due to endonuclease activation [8]. Apoptosis is controlled by two diverse pathways: extrinsic (receptor-mediated) and intrinsic (mitochondrial). The extrinsic pathway is initiated by the interaction of death ligands and agonistic surface receptors able to deliver a death signal from the extracellular microenvironment to the cytoplasm. The intrinsic (mitochondrial) pathway is activated by various agents acting inside the cell, such as DNA damage, cellular distress, or hypoxia, and the death signal converges to mitochondria [8,12].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF (tumor necrosis factor) ligand superfamily of cytokines. TRAIL was discovered independently by two teams, both of which reported sequence homology with the extracellular domain of the other TNF family members: FasL (CD95L/Apo2L) and TNF-α. The death ligand TRAIL induces apoptosis in cancer cells with no toxicity against normal tissues [15–20]. TRAIL is a type II transmembrane protein expressed by cells of the immune system, such as T lymphocytes, natural killer cells, dendritic cells, neutrophils, monocytes, and macrophages. The extracellular domain of TRAIL can be shed from the cell surface by cysteine proteases to produce soluble TRAIL. Soluble or membrane-bounded TRAIL plays an important role in surveillance and defense against tumor cells [21–23]. TRAIL binds to multiple receptors, including two death receptors (DR4 [TRAIL-R1] and DR5 [TRAIL-R2]) and three decoy receptors (TRAIL-R3 [DcR1], TRAIL-R4 [DcR2], and osteoprotegerin [OPG]) [24,25]. Endogenous TRAIL triggers apoptosis via receptor-mediated death (extrinsic pathway) through interaction with the death receptors (DRs) in cancer cells. Only DRs contain complete and functional intracellular death domains (DDs) responsible for the activation of apoptosis pathway in cancer cells. Therefore, death receptor 4 (DR4/TRAIL-R1) and death receptor 5 (DR5/TRAIL-R2) are able to transmit an apoptotic signal [26–29].

The failure to undergo apoptosis has been implicated in the resistance of cancer cells to TRAIL surveillance and, therefore, in tumor development [10–12]. The expression of DRs and pro-apoptotic (Bid, Bax, Bak, Bad, Diablo/Smac) or anti-apoptotic (FLIP, Bcl-2, Bcl-xL, Mcl-1, Akt, IAP-1, IAP-2, XIAP, survivin) proteins in cancer cells is involved in TRAIL resistance [28–35].

Binding of TRAIL to DRs is the first step of the extrinsic apoptotic pathway, also known as the death receptor pathway [30,32]. The decreased expression of DRs in the cancer cell surface causes TRAIL resistance [22,28]. Ligation of trimerized TRAIL to DR4 and/or DR5 receptor leads to conformational change in their DDs along with the subsequent oligomerization and clustering of the DRs. This DR activation allows for the recruitment of the adaptor molecule FADD (Fas-associated death domain) with formation of the DISC (death inducing signaling complex), activation of initiator caspases (caspase-8 and -10), cleavage of effector caspases (caspase-3, -6, and -7), and, finally, DNA fragmentation [12,27,36]. The anti-apoptotic protein FLIP (FLICE [FADD-like IL-1β-converting enzyme] inhibitory protein) can also be part of the DISC to replace caspase-8 and form an inactive complex. Overexpression of FLIP in cancer cells blocks the activation of caspase-8 [13,14]. In some cancer cells activated caspase-8 is sufficient to trigger apoptosis, while other cells require activation of the mitochondrial (intrinsic) pathway to amplify the apoptotic signal. In the mitochondrial pathway, caspase-8 leads indirectly to the activation of effector caspases through the cleavage of the BH3-interacting domain death agonist (Bid), along with the mitochondrial membrane potential (MMP) disruption [10]. Crosstalk between the extrinsic (receptor-dependent) and intrinsic (mitochondrial-dependent) apoptosis pathways is linked by caspase-8 mediated Bid cleavage and subsequent translocation of tBid (truncated Bid) to the mitochondria to initiate the intrinsic apoptosis pathway [11]. Truncated Bid interacts with pro-apoptotic mitochondrial proteins from the Bcl-2 (B-cell leukemia 2) family (Bax, Bak, Bad), stimulating the decrease in the MMP. The loss of integrity of the mitochondrial membrane leads to the release of cytochrome c and Diablo/Smac (direct inhibitor of apoptosis binding protein with low isoelectric point/second mitochondrial activator of caspases) [12]. Among the cellular signaling pathways that promote cell survival, anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-xL, Mcl-1) could inhibit the liberation of cytochrome c from mitochondria [13,14]. Akt, a serine/threonine protein kinase, is another important factor contributing to TRAIL resistance. Akt can also prevent cytochrome c escape to cytosol [14,32]. Furthermore, cytochrome c, in the presence of Apaf-1 (apoptotic protease-activating factor-1) and procaspase-9, forms the apoptosome. Activated caspase-9 in turn stimulates executioner caspases (caspase-3, -6, -7), leading to cell death [35,36]. Effector caspase activity is controlled by IAPs (inhibitor of apoptosis proteins) IAP-1, IAP-2, XIAP (X-linked inhibitor of apoptosis protein), and survivin. Diablo/Smac augments apoptosis by binding to the cellular IAP members, which are potent caspase inhibitors [13,14]. TRAIL-resistant cancer cells can be sensitized to TRAIL-mediated apoptosis by certain natural polyphenols [9–12].

13.3 POLYPHENOLS ENHANCE TRAIL-INDUCED APOPTOSIS IN CANCER CELLS

Epidemiological data support the concept that polyphenols in the diet are safe and non-toxic, and have long-lasting beneficial effects on human health [37–39]. These compounds are divided into several classes on the basis of their chemical structure, namely phenolic acids derivatives (for example, cinnamic acid derivatives), flavones, flavonolignans, flavanols, flavanones, isoflavones, chalcones, and stilbenes [2]. Table 13.1 [1,2,10–12,33,37,38,40–63] presents the classification and the major sources of polyphenols targeting the TRAIL-induced apoptotic

TABLE 13.1 Classification and Major Dietary Sources of Polyphenols Targeting the TRAIL-induced Apoptotic Pathway

Class of polyphenols	Compound	Major sources	References
Cinnamic acid derivative	Artepillin C	Propolis, leaf of *Baccharis dracunculifolia*	[40,41]
Cinnamic acid derivative	Curcumin	Spice turmeric (derived from the rhizome of *Curcuma longa*)	[11,38]
Cinnamic acid derivative	Cycloartenyl ferulate	Rice bran	[42]
Flavone	Chrysin	Carrot, cherry, passion flower (*Passiflora species*), poplar (*Populus balsamifera*, *Populus nigra*), root of *Scutellateria baicalensis*, *Choerospondiasis axillaries*, *Chrysanthemum morifolium*, propolis	[43,44]
Flavone	Apigenin	Celery, parsley, onion, orange, black tea, propolis	[2,33,45]
Flavone	Luteolin	Cabbage, pepper, garlic, guava, basil, oregano	[10,11]
Flavone	Baicalein	Celery, broccoli, carrot, root of *Scutellateria baicalensis*	[37,38]
Flavone	Wogonin	Root of *Scutellateria baicalensis*	[1,11]
Flavone	Casticin	Fruit of *Vitex trifolia* (fructus *Viticis*)	[46]
Flavone	Hispidulin	*Saussurea involucrata*	[47]
Flavone	5,7-Dimethoxyflavone	*Leptospermum scoparium*, *Kaempferia parviflora*, *Piper methysticum* (Kava)	[48]
Flavonolignan	Silibinin	Milk thistle (*Silybum marianum*)	[49]
Flavanol	Epigallocatechin -3-gallate (EGCG)	Green tea	[50]
Flavanone	Naringenin	Orange, grape, tomato, propolis	[12,51]
Isoflavone	Daidzein	Soybean	[52–54]
Isoflavone	Genistein	Soybean	[52–54]
Isoflavone	Biochanin A	Soybean, red clover (*Trifolium pretense*), propolis	[52–54]
Flavonol	Kaempferol	Berries, tomato, broccoli, red wine, green tea, black tea, propolis	[10,12]
Flavonol	Quercetin	Apple, cherry, onion, broccoli, red wine, green tea, black tea, propolis	[41,43,55]
Flavonol	Myricetin	Berries, red wine, green tea, black tea	[10,11]
Flavonol	Fisetin	Apple, strawberry, grape, kiwi fruit, persimmon, cucumber, onion	[56]
Flavonol	Dihydroflavonol BB-1	*Blumea balsamifera*	[57]
Chalcone	Isobavachalcone	*Psoralea corylifolia*, *Broussonetia papyrifera, Angelica keiskei*	[58]
Chalcone	Licochalcone A	Root of licorice (*Glycyrrhiza glabra*, *Glycyrrhiza uralensis*)	[58]
Chalcone	Xanthohumol	Hops (*Humulus lupulus)*	[58]
Chalcone	Flavokawain B	*Piper methysticum* (Kava)	[59]
Chalcone	Butein	Stem bark of *Semecarpus anacardium*, heartwood of *Dalbergia odorifera*, *Caragana jubata*, *Rhus verniciflua*	[60]
Chalcone	Cardamomin	Cardamom (fruit of *Amomum subulatum*), *Catimbium speciosum* (*Alpinia speciosa*)	[61]
Chalcone	Isoliquiritigenin	*Glycyrrhiza uralensis* (licorice), *Glycine max*, *Dalbergia odorifera*, *Sinofranchetia chinensis*, *Allium ascalonicum*, soybean, propolis	[62]
Stilbene	Resveratrol	Red wine, red grape, peanuts, rhizome of *Polygonum cuspidatum*, *Veratrum grandiflorum* (*Helleborus)*	[63]

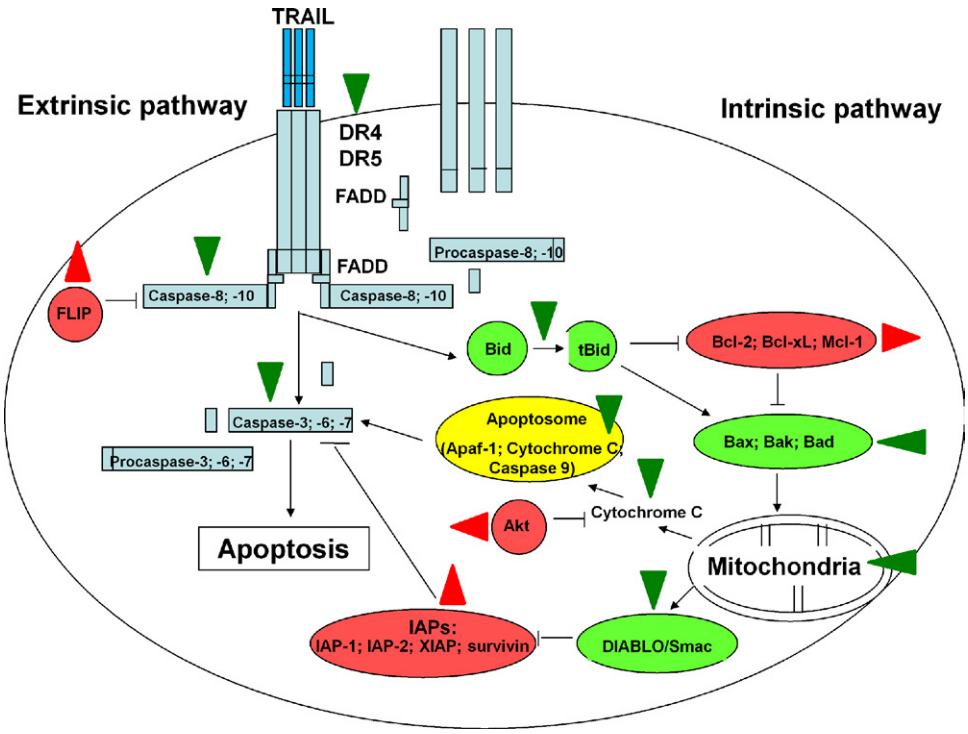

FIGURE 13.1 The molecular targets in TRAIL-mediated apoptotic pathways in cancer cells for polyphenols (the green arrowheads signing activation and red arrowheads signing inhibition). TRAIL binds to death receptors DR4 and/or DR5 and promotes the recruitment of the adaptor molecule FADD (Fas-associated death domain) to activate caspase-8 and/or caspase-10, which trigger activation of downstream effector caspases (caspase-3, -6, -7). FLIP can block activation of caspase-8 or casapase-10. Caspase-8 also mediated cleavage of Bid (BH3-interacting domain death agonist). Truncated Bid (tBid) translocates to the mitochondria, where it interacts with pro-apoptotic Bax, Bak, and Bad, stimulating disruption of MMP (mitochondrial membrane potential) and the release of cytochrome c and Diablo/Smac (direct inhibitor of apoptosis binding protein with low isoelectric point/second mitochondrial activator of caspases). Anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-xL, and Mcl-1) could inhibit loss of MMP. Akt may prevent cytochrome c escape to cytosol. Cytochrome c liberated from the mitochondria binds to the adaptor protein Apaf-1 (apoptotic protease-activating factor-1) and procaspase-9, forming the apoptosome and activating caspase-9, which in turn activates executioner caspases (caspase-3, -6, -7), leading to cell death. Activity of executioner caspases is inhibited by IAPs (inhibitor of apoptosis proteins): IAP-1, IAP-2, XIAP, and survivin. Diablo/Smac blocks IAPs.

pathway. Resistance to apoptosis is a hallmark of cancer, with both the loss of pro-apoptotic signals and the gain of anti-apoptotic mechanisms contributing to tumorigenesis [10]. Polyphenols act through the induction of apoptosis to prevent tumor promotion and progression [7,8]. Various studies have shown that many types of cancer cells are resistant to TRAIL-induced death, but combinatorial approaches based on TRAIL and natural polyphenols have been discovered to overcome the resistance of cancer cells to TRAIL [12]. The effects of cotreatment with natural polyphenols and TRAIL on cancer cells have been investigated. Figure 13.1 shows the molecular targets for polyphenols in TRAIL-mediated apoptotic pathways in cancer cells. The experimental data suggest that polyphenols augment the anticancer immune effector response through restored sensitivity of cancer cells to TRAIL-mediated apoptosis. Table 13.2 [42,46–48,51,56–62,64–129] demonstrates the mechanism by which polyphenols enhance TRAIL-induced apoptosis in cancer cells. TRAIL is one of several members of the TNF superfamily that induce apoptosis through engagement of death receptors. The extrinsic pathway is initiated by interaction between death ligand TRAIL and agonistic surface receptors. Expression levels of DR4/TRAIL-R1 and/or DR5/TRAIL-R2 on the cancer cell surface may play a critical role in intensity and/or duration of death receptor-mediated signaling in response to death ligand. The decreased expression of DRs on the cancer cell surface causes TRAIL resistance. Numerous natural polyphenols have been reported to upregulate expression of DRs (at mRNA and/or protein levels) and sensitize tumor cells to TRAIL-mediated apoptosis [13,28]. The cinnamic acid derivatives (artepillin C, curcumin, cycloartenyl ferulate), flavones (chrysin, apigenin, luteolin, baicalein, wogonin, casticin, 5,7-dimethoxyflavone), flavonolignan (silibinin), flavanol (EGCG), flavanone (naringenin), isoflavone (biochanin A), flavonols (kaempferol, quercetin, myricetin, fisetin, dihydroflavonol BB-1), chalcones (chalcone, isobavachalcone, licochalcone A, xanthohumol,

TABLE 13.2 Schematic Presentation of the Mechanisms by which Polyphenols Modulate TRAIL-induced Apoptotic Signaling in Cancer Cells

Class of polyphenols	Compound	Targets	Cell lines	References
Cinnamic acid derivative	Artepillin C	↑ DR5	Prostate cancer LNCaP	[64]
		↑ caspase-8		
		↑ loss of MMP		
		↑ caspase-3		
	Curcumin	↑ DR4	Prostate cancer LNCaP	[65–80]
		↑ DR5	Prostate cancer DU145	
		↑ caspase-8	Prostate cancer PC3	
		↑ Bid cleavage	Colon cancer HT-29	
		↑ Bax	Colon cancer HT-116	
		↑ Bak	Hepatocellular cancer HepG2	
		↓ Bcl-2	Lung cancer NCI-H446	
		↓ Bcl-xL	Renal cancer Caki	
		↑ loss of MMP	Bladder cancer MBT-2	
		↑ cytochrome c release	Bladder cancer 253J-BV	
		↑ caspase-9	Bladder cancer KU-7	
		↓ Akt	Bladder cancer RT4V6	
		↓ XIAP	Ovarian cancer SKOV3	
		↓ survivin	Ovarian cancer ES-2	
		↑ caspase-3	Glioma U87-MG	
			Neuroblastoma SK-N-AS	
			Neuroblastoma SHEP1	
			Lymphoma B CA46	
			Lymphoma B KK124	
			Lymphoma B AS283A	
			Lymphoma B PA862PB	
	Cycloartenyl ferulate	↑ DR4	Colon cancer SW480	[42]
		↑ DR5	Colon cancer SW620	
		↑ caspase-8	Colon cancer Colo-201	
		↑ caspase-10		
		↑ Bid cleavage		
		↑ Bak		
		↓ Bcl-2		
		↑ loss of MMP		
		↑ cytochrome c release		
		↑ DIABLO release		
		↑ caspase-9		
		↑ caspase-3		
		↑ caspase-6		
		↑ caspase-7		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Flavone	Chrysin	↑ DR5	Colon cancer HT-29	[81,82]
		↓ FLIP	Hepatocellular cancer HepG2	
		↑ caspase-8	Pancreatic cancer Capan-1	
		↑ caspase-3	Breast cancer MDA-MB-231	
			Nelanoma SK-MEL-37	
			Nasopharyngeal cancer CNE1	
	Apigenin	↑ DR5	Prostate cancer DU145	[82,83]
		↑ FLIP	Colon cancer HT-29	
		↑ caspase-8	Colon cancer DLD-1	
		↑ caspase-10	Hepatocellular cancer HepG2	
		↑ Bid cleavage	Pancreatic cancer Capan-1	
		↑ caspase-9	Breast cancer MDA-MB-231	
		↑ caspase-3	Melanoma SK-MEL-37	
			Leukemia Jurkat	
	Luteolin	↑ DR4	Colon cancer HT-29	[84–87]
		↑ DR5	Hepatocellular cancer HepG2	
		↑ caspase-8	Lung cancer A549	
		↑ caspase-10	Nasopharyngeal cancer CNE1	
		↑ Bid cleavage	Cervical cancer HeLa	
		↑ caspase-9		
		↓ XIAP		
		↑ caspase-3		
	Baicalein	↑ DR5	Prostate cancer PC3	[88]
		↑ caspase-8	Colon cancer SW480	
		↑ caspase-10		
		↑ caspase-9		
		↑ caspase-3		
	Wogonin	↑ DR5	Prostate cancer LNCaP	[82,89–91]
		↑ caspase-8	Colon cancer HT-29	
		↓ FLIP	Hepatocellular cancer HepG2	
		↑ caspase-9	Pancreatic cancer Capan-1	
		↓ IAP-1	Lung cancer A549	
		↓ IAP-2	Breast cancer MDA-MB-231	
		↓ XIAP	Melanoma SK-MEL-37	
		↑ caspase-3		
	Casticin	↑ DR5	Hepatocellular cancer HepG2	[46]
		↑ caspase-8	Hepatocellular cancer PLC/PFR	
		↑ caspase-9		
		↑ caspase-3		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Flavone	Hispidulin	↑ caspase-8	Ovarian cancer SKOV3	[47]
		↓ Bcl-2		
		↓ Bcl-xL		
		↓ Mcl-1		
		↑ caspase-9		
	5,7-Dimethoxyflavone	↑ DR5	Hepatocellular cancer HepG2	[48]
		↑ caspase-8	Hepatocellular cancer Hep3B	
		↑ caspase-9	Hepatocellular cancer Huh-7	
		↑ caspase-3		
Flavonolignan	Silibinin	↑ DR4	Colon cancer SW480	[92–95]
		↑ DR5	Colon cancer SW620	
		↑ caspase-8	Hepatocellular cancer Hep55-1C	
		↑ caspase-10	Glioma U87-MG	
		↓ FLIP	Glioma U251-MG	
		↑ Bid cleavage	Glioma A172	
		↓ Mcl-1	Glioma U251N	
		↑ loss of MMP		
		↑ cytochrome c release		
		↑ caspase-9		
		↓ Akt		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
Flavanol	Epigallocatechin -3-gallate (EGCG)	↑ DR4	Prostate cancer LNCaP	[96–100]
		↑ DR5	Hepatocellular cancer HepG2	
		↑ caspase-8	Pancreatic cancer MIAPaCa-2	
		↓ FLIP	Melanoma A375	
		↑ Bid cleavage	Glioma U87	
		↑ Bax	Glioma U87-MG	
		↑ Bak	Glioma A172	
		↓ Bcl-2		
		↓ Bcl-xL		
		↑ caspase-9		
		↓ Akt		
		↓ IAP-1		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
		↑ caspase-6		
		↑ caspase-7		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Flavanone	Naringenin	↑ DR5	Lung cancer A549	[51]
		↑ Bid cleavage		
		↑ loss of MMP		
Isoflavone	Daidzein	↓ Bcl-2	Prostate cancer LNCaP	[101,102]
		↑ loss of MMP	Glioma LN229	
		↑ caspase-9		
	Genistein	↓ FLIP	Hepatocellular cancer HepG2	[102–108]
		↑ caspase-8	Hepatocellular cancer Hep3B	
		↑ Bid cleavage	Pancreatic cancer AsPC1	
		↑ Bax	Gastric cancer AGS	
		↓ Bcl-2	Lung cancer A549	
		↓ Bcl-xL	Glioma LN229	
		↑ loss of MMP	Glioma NCH89	
		↑ caspase-9		
		↓ Akt		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
		↑ caspase-7		
	Biochanin A	↑ DR5	Prostate cancer LNCaP	[109]
		↑ loss of MMP	Prostate cancer DU145	
Flavonol	Kaempferol	↑ DR4	Colon cancer SW480	[110,111]
		↑ DR5	Glioma U87	
		↑ caspase-8	Glioma U251	
		↑ caspase-10		
		↓ Bcl-2		
		↓ Bcl-xL		
		↓ Mcl-1		
		↑ caspase-9		
		↓ Akt		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Flavonol	Quercetin	↑ DR4	Prostate cancer LNCaP	[112–119]
		↑ DR5	Prostate cancer DU145	
		↑ caspase-8	Prostate cancer PC3	
		↓ FLIP	Colon cancer HT-29	
		↑ Bid cleavage	Colon cancer SW620	
		↓ Mcl-1	Colon cancer Caco-2	
		↑ cytochrome c release	Hepatocellular cancer HepG2	
		↑ caspase-9	Hepatocellular cancer SK-Hep	
		↓ Akt	Hepatocellular cancer SNU-387	
		↓ XIAP	Hepatocellular cancer SNU-423	
		↓ survivin	Hepatocellular cancer SNU-449	
		↑ caspase-3	Hepatocellular cancer SNU-475	
			Lung cancer H460	
			Lung cancer H2009	
			Lung cancer H1299	
			Lung cancer A549	
			Lymphoma B VAL	
			Lymphoma B RL	
			Lymphoma B SUDHL4	
			Glioma U87-MG	
			Glioma U251	
			Glioma A172	
			Glioma LN229	
	Myricetin	↑ DR5	Glioma U251	[120]
		↑ caspase-8	Glioma LN229	
		↓ FLIP	Glioma NCH89	
		↓ Bcl-2		
		↑ caspase-9		
		↓ survivin		
		↑ caspase-3		
		↑ caspase-7		
	Fisetin	↑ DR4	Prostate cancer LNCaP	[56]
		↑ caspase-8		
		↑ loss of MMP		
		↑ caspase-3		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Flavonol	Dihydroflavonol BB-1	↑ DR5	Leukemia U937	[57]
		↑ loss of MMP	Leukemia K562	
		↑ caspase-9	Leukemia KOB	
		↓ Akt		
		↓ survivin		
		↑ caspase-3		
Chalcone	Chalcone	↑ DR4	Cervical cancer HeLa	[58,121]
		↑ DR5		
		↑ loss of MMP		
	Isobavachalcone	↑ DR4	Cervical cancer HeLa	[58]
		↑ DR5		
	Licochalcone A	↑ DR4	Cervical cancer HeLa	[58]
		↑ DR5		
	Xanthohumol	↑ DR4	Cervical cancer HeLa	[58]
		↑ DR5		
	Flavokawain B	↑ DR5	Prostate cancer DU145	[59]
		↑ Bax	Prostate cancer PC3	
		↑ caspase-9		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
		↑ caspase-7		
	But-ein	↑ DR5	Colon cancer HCT-116	[60,122]
		↑ caspase-8	Hepatocellular cancer HepG2	
		↑ Bid cleavage	Hepatocellular cancer Hep3B	
		↑ Bad	Leukemia U937	
		↓ Bcl-2	Leukemia Jurkat	
		↑ loss of MMP	Leukemia K562	
		↑ cytochrome c release		
		↑ caspase-9		
		↓ IAP-1		
		↓ IAP-2		
		↓ XIAP		
		↑ caspase-3		

(*Continued*)

TABLE 13.2 (Continued)

Class of polyphenols	Compound	Targets	Cell lines	References
Chalcone	Cardamomin	↑ DR4	Prostate cancer DU145	[61,123]
		↑ DR5	Prostate cancer PC3	
		↓ DcR1	Colon cancer DLD-1	
		↓ DcR2	Colon cancer HCT-116	
		↑ caspase-8	Pancreatic cancer MIAPaCa-2	
		↓ FLIP	Leukemia KMB-5	
		↑ Bid cleavage	Multiple myeloma U266	
		↑ Bax		
		↓ Bcl-2		
		↓ Bcl-xL		
		↑ caspase-9		
		↓ IAP-1		
		↓ IAP-2		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
	Isoliquiritigenin	↑ DR5	Colon cancer HT-29	[62]
Stilbene	Resveratrol	↑ DR4	Prostate cancer LNCaP	[124–129]
		↑ DR5	Prostate cancer DU145	
		↑ caspase-8	Prostate cancer PC3	
		↓ FLIP	Colon cancer HT-29	
		↑ Bid cleavage	Colon cancer HCT-116	
		↑ Bax	Melanoma LU1205	
		↑ Bak	Neuroblastoma SHEP	
		↓ Bcl-2	Osteosarcoma Saos	
		↓ Bcl-xL		
		↑ loss of MMP		
		↑ cytochrome c release		
		↑ DIABLO release		
		↑ caspase-9		
		↓ XIAP		
		↓ survivin		
		↑ caspase-3		
		↑ caspase-6		
		↑ caspase-7		

flavokawain B, butein, cardamomin, isoliquiritigenin) and stilbene (resveratrol) increase expression of DR4 and/or DR5 [42,46,48,51,56–62,64,66,67,70,73–75,77,82–85,88,92–96,99,100,109,110,112,113,116,118,120,122,123,125,127,128]. Recent studies have revealed that DR5, called the "KILLER" receptor, plays a more prominent role than DR4 in TRAIL-mediated apoptotic signaling, especially in cancer cells derived from solid tumors. The use of DR5/Fc chimera protein, which has a dominant negative function against DR5 receptor or DR5 gene silencing by transfection of cells with small interfering RNA (siRNA), efficiently blocked apoptosis caused by the co-treatment with polyphenols and TRAIL. These data suggested that polyphenols sensitize cancer cells to TRAIL through the extrinsic (receptor) apoptotic pathway first of all via affecting DR5 [12,14,35].

Polyphenols could also enhance the cytotoxic effect of TRAIL in cancer cells through caspase activation. Caspases are crucial players in the induction of apoptosis. TRAIL-mediated apoptosis is primarily executed by the extrinsic death receptor pathway [10]. This pathway involves caspases-8 and -10 as the initiator caspases. The cinnamic acid derivatives (artepillin C, curcumin, cycloartenyl ferulate), flavones (chrysin, apigenin, luteolin, baicalein, wogonin, casticin, hispidulin, 5,7-dimethoxyflavone), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein), flavonols (kaempferol, quercetin, myricetin, fisetin), chalcones (butein, cardamomin), and stilbene (resveratrol) reverse TRAIL resistance in cancer cells via activation of caspase-8 and/or -10 [42,46–48,56,60,61,64,65,68,69,71,81,83–91,93–96,98,99,106–108,110,111,114–117,119,120,122–124,127,128]. A well-known inhibitor of this route is FLIP, a catalytically inactive procaspase-8 and -10 homolog that, after recruitment to the DISC, prevents apoptosis. Cellular FLIP suppresses the activation of caspase-8 and -10 [13]. Flavones (chrysin, apigenin, wogonin), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein), flavonols (quercetin, myricetin), chalcone (cardamomin), and stilbene (resveratrol) sensitize cancer cells to TRAIL-induced death through down-regulation of FLIP expression [82,91,92,96,106,116,120,123,129]. Caspase-9 is engaged in the mitochondrial pathway. The cinnamic acid derivatives (curcumin, cycloartenyl ferulate), flavones (apigenin, luteolin, baicalein, wogonin, casticin, hispidulin, 5,7-dimethoxyflavone), flavonolignan (silibinin), flavanol (EGCG), isoflavones (daidzein, genistein), flavonols (kaempferol, quercetin, myricetin, dihydroflavonol BB-1), chalcones (flavokawain B, butein, cardamomin), and stilbene (resveratrol) induce caspase-9 activation and in this way augment TRAIL-mediated apoptosis in cancer cells [42,46–48,57,61,65,68,69,71,73,75,78,83,85,87,88,90,93,94,96,101,106,107,110,114–120,122–124,127,128]. Caspases-3, -6, and -7 are the executor caspases [34]. The cinnamic acid derivatives (artepillin C, curcumin, cycloartenyl ferulate), flavones (chrysin, apigenin, luteolin, baicalein, wogonin, casticin, 5,7-dimethoxyflavone), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein), flavonols (kaempferol, quercetin, myricetin, fisetin, dihydroflavonol BB-1), chalcones (flavokawain B, butein, cardamomin), and stilbene (resveratrol) overcome TRAIL-resistance in cancer cells by activation of caspases-3 or -6 and -7 [42,46,48,56,57,59–61,64–66,68–71,73,75,78,80,81,83,85–88,90–92,94–100,104–108,110,114–116,118–120,122–124,127,128].

Bid links the extrinsic and intrinsic apoptotic pathways [29]. The cinnamic acid derivatives (curcumin and cycloartenyl ferulate), flavones (apigenin and luteolin), flavonolignan (silibinin), flavanol (EGCG), flavanone (naringenin), isoflavone (genistein), flavonol (quercetin), chalcones (butein and cardamomin), and stilbene (resveratrol) cooperate with TRAIL through induction of Bid cleavage in cancer cells [42,51,65,69,83–85,93,98,107,108,117,122–124].

Bcl-2 family members are key regulators of mitochondrial apoptosis in which the balance between anti-apoptotic proteins (Bcl-2, Bcl-xL, Mcl-1) and pro-apoptotic proteins (Bax, Bak, and Bad) determines the mitochondrial membrane permeabilization [14,30]. Truncated Bid engages the intrinsic pathway by binding to Bax, Bak, and Bad, resulting in their oligomerization and translocation to the mitochondrial outer membrane. These pro-apoptotic proteins promote the decrease of mitochondrial membrane potential with subsequent cytochrome c and Dablo/Smac release [10]. The cinnamic acid derivatives (curcumin and cycloartenyl ferulate), flavanol (EGCG), isoflavone (genistein), chalcones (flavokawain B, butein, and cardamomin), and stilbene (resveratrol) restore tumor cells sensitivity to TRAIL-induced death by up-regulation of Bax or Bak and Bad [42,59,73–75,78,96,107,122–124,127,128]. In contrast, anti-apoptotic Bcl-2 proteins inhibit TRAIL-mediated apoptosis in cancer cells. The cinnamic acid derivatives (curcumin and cycloartenyl ferulate), flavone (hispidulin), flavonolignan (silibinin), flavanol (EGCG), isoflavones (daidzein, genistein), flavonols (kaempferol, quercetin, myricetin), chalcones (butein, cardamomin), and stilbene (resveratrol) induce synergistic anticancer effect with TRAIL through down-regulation of Bcl-2 or Bcl-xL and Mcl-1 [42,47,61,73,74,78,94,96,100,101,103,107,111,119,120,122–124,127–129].

The analyses of the mitochondrial membrane potential (MMP) show that TRAIL and polyphenol co-treatment affects the intrinsic pathway in cancer cells via a significant reduction of MMP [33]. The cinnamic acid derivatives (artepillin C, curcumin, cycloartenyl ferulate), flavonolignan (silibinin), flavanone (naringenin), isoflavones (daidzein, genistein, biochanin-A), flavonols (fisetin, dihydroflavonol BB-1), chalcones (chalcone and

butein), and stilbene (resveratrol) increase the loss of MMP in cancer cells [42,51,56,57,64,73,75,78,93,94,101,102,107,109,121,122,127].

The polyphenolic compounds could promote cytochrome c release from mitochondria and in this way interact with TRAIL in the induction of programmed death in tumor cells. The cinnamic acid derivatives (curcumin and cycloartenyl ferulate), flavonolignan (silibinin), flavonol (quercetin), chalcone (butein), and stilbene (resveratrol) activate translocation of cytochrome c from mitochondria into cytoplasm [42,65,68,69,75,78,93,119,122,127,128]. Akt, a serine/threonine protein kinase, blocks cytochrome c escape to cytosol [32]. The cinnamic acid derivative (curcumin), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein) and flavonols (kaempferol, quercetin, and dihydroflavonol BB-1) sensitize cancer cells to TRAIL-mediated apoptosis via suppression of Akt expression [57,72,76,79,92,97,103,111,112,114,117].

Diablo/Smac binds to the cellular IAP members, which are potent caspase inhibitors [12]. The cinnamic acid derivative (cycloartenyl ferulate) and stilbene (resveratrol) enhance TRAIL-mediated apoptosis in cancer cells though increased liberation of Diablo/Smac from mitochondria into cytoplasm [42,127]. IAPs (IAP-1, IAP-2, XIAP, and survivin) have been shown to neutralize the activity of effector caspases [22]. Polyphenols restore TRAIL-sensitivity in cancer cells by decreasing IAPs expression. The flavone (wogonin), flavanol (EGCG), and chalcones (butein and cardamomin) downregulate the expression of IAP-1 and/or IAP-2 in cancer cells [91,96,122,123]. The cinnamic acid derivative (curcumin), flavones (luteolin and wogonin), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein), flavonols (kaempferol and quercetin), chalcones (flavokawain B, butein, cardamomin) and stilbene (resveratrol) block the XIAP expression in cancer cells [59,70,72,73,80,86,91,94,96,106,107,111,117,122,123,128]. The cinnamic acid derivative (curcumin), flavone (wogonin), flavonolignan (silibinin), flavanol (EGCG), isoflavone (genistein), flavonols (kaempferol, quercetin, myricetin, and dihydroflavonol BB-1), chalcones (flavokawain B and cardamomin), and stilbene (resveratrol) suppress the expression of survivin in cancer cells [57,59,73,92,96,97,106,111,112,115,117,119,120,123,124,126–129].

Taken together, polyphenols used in combination with TRAIL increased the expression of pro-apoptotic proteins or decrease the expression of anti-apoptotic proteins in cancer cells. This modulation of pro-apoptotic and anti-apoptotic regulatory factors by polyphenols contributed to sensitization of cancer cells to TRAIL-mediated apoptosis. Based on the evidence from laboratory studies, malignant diseases can be prevented by natural, dietary polyphenols, which induced apoptosis synergistically with TRAIL in cancer cells [130].

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CHAPTER

14

Use of the Ayurvedic Drug Triphala in Medical Conditions Afflicting Older Adults

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14.1 INTRODUCTION

Before the advent of modern medicine, Ayurveda, the traditional system of Indian medicine, which when translated literally means "science of life," was the cornerstone of the healthcare system in ancient India. Ayurveda has its roots in the *Vedas*, which dates back to nearly 5000 years BC, and ancient literatures like the *Charaka Samhita* and *Sushruta Samhita*, written about 1000 years BC, which give a detailed account on the diagnostic procedures and treatment needed to manage various ailments and diseases of both humans and domesticated animals [1,2].

Ayurveda is an integral part of the Indian culture and materia medica, and even today remains an influential system of medicine. Estimates are that nearly 70% of India's population relies on the use of Ayurvedic medications and its proprietary drugs for the treatment and prevention of various ailments and diseases. Ayurveda and its variations are also practiced around the world, and in Europe and the United States it is considered to be a form of complementary and alternative medicine [3].

The concept and treatment principles of Ayurveda are different from those of modern medicine. While modern medicine is evidence-based and makes use of a distinct well-defined chemical entity for treatment, emphasis in Ayurveda is mainly on disease prevention and promotion of good health by following a proper lifestyle and adopting measures that rejuvenate the cells of the body [1,4].

According to Ayurvedic philosophy, the body is made up of *Tridoshas* (three humors), *Saptha Dhatus* (seven types of tissues), and *Malas* (waste products). All three are made up of *Panchamaha boothas*, the five basic elements of the universe: earth, water, fire, air, and ether [1,5]. The three *Doshas* constitute the *Vata* (wind or air in Sanskrit), the *Pitta* (fire or bile), and the *Kapha* (water or mucus).

According to Ayurveda, *Vata* is related to physical movement and the nervous system, and is responsible for cellular differentiation [5,6]. *Pitta* refers to the gastric juices and other cellular enzymes responsible for the digestion and biotransformation of food, drugs, and xenobiotics [5]. It is also thought to be involved in the generation and preservation of body heat [6]. *Kapha* relates to moisture in the tissues and sensory organs of the body [5]. *Kapha* and *Pitta* are more anabolic in nature, whereas *Vata* is catabolic in nature [5].

The concept of *Tridoshas* is similar to that of Greek philosophy, and may be equated to air (*Vata*), bile (*Pitta*), and phlegm (*Kapha*), respectively [6]. Tripathi [7] proposes

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that these *Tridoshas* at the molecular level are responsible for various functions: *Vata* for membrane-bound signal transduction, *Pitta* for signaling moieties and enzymes, and *Kapha* for gene and protein expression. For optimal functioning of the body, the *Tridoshas* need to be in a state of equilibrium with each other, and any alterations affect the normal functions and health of the individual. The balance in *Tridosha* is highly sensitive, and is susceptible to variation due to environmental changes (diurnal, nocturnal, and seasonal modifications), altered lifestyle (changes in food habits, physical activity), and age [6]. The other important aspect of Ayurvedic philosophy is the concept of the *Saptha Dhatus*, or seven tissue system. These are *Rasa* (plasma or lymph), *Raktha* (blood cells), *Mamsa* (muscle), *Medas* (fat), *Asthi* (bones), *Majja* (marrow), and *Sukra* (semen) [5,6]. According to Ayurveda, *Rasa Dhatu* is the most important as it nourishes all the other tissues, and *ojas* is the end result of the ensuing anabolic process [5,6]. *Ojas* is an excellent body element, and its quantity and quality are responsible for optimal functioning of the body. It is also believed that *ojas* enhances the performance of the body and increases resistance against infections and diseases, thereby resulting in a healthy life [5,6].

Ayurveda assesses a person's body constitution (*Prakruti*) based on the *Doshas*, which in turn help in offering a personalized treatment. This classification is based on the physical, physiological, and psychological characteristics of the individual. The *Prakruti* is correlated to phenotype of the individual, and accordingly is classified as *Vata Prakruthi*, *Pitta Prakruthi*, *Kapha Prakruthi*, *Dwanda Prakruthi* (a combination of two *Doshas*), or *Sama Doshaja Prakruthi* (a combination of all three) [8].

In order to stay healthy, the *Dosha*, *Dhatu*, and *Malas* (feces, urine, and sweat) need to function in harmony with one another. An imbalance in one or more of the *Doshas* would have a direct impact on the *Dhatus* or *Malas*, leading to the onset of, or aggravating, a disease. Ayurveda strongly suggests that lifestyle and diet have an important role in altering the delicate tridoshic balance and necessary remedial measures need to be adopted or followed accordingly [5,6].

Ayurveda has eight branches/specializations, called *Ashtanga Ayurveda*, dealing with different aspects of prevention and treatment. These are *Kaya Cikitsa* (internal medicine), *Salya Tantra* (surgery), *Salakya Tantra* (treatment of diseases of the head and neck region), *Agada Tantra* (toxicology), *Bhuta Vidya* (psychiatry), *Bala Roga* (pediatrics), *Rasayana Tantra* (rejuvenation therapy and geriatrics), and *Vajikarana Tantra* (the science of aphrodisiacs) [9,10].

14.2 RASAYANA DRUGS IN AYURVEDA

In Ayurveda, maintenance of good health is considered just as important as the treatment of disease, and this aspect is addressed in the subdiscipline of *Rasayana Tantra/Shastra*. In the classical Ayurvedic text *Charaka Samhita* there is an exclusive chapter on Rasayana, while in *Ashtanga Hridaya* a detailed description of Rasayana, with procedures and care that need to be followed, is suggested [10,11]. According to *Charaka Samhita*, Rasayana means "the path of juice" or "juice-incorporate" (*Rasa* = juice and *Ayana* = path), or *Elixir vitae*, which literally means augmentation of *Rasa* in the cell and body. The word therefore signifies the property of the plant that helps to rejuvenate the system – i.e., adaptogenic activity [11].

Rasayana increases *ojas* (life's vitality) and *Sattva Guna* (mental/spiritual clarity), balances the humors – *Tridosha* (*Vata, Pitta, Kapha)* – and maintains *Rasa*, the vital fluid produced by digestion, which sustains life, leading to enhanced immunity [1]. Rasayana is deemed beneficial for nearly all diseases, with a special emphasis on the disorders of aging, where the body is deprived of adequate nutrition and vigor by means of optimization or homeostasis. It is used either to rejuvenate the general health of the body, or to aid the body in attaining its maximum functional potential [10].

According to *Charaka Samhita* [12,13]:

> administration of *Rasayana* nourishes blood, lymph, muscles, adipose tissue, bones, bone marrow and semen. On account of this, it prevents any kind of degenerative changes and illness, increases life span and arrests the signs of aging. Studies suggest that the Rasayana therapy acts by modulating the neuro-endocrine–immune systems and in turn rejuvenating the complete functional dynamics of the organs by delaying aging and enhancing intelligence, memory, strength, youth, luster, sweetness of voice and vigor.

Rasayana is beneficial to people irrespective of their age, sex, or ethnicity. Rasayana treatment defers old age and, when taken in good health, optimizes all aspects of the physiology and maintains youthfulness, vigor, and vitality of the body. This has a major role in increasing vitality and keeping diseases at bay. The health benefits with consuming Rasayana drugs are cumulative with time and regularity, and the drugs are devoid of side effects even when taken indefinitely [1,10].

The Rasayana drugs or preparations used to achieve this are customarily a complex mixture of medicinal plants with miniscule amounts of minerals, pearls, corals, gems, and *Shilajit* (mineral exudates) [1]. Several recipes for Rasayana are presented in Ayurveda and, depending on the composition of the plants and their ratio, they may be organ-/tissue-specific (for example, for brain, heart, reproductive organs, etc.) or for general/whole body use [1,9,10].

Among all Rasayana formulations, Triphala is the simplest. As the name suggests, Triphala in Sanskrit means three fruits (*Tri* = three and *Phala* = fruits) of the myrobalans *Terminalia chebula* (Haritaki, family Combretaceae),

Terminalia bellirica (Bibhitaki, family Combretaceae), and *Phyllanthus emblica* Linn. or *Emblica officinalis* Gaertn. (Amalaki or the Indian gooseberry, family Euphorbiaceae), with each of the individual myrobalans and the combination possessing Rasayana properties (Figure 14.1) [2,14,15]. Of the three myrobalans, Haritaki and Bibhitaki have a warm potency (veerya) while Amalaki is cool. Triphala, being a combination of all three, is therefore balanced [16,17].

Triphala is known to preserve the harmony of the three *Doshas*, increase the life force (*ojas*) and immunity of a person, enhance regeneration of cells and tissues in the body, and halt senescence [10]. Each of the individual ingredients of Triphala mitigates the three *Doshas*; the *Vata* by Haritaki, *Pitta* by Amalaki, and *Kapha* by Bibhitaki [18]. Triphala is normally formulated with equal proportions of the three; however, variations in the ratio of 1:2:4 of Haritaki, Bibhitaki, and Amalaki were followed by some ancient Ayurvedic physicians. The reason may be due to the need for rectifying the severely vitiated *Pitta* and *Kapha Dosha* in certain conditions [19]. Haritaki, which possesses all the rasas except Lavana (salty), decreases the *Doshas*. It is light in quality, and is a digestive, nutritious, and stimulates hunger. It cures all diseases, increases intellectual power, strengthens sensory organs, and promotes longevity. Bibhitaki (*Terminalia belerica*) is a powerful rejuvenator, and reduces the risk of liver and heart diseases. It also improves voice, vision, and growth of hair, and balances *Kapha.*

Amalaki is described as possessing *Kashaya* (astringent), *Amla* (sour), and *Madhura* (sweet), to increase Veerya (potency), and to be *Aghu* (light) in *Gun* (quality). It is helpful in burning, bilious vomiting, and urinary disorders; in diabetes and edema; and is a Rasayana (rejuvenator). Haritaki (*Terminalia chebula*), the Tibetan "king of medicine," is standard tonic for the heart and brain, and increases longevity. According to Ayurveda, it pacifies *Vata*.

When combined, the Triphala constituents are believed to maintain the equilibrium of all the three *Doshas*. They improve the *Agni* or metabolism, appetite, digestion, and excretion. This also enhances the biotransformation and excretion of the toxic substances and ultimately helps in nourishing *Rasa*, *Raktha*, *Mamsa*, and other *Dathus*, which will together improve anabolic activity, maintain youthfulness, and prevent degeneration [10,16,17].

14.2.1 Common Forms of Triphala

In Ayurvedic practice, depending on the *Dosha*, Triphala is used for various ailments – for example, to prevent constipation, as a colonic and gastrointestinal tract tonifier, an intestinal cleanser and a digestive; for food assimilation support; to maintain serum cholesterol level, improve the circulation, and relax the bile duct; for sluggish peristalsis; as an antioxidant; for headaches; to protect the kidney; and also as a hepatoprotective agent [2,14]. Various preparations of Triphala are available – in the form of *churna* (fine powder), *kwatha* (decoction), *mashi* (ash), *taila* (oil), or *gritha* (Triphala cooked with clarified butter or ghee) – depending on the condition of the patient and the disease [16,17].

Triphala churna and *kwatha* are the simplest and most commonly used forms, made by powdering the three constituents. In the case of *churna*, the dry fruit pulps of all the three myrobalans are mixed in the ratio of either 1:1:1 or 1:2:4 of Haritaki, Bibhitaki, and Amalaki, respectively, and is finely powdered. It is recommended that it be administered with ghee, honey, or milk according to the vata and pitta predominance [6]. In the Ayurvedic traditions, *kwatha* is conventionally prepared by boiling 1 part of the powder with 16 parts of water and reducing it to one-eighth of the total. The decoction is then strained using a clean cloth. The filtrate may be used internally in cases of erysipelas, eruptions, scrotal swelling, various refractory errors, colic pain, worm infestation, and urinary disorders. It is also gargled in stomatitis and sore throat. It is used externally to wash wounds and the eyes, and to prevent and treat eye diseases [16,17].

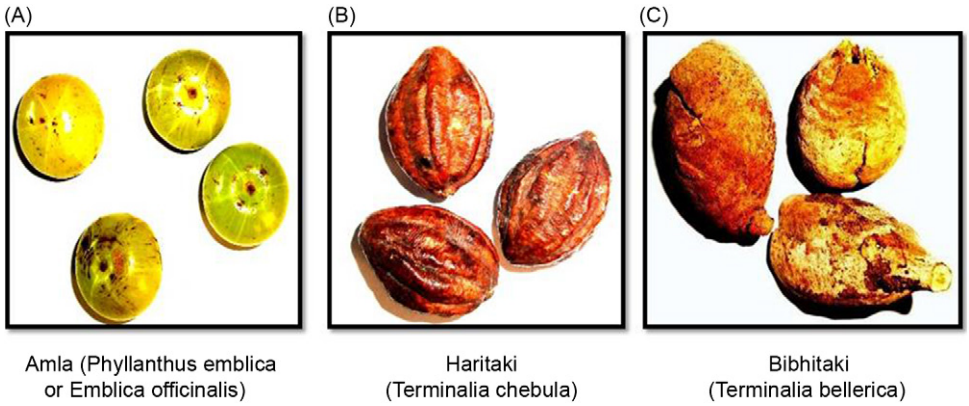

FIGURE 14.1 The individual constituents of triphala: (A) Amla/amalaki (*Phyllanthus emblica* or *Emblica officinalis*); (B) Haritaki (*Terminalia chebula*); and (C) Bibhitaki (*Terminalia bellerica*).

Triphala Masi is prepared by heating the Triphala powder at lower temperatures for a long time in a controlled environment (generally below 450°C). If heating is continued further at higher temperatures (above 450°C), it forms *Bhasma* (white ash). *Mashi/Masi* is an intermediate product containing both organic and inorganic constituents, unlike *Bhasma*. Due to thermal degradation, the thermolabile constituents are lost and the bulk of raw material is reduced [20,21]. The *Masi* is black in color and contains high percentages of carbon and oxides [20,21]. References to the use of Triphala Masi to cure illnesses are found in *Bhaisajyaratnavali*, *Bharat Bhaisjya Ratnakar*, and *Sharangdhar Samhita – Uttar Khanda* [20,21]. The Triphala Masi can be mixed with honey and applied in *upadamsha* (soft chancre); it heals the wound quickly [10,16,17].

Triphala Gritha is commonly used in the treatment of eye diseases, including refractory errors, itching, conjunctivitis, blindness, and cataract. It is mentioned in the ancient texts as alleviating eye disease, and being useful in erysipelas, leucorrhea, jaundice, and *Arbuda* (tumor), and in preventing graying and loss of hair [16,17]. Conventionally, Triphala Gritha is prepared by cooking the paste of Triphala, Trikatu (an herbal compound of Indian long pepper [*Piper longum*], black pepper [*Piper nigrum*], and ginger [*Zingiber officinale*]), grapes (*Vitis vinifera*), Yestamadhu (*Glycerrhiza glabra*), Kutki (*Picorrhyza korroa*), and elaichi (cardamom, *Elettaria cardamom*) in ghee and milk. The ratio of the Triphala decoction is very important and should be three-fold more than the other ingredients [16,17].

Triphala Taila is prepared by boiling Triphala powder with oil. It is used as a gargle, for local application, as snuff, as an enema, and orally for the treatment of obesity, lassitude, itching, and other diseases due to vitiation of *Kapha* [16,17].

14.3 TRADITIONAL USES OF TRIPHALA

Triphala is a highly valued Ayurvedic formulation, utilized widely for the treatment of various illnesses. It is credited with diverse beneficial properties, ranging from gastroprotection to immunomodulation, and is known as a universal panacea against various disease conditions [2,14]. The most important use of Triphala in Ayurveda is in various ailments of the gastrointestinal tract and as a colon cleanser. It is believed that Haritaki acts as a bowel toner, Amalaki prevents and enhances repair of the damaged intestinal cells, and Bibhitaki reduces the mucus build-up that can aid the growth of bacteria, yeast, and parasites.

Triphala is a useful internal cleanser and detoxifying formula for everyone, including more sensitive individuals. According to Ayurveda, daily use of Triphala promotes normal appetite, good digestion, the increase of red blood cells and hemoglobin, and the removal of undesirable fat [16,17]. Triphala is a very useful formulation for creating a favorable chemical environment for beneficial intestinal bacteria, and an unfavorable environment for pathogenic bacteria. Triphala is a bowel regulator. It is considered safe as a food, and is not habit-forming even when taken on a regular basis. It improves vision and is also useful for cataracts, conjunctivitis, and refractory errors. Amalaki is one of the greatest rejuvenators, as a powerful natural antioxidant, and also helps to boost the immunity system and balances *Pitta* [16,17].

Triphala tones and cleanses blood. It is a mild laxative, and has the ability to regulate the processes of digestion and elimination. Ayurveda designates Triphala as Rasayana, the medicine that gives better health, prolongs life, and is useful in the treatment of variety of diseases: "When in doubt use Triphala," as it is so safe and effective. It includes all the tastes except Lavana (salty) and acts to promote a state of balance between the three *Doshas* (humors).

These three drugs can also be powdered and taken together. If this formula is taken for 1 year, the person will live for 100 years without signs of old age and without any disease. Another type of Triphala Rasayana is as follows: Triphala paste is applied inside a new iron vessel, and after 24 hours this paste is removed and taken in honey and water. During this, rice with ghee is also consumed. If this Rasayana is taken for 1 year it gives longevity [2]. Rightfully, according to a popular folk saying in India, just as a mother cares for her child so too does Triphala take care of the internal organs of the body [2]. Triphala is also incorporated into other Rasayana and complex Ayurvedic formulations, like *Chyavanprash* [2].

In consortium the three myrobalans promote internal cleansing, optimize peristaltic movement, and facilitate easy passage of food [2,14]. Triphala is also an appetizer and aids in the digestion of food. As it is a mild laxative, it helps in emptying the bowel and in the expulsion of toxins from the body. Triphala brings about colonic cleansing in order to maintain a healthy colon, without causing any undesirable side effects or irritations [2,14].

Triphala also aids in curing ophthalmic problems. In traditional practice, the eyes are washed regularly with filtered Triphala water. A variety of Ayurvedic preparations that aim at improving eyesight and preventing cataract have Triphala as one of their components. The anti-inflammatory and antimicrobial properties possessed by Triphala are useful in the treatment of ophthalmic ailments. Triphala improves vision, strengthens the eye muscles, and prevents development of cataracts – especially in diabetic patients. Triphala is also useful in the treatment of conjunctivitis and progressive myopia, and in the initial stages of glaucoma and blindness [16,17].

Moreover, Triphala provides cosmetic benefit and improves skin tone and complexion. Amalaki, present in Triphala, is rich in vitamin C, and this helps in the production of collagen, which results in providing luster to the skin. Its other ingredient, Bibhitaki, helps to improve skin pigmentation, and hence is used in the treatment of leucoderma [18]. Amalaki has also been observed to improve immune function and prevent skin and body infections [2,14]. Triphala, when used externally as a hair tonic/hair wash, or when consumed orally, ensures a lustrous mane and prevents graying of the hair [2,14,16,17].

Triphala is believed to maintain proper secretions of the endocrinal system, responsible for maintaining appropriate homeostasis in the body and helping the vital physiological processes. It also prevents senescence, increases immunity, stimulates hematopoiesis, purifies blood, and prevents urinary tract infections [2,10,14]. Amalaki and Haritaki are hematopoietic and are used in various disorders of the blood, such as anemia and blood dyscrasias. Bibhitaki possesses mild coagulant and styptic properties, and therefore is used to stop bleeding [18]. Triphala is also believed to improve mental faculties and two of its constituents, Amalaki and Haritaki, are used to enhance memory and prevent/ameliorate various neurological disorders. Bibhitaki has a sedative hypnotic action, and is used to treat insomnia and various neurological and psychiatric disorders [18]. The following sections address the usefulness of Triphala in various conditions afflicting older adults.

14.3.1 Antimicrobial Activity

Reports indicate that microbial infections in elderly adults lead to increased morbidity and slower recovery. Preclinical studies have shown that Triphala and its constituents, *Terminalia chebula*, *Terminalia bellirica*, and *Emblica officinalis*, possess significant antibacterial effects against diverse pathogenic bacteria. Experiments by Srikumar *et al*. [22] have shown that the aqueous and ethanol extracts of the individual components as well as Triphala *churna* possess significant antibacterial activity against the isolates obtained from HIV-infected patients, with optimal effects being observed in the ethanol over aqueous extracts [22]. Subsequent studies showed that the ethanolic and aqueous extracts of Triphala and Triphala *Mashi/Masi* were also effective on clinical isolates, and that the aqueous extract was better than the ethanolic extract on *E. coli* and *S. aureus*, and also against the antibiotic-resistant isolates [21]. A recent report also suggests that hydroalcoholic extracts of Triphala were effective against multidrug-resistant uropathogenic bacteria. Together, all these observations clearly indicate Triphala's usefulness as a novel antibacterial and non-toxic agent [23].

14.3.2 Wound Healing

In older populations wound healing is delayed, which can lead to secondary infection and the risk of various complications. Animal studies with laboratory rats have shown that the Triphala extract ointment (10% w/w) was effective in promoting the healing of full-thickness dermal wounds in both clean as well as infected (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*) conditions [24]. Topical application of Triphala ointment reduced bacterial counts and improved wound healing by reducing matrix metalloproteinase expression; enhancing wound closure; and improving levels of collagen, hexosamine, uronic acid, and superoxide dismutase [24].

14.3.3 Dental Care

Dental caries is an important problem in older populations, and the lack of dentition compromises quality of life. Preclinical studies have shown that, when compared to doxycycline (300 μg/ml), Triphala (1500 μg/ml) was effective in reducing the MMP-9 activity expressed in adult periodontitis patients. Triphala also caused inhibitory activity on PMN-type MMPs involved in extracellular matrix (ECM) degradation during periodontitis, indicating its usefulness for this condition [25]. Triphala was also shown to be less effective than sodium hypochlorite on *E. faecalis*, a bacteria involved in biofilm formation on tooth substrate [26]. Recently, Srinagesha and co-workers [27] observed that 6% Triphala in a mouthwash formulation was effective in reducing the microbial population of the oral streptococci in healthy volunteers, and that the beneficial effects were comparable to the positive control chlorhexidine (0.2%) [27].

14.3.4 Anti-stress Activity

Older adults are reported to suffer increased stress, which can contribute to or aggravate many ailments. Older adults cannot adapt to temperature changes efficiently and are distressed when exposed to colder temperatures (below 18°C). Seminal studies by Dhanalakshmi and co-workers [28] showed that Triphala was effective in ameliorating the cold stress-induced alterations in behavioral and biochemical changes in rats [28]. The authors observed that cold stress (8°C for 16 hours per day for 15 days) caused both behavioral (decreased rearing, grooming, and ambulation) and biochemical (increase in the lipid peroxidation and corticosterone levels) changes, and that administration of Triphala (1 g/kg animal body weight) orally for 48 days reversed these effects, thus indicating its usefulness [28].

14.3.5 Immunomodulatory Activity

Older populations are affected by high-decibel noise, both psychologically and physically. Animal studies by Srikumar and associates [29] have shown that administering Triphala before exposure to 100 decibels for 4 hours per day for 15 days prevented noise stress-induced changes in hormonal, antioxidant, and cell-mediated immune response [29]. Noise stress significantly suppressed the cell-mediated immune response, and supplementation with Triphala prevented these changes [29,30]. Additionally, seminal studies by Phetkate and co-workers [31] have shown that intake of Triphala by HIV/AIDS-positive patients causes a significant increase in cytotoxic T lymphocytes and natural killer cells, thereby indicating it to be a novel adjuvant therapy for immunological improvement [31].

14.3.6 Anti-arthritic Effects

Arthritis a major problem in older adults, and studies have shown that oral administration of Triphala (1 g/kg body weight) was effective in preventing Freund's adjuvant-induced arthritis and inflammation in paws of Swiss albino mice better than the standard drug indomethacin (3 mg/kg body weight). Mechanistic studies indicated that administering Triphala altered the levels of lysosomal enzymes; the tissue marker enzymes, and glycoproteins [32]. Subsequent studies also showed that Triphala was better than the non-steroidal anti-inflammatory drug indomethacin in reducing monosodium urate crystal-induced inflammation in mice, and to mediate these effects by decreasing the levels of lysosomal enzymes, lipid peroxidation, and tumor necrosis factor-α (TNF-α) [32].

14.3.7 Hepatoprotective Effects

Liver function is compromised in older adults, and this can affect the health and quality of life of the individual. Animal studies with laboratory mice showed that Triphala was effective in ameliorating acetaminophen-induced liver toxicity [33]. Mechanistic studies indicate that administration of Triphala reduced the levels of pro-inflammatory cytokines TNF-α and lipid peroxides; restored the levels of free glutathione and the antioxidant enzymes superoxide dismutase, catalase, gluthathione peroxidase, gluthathione reductase, and gluthathione S-transferase; and decreased the level of hepatic damage as observed by lower levels of the biochemical markers alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase, as confirmed by histopathological observations [33].

14.3.8 Anti-hyperglycemic Effects

Diabetes is globally a major health problem, and experimental studies with laboratory rats have shown that administration of an equiconcentration of Triphala and its individual constituents at 100 mg/kg body weight was effective in reducing serum glucose levels in both normal as well as alloxan-induced diabetic rats [34]. Investigations indicated that the antihyperglycemic effect was significant, irrespective of whether given once or for an extended period, and that this property could be of immense use in day-to-day care [34].

14.3.9 Anti-hyperlipidemic Effects

Dyslipidemia, characterized by increased levels of triglycerides, total cholesterol and LDL, and decreased level of HDL, is the major risk factor for atherosclerosis [35]. Studies with laboratory rats have shown that Triphala possesses antihypercholesteremic effects [35]. The investigators observed that feeding rats with a high-fat diet (4% cholesterol, 1% cholic acid, and egg yolk) for 48 days caused significant increases in total cholesterol, LDL, VLDL, and free fatty acid, and that co-administration of Triphala at 1 g/kg body weight mediated the hypolipidemic effects [35]. Additionally, studies showed that Triphala and its constituents were effective in reducing obesity and symptoms of visceral obesity syndrome in high fat-diet fed mice [36].

14.3.10 Triphala in the Prevention and Treatment of Cancer

The incidence of cancer increases with age, and its prevention/effective treatment is vital. Animal studies have shown that feeding mice a Triphala-incorporated diet was effective in reducing benzo(a)pyrene-induced forestomach papillomagenesis, and that its protective effects were better than with the individual constituents [37]. Mechanistic studies indicate that administering Triphala increased the levels of antioxidants and prevented lipid peroxidation [367]. Together these properties might have contributed to the observed chemoprevention, at least in part [37].

Triphala has also been shown to be devoid of any cytotoxic effects in the normal human breast cell line MCF-10F [38], normal human pancreatic ductal epithelial cells (HPDE-6) [39], and normal murine spleen [38] and liver cells [38], while at the same concentration to possess significant antineoplastic effects in various neoplastic cells such as MCF-7 and T47D (human breast cancer cells) [38,40,41], PC-3 and DU-145 (prostate cancer cells) [40], Capan-2 and BxPC-3 (human pancreatic cancer) [39], Shionogi 115 (mouse breast cancer cells) [40], and barcl-95 transplantable mouse thymic lymphoma [41].

Additionally, oral administration of Triphala (50 mg/kg or 100 mg/kg for 5 days/week) significantly suppressed the growth of Capan-2 pancreatic tumor xenograft. Reduced tumor growth in Triphala-fed mice was due to increased apoptosis in tumor cells, and was associated with increased activation of p53 and ERK [39].

14.4 CONCLUSION

Triphala, an ancient Ayurvedic formulation, has been used for centuries to treat and prevent ailments that afflict the elderly population, and preclinical studies have validated many of its ethnomedicinal properties. Many of the beneficial effects seem to be mediated by myriad biochemical mechanisms. Triphala is reported to be a scavenger of free radicals in cell free assays [42,43,44]; to be effective in preventing superoxide-induced hemolysis of red blood cells [42]; to increase the levels of antioxidant molecules such as glutathione and ascorbic acid [45], and antioxidant enzymes such as superoxide dismutase [29,41,45], catalase [29,45], glutathione peroxidase [29,30,45], glutathione reductase [45], and glutathione-S-transferase [45]; to decrease the concentrations of malondialdehyde [45], lipofuscin [45], and protein carbonyls [45]; and to decrease lipid peroxidation [28,29,37] and the activities of myeloperoxidase [20,41] and xanthine oxidase [20,41,45] in laboratory animals. Additionally, studies have also shown that Triphala possesses antimutagenic effects in the Ames histidine reversion assay against various chemical mutagens [46], and anticlastogenic effects against the gamma radiation-induced DNA strand breaks in plasmids (pBR322) [47] and in mice [41]. Humans have been using Triphala for a long time, and this in itself supports its easy acceptability. Studies have shown Triphala to be devoid of any toxic effects at its recommended and effective concentrations in humans [14]. The most advantageous aspect of Triphala in human applications is its easy affordability, at around US$1 for 100 g powder. For optimal use and application, detailed studies are required to understand the use of Triphala in ameliorating various geriatric ailments in human volunteers. The results of these experiments will be of immense use for the future in both treatment and prevention of aging and associated ailments.

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CHAPTER

15

Use of Ayurvedic Medicinal Plants as Immunomodulators in Geriatrics: Preclinical Studies

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15.1 INTRODUCTION

The immune system is an important physiological system that regulates homeostasis of the body in vertebrates. It is a highly complex defense system that protects vertebrates from invading microorganisms, and hence forms an important component of an individual from the time of conception till death. Any alterations in the desired homeostasis in the system can lead to infectious diseases, autoimmune conditions, inflammation, or neoplasia [1]. A wide range of organs and cells form the basic components for the lymphoreticular system, which includes lymphatic vessels and organs (lymph nodes, thymus, spleen, and tonsils), white blood cells such as lymphocytes and monocytes, and specialized cells residing in various tissues that are responsible for phagocytosis (macrophages). However, for optimal immune response the timely interaction of the humoral and cellular immune response is required, and this is ensured by the specific cytokines and their receptors [2].

15.1.1 The Immune System in the Elderly

Due to advances in medical science the life expectancy of humans has increased, and as a consequence of their increased age the geriatric population is prone to severe community acquired infections. Such infections cause high morbidity and mortality as a direct result of changes that occur in the innate and acquired immunity of the aged, including:

1. *Changes in innate immunity*. There is an increase in the production of immune cells such as macrophages, neutrophils, and natural killer (NK) cells. However, these cells have decreased functional ability. In addition, the production of major cytokines, including tumor necrosis factor (TNF), interleukin (IL)-6, IL-11, and colony stimulating factors (CSFs), are reduced. Furthermore, the functional capacity of receptors such as toll-like receptors (TLRs) are reduced, leading to a reduction in lymphocyte production. Natural killer cell-mediated cytotoxicity is also reduced, leading to susceptibility to viral and intracellular infections [3].
2. *Changes in adaptive immunity*. The functionality of the adaptive immune system is based on the production of naïve lymphocytes in bone marrow and thymus, and the competency of memory cells in the system. However, in the elderly population there is a reduction in counts of these cells, which explains the reduction in adaptive immunity [3].

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In the geriatric population, the function of NK and regulatory T (T reg) cells and the response of T cells are reduced due to altered cytokine response. This causes fast progression of the disease to a more severe status, even in infections with trivial pathogens, and also causes an increase in the incidence of cancer [3].

3. *Changes in cellular immunity*. The T cells play a major role in cellular immunity. Thymic involution in the elderly causes a reduction in naïve T lymphocytes, and the activated T lymphocytes that are produced are less responsive to stimulation. This in turn causes reduced T helper (Th) cells, leading to a decrease in antibody production by B cells. Alteration in IL-2 and IL-6 receptor signaling in T cells is also observed, which causes changes in the JAK/STAT pathway. In addition, there is a decrease in the absolute number of peripheral blood lymphocytes in elderly individuals [4].
4. *Changes in humoral immunity*. With aging, there is shift in the antibody produced: isotypes from immunoglobulin (Ig)G to IgM, specificity from foreign to autoantigens, and antibody affinity from high to low. This can be attributed to impaired T cell function in maturing B cells with respect to production of antibody isotype and affinity, and alteration in B cell precursors in bone marrow for specificity [5].

As with any system, the immune response can be modulated by certain drugs and chemicals known as immune modulators. Depending on their effect, the immunomodulators are broadly classified as immunosuppressants (depression of the immune response) or immunostimulators (immunopotentiation or strengthening of immune reactions). Clinically, immunostimulating drugs are used to alleviate immunodeficiency (as in the treatment of AIDS), while immunosuppressive agents are used to suppress or decrease excessive immune responses (as in the treatment of graft rejection or autoimmune disease).

Unfortunately, most conventional immunomodulators have severe adverse effects on the liver, bone marrow, and other vital organs, thus negating their therapeutic value [2]. This has necessitated the need for alternatives that are non-toxic at an effective concentration, and are devoid of any cumulative toxicity when administered for extended periods of time [1].

15.2 PLANTS AS IMMUNOMODULATORS

Herbal medicine is generally considered a well-established form of complementary medicine, and studies show the that majority of the world's population, especially in the developing countries, still use traditional plant medicine for their primary health care. Ayurveda, the traditional Indian system of medicine, has suggested means to increase the body's natural resistance to disease by using plant-based polyherbal formulations. Experimental studies carried out in the past two decades on some of the medicinal plants used in various traditional and folk systems of medicine in India have validated their ethnomedicinal claims. In this chapter we will focus on the importance of Indian medicinal plants that have been shown scientifically to possess immunomodulatory effects according to the norms followed in the modern system of medicine (Figure 15.1) [1,2].

15.2.1 *Ocimum sanctum* Linn. or *Ocimum tenuiflorum* L. (Family Lamiaceae)

Ocimun sanctum, commonly known as tulsi in Hindi and holy basil in English, is a very important plant in India and is used extensively in Ayurveda. Reports have suggested the presence of antipyretic, anti-stress, immunomodulatory, anti-inflammatory, analgesic, and other beneficial properties [6,7]. Preclinical studies with non-stressed and stressed (deprived of food and water) animals have shown that tulsi oil was effective as an immunomodulator in both non-stressed and stressed rats. The administration of tulsi oil (3 ml/kg) intraperitoneally to normal animals produced a significant increase in anti-sheep red blood cell antibody titer and concomitantly decreased the histamine release from peritoneal mast cells of sensitized rats, indicating the triggering of humoral immune responses [6]. The oil was also effective in decreasing footpad thickness and the percentage of leukocyte migration inhibition, suggesting induction of a cell-mediated immune response [6].

Additional experiments have shown that pretreatment with the oil effectively suppressed humoral as well as cell-mediated responses in retained animals. The oil was effective when combined with diazepam (an anxiolytic-sedative drug), suggesting its usefulness as an adjuvant to the conventional agent. Mechanistic studies showed that the immunomodulatory effect of the oil was negated when the animals were administered flumazenil, a central benzodiazepine receptor antagonist, confirming the role of GABA-ergic pathways in mediating these beneficial effects [6]. Experiments with rats have also shown that the oil was effective in attenuating the immunotoxic effects of lindane. Mechanistic studies indicated that these beneficial effects were mediated through immune modulation of cells of the humoral and cellular immune responses, and ameliorating the oxidative stress [7].

15.2.2 *Phyllanthus emblica* L. or *Emblica officinalis* Gaertn. (Family Phyllanthaceae)

Emblica officinalis, commonly known as amla, is arguably one of the most important plants in various

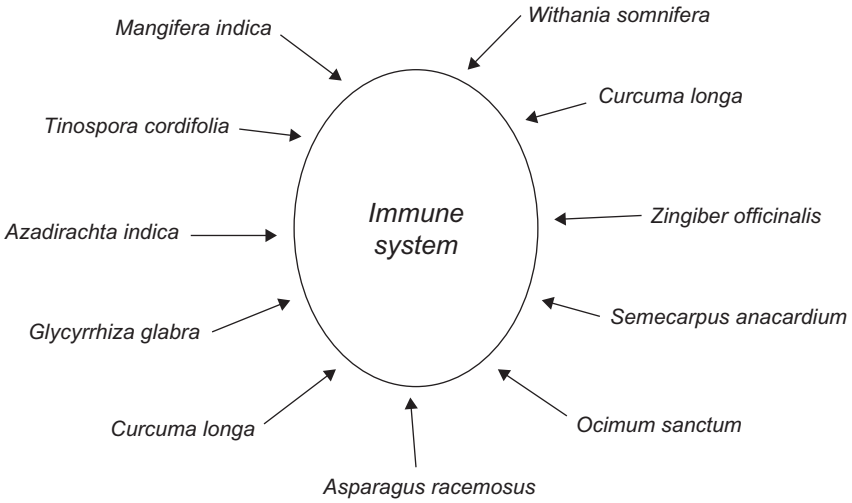

FIGURE 15.1 Indian medicinal plants with specifically validated immodulatory properties.

traditional and folk systems of medicine in India. In Ayurveda, amla is considered to be a potent rejuvenator and immunomodulator effective in stalling degenerative processes and senescence, and to promote longevity, enhance digestion, treat constipation, reduce fever and cough, alleviate asthma, strengthen the heart, benefit the eyes, stimulate hair growth, enliven the body, and enhance intellect [8,9].

Oral administration of amla to tumor-bearing mice (Dalton's lymphoma ascites) has been reported to enhance natural killer cell (NK) activity and antibody-dependent cellular cytotoxicity (ADCC). Amla increased the lifespan of tumor-bearing animals by 35%, and co-administration of cyclophosphamide or anti-asialo-GM1 antibody treatment abrogated the survival time, clearly indicating that the observed effects were dependent on the activities of NK cells and killer cells. Amla mediated antitumor activity and subsequent reduction in tumor size by augmenting natural cell mediated cytotoxicity [8].

Preclinical studies also showed that amla was effective in preventing bacterial colonization while decreasing the bacterial load. Amla was more effective when administered for a longer (30 days) rather than shorter (15 days) period. When compared to the controls (non-amla treated), long-term feeding of amla caused a decrease in the levels of malondialdehyde while increasing phagocytic activity and nitrite levels in the broncheoalveolar lavage fluid [9]. Together, these observations clearly suggest that amla has antibacterial activity.

15.2.3 *Withania somnifera* (L.) Dunal (Family Solanaceae)

Ashwagandha, scientifically known as *Withania somnifera Dunal*, is an important medicinal plant in the Ayurvedic system of medicine. The roots are highly valued, and are used either alone or in combination with other medicinal plants to treat a variety of ailments. It is also used as a general tonic to increase and to improve overall health and longevity. Regular consumption of ashwagandha is believed to prevent diseases in individuals of different ages and with various health conditions [10–12]. Preclinical studies showed that ashwagandha was an effective immunomodulatory agent and inhibited the myelosuppression induced by diverse immunotoxins (namely, cyclophosphamide, azathioprin, and prednisolone) in mice [10]. Ashwagandha was effective in preventing myelosuppression with all three immunotoxins. The body weight, along with the levels of hemoglobin, red blood cells, white blood cells, and platelets, was restored [10].

Studies have also shown that ashwagandha was effective in modulating cytokines of both Th1 (IFN-γ, IL-2) and Th2 (IL-4) profiles [11]. In a study to determine whether aqueous extracts of ashwagandha selectively upregulates Th1 activity, administering the extract increased the CD4+ and CD8+ counts as compared to both the control and cyclosporin A, with a faster recovery of CD4+ T cells in immune-suppressed animals. The immunopotentiation effects were comparable to that of levamisole (a synthetic immunomodulator) under immunosuppressed conditions [11].

Ashwagandha is also shown to selectively stimulate Th1 immunity, as evidenced by enhanced secretion of IFN-γ and IL-2 in tumor-bearing animals. Concomitantly, it increased the proliferation of CD4+/CD8+ and NK cells and increased the expression of co-stimulatory molecules, namely CD40/CD40L/CD80 [12]. Additionally, administration of ashwagandha to (HL-60, human promyelocytic leukemia cells) tumor-bearing mice along with the anticancer drug camptothecin also led to enhancement in T cell activation [12]. All these observations indicate the usefulness of ashwagandha as an

immunopotentiating agent in both normal and tumor-bearing animals, and validate Ayurvedic observations.

15.2.4 *Tinospora cordifolia* (Thunb.) Miers (Family Menispermaceae)

Tinospora cordifolia, commonly known as guduchi or amrita, is another important medicinal plant in Ayurveda. The stems and roots are an integral constituent of several compound preparations. Guduchi is a potent tonic, and is effective for chronic debilitating ailments, dyspepsia, fever, and urinary diseases. Preclinical studies with rats for cholestasis showed that guduchi was effective in ameliorating cholestasis-induced immunosuppression [13]. Studies have also shown that guduchi deleted the carbon tetrachloride (CCl_4)-induced immunosuppression and increased the functional capacities of peritoneal macrophages [14]. *In vitro* studies with (1,4)-α-D-glucan (RR1), a phytochemical of guduchi, showed it to possess immunostimulatory properties [15].

15.2.5 *Semecarpus anacardium* Linn. (Family Anacardiaceae)

Semecarpus anacardium, commonly known as "marking nut," has important applications in indigenous systems of medicine [16,17]. The fruits and nut are reported to possess various characteristics, such as antiatherogenic, anti-inflammatory, antioxidant, antimicrobial, and antireproductive activity, and to act as a central nervous system stimulant, hypoglycemic, and anticarcinogenic [16,17]. Preclinical studies have shown that ballataka mediates the (adjuvant-induced) antiarthritic effects in rats via modulation of humoral and cell-mediated immune responses, and also its anti-inflammatory effects. When compared with the adjuvant-induced arthritic cohorts, administration of ballataka decreased paw edema along with the levels of TNF-α, nitric oxide, and myeloperoxidase. Ballataka also possesses anti-inflammatory (in xylene-induced ear edema and formalin-induced inflammatory models) and analgesic effects [17]. It also increased the antioxidant status in lymphocytes and lymphoid organs (namely, the spleen and thymus) of adjuvant-induced arthritic rats [16]. Together these observations clearly suggest that ballataka is useful both as an antioxidant as well as an immunomodulatory agent.

15.2.6 *Azadirachta indica A. Juss* (Family Meliaceae)

Neem (*Azadirachta indica A. Juss*) is arguably the most useful traditional medicinal plant in India, and almost all parts of the tree are known to possess myriad medicinal uses in various traditional and folk systems of medicine. The aqueous preparation of neem leaves is shown to be effective in activating immune responses against tumor antigens. Studies in both mice and rats have shown that the extract, in combination with a breast tumor-associated antigen, was effective in initiating a strong IgG response when compared to the response generated by the breast cancer-associated antigen alone. This indicates that the extract is capable of enhancing immune responses to tumor vaccines. Additionally, the released IgG had the potential to mediate antibody-dependent cellular toxicity and also initiated the cytotoxic T cell response. This process was aided by the secretion of IFN-γ, which drove a Th1 response while decreasing IL-10, thereby mediating cytotoxicity. Furthermore, neem extract is non-toxic and abundantly available, making it a cost-effective immune enhancer in breast tumor-associated antigen vaccine [18].

15.2.7 *Curcuma longa* Linnaeus (Family Zingiberaceae)

The rhizome of *Curcuma longa*, commonly known as turmeric, is arguably the most important Indian spice, with immense medicinal properties. Preclinical studies by Yarru *et al*. [19] have shown that co-administration of a turmeric-based diet (0.5%) to broiler chicks feeding on aflatoxin (1.0 mg/kg of diet) resulted in a decrease of IL-6, a pro-inflammatory cytokine. Recent studies with the cultured human peripheral blood mononuclear cells *in vitro* have shown that polar fractions of an aqueous extract of turmeric also possess immunostimulating effects and modulate the cytokines TGF (transforming growth factor)-β, TNF-α, granulocyte macrophage-colony stimulating factor (GM-CSF), IL-1α, IL-5, IL-6, IL-8, IL-10, and IL-13 [20]. Experiments have also shown that curcumin (Figure 15.2) was effective in inhibiting IL-12 production by macrophages stimulated *in vitro* with either lipopolysaccharide (LPS) or heat-killed *Listeria monocytogenes*, thereby leading to the inhibition of the Th1 cytokine profile (decreased IFN-γ and increased IL-4 production) in CD4+ T cells [21]. These observations point to the usefulness of turmeric and its active principal constituent, curcumin, as an immunomodulatory agent.

15.2.8 *Zingiber officinale* Roscoe (Family Zingiberaceae)

The rhizome of *Zingiber officinale*, commonly known as ginger, is an important culinary and medicinal agent in various traditional and folk systems of medicine. The volatile oil of ginger (0.001–10 ng/ml) caused a concentration-dependent inhibition in lymphocyte proliferation

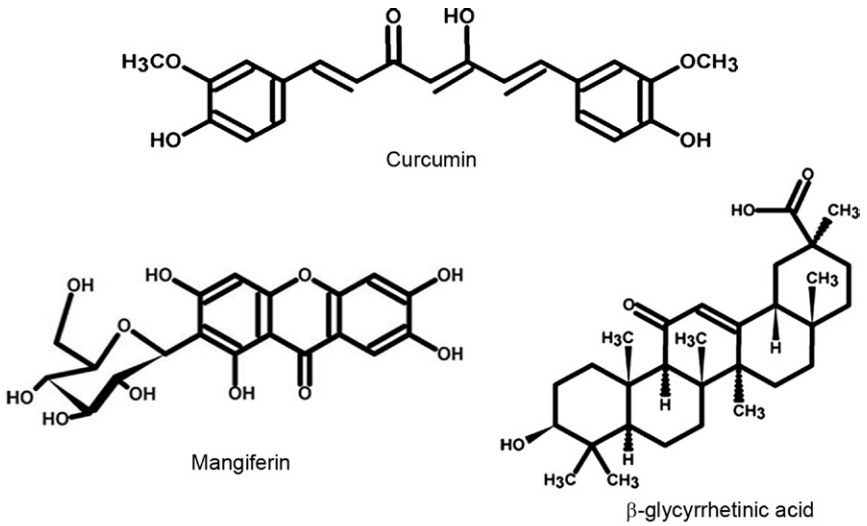

FIGURE 15.2 Phytochemicals with immodulatory properties.

and decreased the number of the total T lymphocytes and T helper cells, but increased the percentage of T suppressor cells in the total population of T lymphocytes. It was also effective in causing a concentration-dependent inhibition of IL-1α secretion in mice peritoneal macrophages *in vitro*. Animal studies also showed that the volatile oil of ginger (doses 0.125, 0.25, and 0.5 g/kg body weight) caused a concentration-dependent decrease in the delayed type of hypersensitivity response to 2,4-dinitro-1-fluorobenzene in sensitized mice [22].

Studies with LPS-stimulated macrophages have also shown that ginger was effective in inhibiting the production of the pro-inflammatory cytokines (IL-12, TNF-α, and IL-1β) and the pro-inflammatory chemokines (RANTES [Regulated upon Activation, Normal T-cell Expressed, and Secreted], MCP-1 [monocyte chemotactic protein-1]/chemokine [C-C motif] ligand 2 [CCl2]), and downregulated the expression of B7.1, B7.2, and MHC class II molecules. Furthermore, ginger affected the antigen presenting function and indirectly inhibited T cell activation [23].

Studies with immune-suppressed mice have also shown that the essential oil of ginger enhanced the humoral immune response [24]. The aqueous extract of ginger was also observed to be effective in reducing ovalbumin-induced inflammation of the lungs in mice [25]. When compared to placebo-treated controls, intraperitoneal administration of an aqueous extract prior to the induction of pulmonary inflammation caused a decrease in the recruitment of eosinophils in the lungs of mice, and was accompanied by a suppression of the Th2 cell-mediated allergic response. Mechanistic studies showed a decrease in the levels of IL-4, IL-5, and eotaxin in the lungs, as well as specific IgE titers in the serum, after allergen sensitization and challenge [25].

15.2.9 *Mangifera indica* Linn. (Family Anacardiaceae)

Mangifera indica, commonly known as mango, is arguably the most important Indian fruit and also possesses diverse medicinal uses. Scientific studies by Makare and co-workers [26] have shown that an alcoholic extract of the stem bark of *Mangifera indica* possesses immunostimulant properties. Administering the extract to normal mice caused an increase in both humoral antibody titer and delayed type hypersensitivity [26]. Administration of the aqueous extract for 4 weeks is also reported to be effective in inhibiting the microsporidian-induced increase in IgG and in reducing the splenomegaly in these animals [27]. Mangiferin (Figure 15.2), the principal compound, was effective in ameliorating cyclophosphamide-induced immunotoxicity in rats and in increasing the lymphoid organ weights, IgM specific to antigen, and cellular immune response [28]. Mangiferin also decreased the cyclophosphamide-induced increase in lipid peroxidation and concomitantly increased the levels of antioxidant enzyme in lymphocytes, macrophages, and polymorphonuclear cells. *In vitro* studies revealed mangiferin's ability to protect lymphocytes from hydrogen peroxide (H_2O_2)-induced cytotoxicity, thereby confirming *in vivo* studies. All the results were compared against levamisole (an immunostimulant drug administered in immunosuppressive conditions), and the effects of mangiferin were significantly higher than those of levamisole, indicating the potential immunostimulant property of mangiferin.

15.2.10 *Asparagus racemosus* (Willd.) Oberm. (Family Asparagaceae)

Asparagus racemosus, commonly called Satavar, Shatavari, or Shatamull, is native to the Himalayas in India. It is successfully used in Ayurveda to cure many diseases. Gautam and co-workers [29] observed the immunoadjuvant properties of Satavar in animal models that were immunized with DPT (diphtheria, tetanus, pertussis) vaccine. It was observed that oral administration of 100 mg/kg of test material for 15 days to Swiss albino mice induced a significant increase in anti-Bordetella pertussis antibodies when compared to the control animals (untreated). The effect was heightened in the drug-treated group when the immunized animals were challenged with a specific strain of *B. pertussis*. A significant increase in antibody titers was observed compared to animals that were untreated. From the results of this study, Gautam *et al*. concluded that *Asparagus racemosus* acts as a potent immunoadjuvant, resulting in reduced mortality and morbidity rates [29].

15.2.11 *Glycyrrhiza glabra* L. (Family Fabaceae)

Glycyrrhiza glabra, also known as licorice, sweetwood, or Mulaithi, is native to parts of Asia and Europe. Preclinical studies have shown that co-administration of polysaccharides isolated from *Glycyrrhiza glabra* to mice fed a high-fat diet enhances the immune response and increases the activities of various antioxidant enzymes [30]. Experiments have also shown that β-glycyrrhetinic acid (Figure 15.2), one of the main constituents of *Glycyrrhiza glabra*, also possesses immunomodulatory properties and affects the level of complement component C2 [31].

15.3 CONCLUSIONS

Scientific studies carried out over the past two decades have shown conclusively that many of the traditionally used Indian medicinal plants possess immunomodulatory effects, and that they could be useful in geriatric populations. As most studies are with experimental animals, the observations help in validating their applicability to humans. The medicinal plants studied have been consumed by the habitants of the Indian subcontinent since time immemorial, and this gives them an advantage over synthetic drugs. Apart from their application in clinics as immunomodulators, many of the plants also possess other beneficial activities, such as free radical-scavenging, antioxidant, anti-inflammatory, gastroprotective, nephroprotective, cardioprotective, and neuroprotective effects, which in total contribute towards improving quality of life for the elderly.

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CHAPTER

16

The Health Benefits of Indian Traditional Ayurvedic Rasayana (Anti-aging) Drugs: A Review

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16.1 INTRODUCTION

With advances in the field of medicine the mean survival age of human beings has increased, leading to a proportionate rise in the percentage of geriatric population. Estimates are that by the year 2050 the number of people aged over 60 years will have increased from the current 1 in 10 to 1 in 5, and that the ratio of people aged 65 years or older to those aged 15–64 years will double in developed nations and triple in developing nations [1]. This increase in the older population combined with possibly a lesser number of carers will greatly affect the global healthcare system. Aging or senescence is fundamentally a complex process where a progressive decline in the efficiency of physiological processes occurs and is manifested within an organism at genetic, molecular, cellular, biochemical, organ, physiological, and system levels. There is also a decrease in the ability of cells to recover from physical or mutagenic damage. This leads to deterioration in physical function, reduction in fecundity, and loss of vitality, concomitantly increasing the risk of various metabolic and degenerative diseases, and cancer (Figure 16.1), and can eventually cause death [1,2].

16.2 HYPOTHESIS OF AGING

Although the fundamental mechanisms for aging are still poorly understood, various hypotheses have been proposed, such as the error catastrophe and genomic instability theory, the neuroendocrine theory, the free radical theory, the membrane dysfunction theory, the hayflick limit theory, the mitochondrial decline theory, the crosslinking (glycation) theory, and the inflammatory theory. Of these, the free radical theory proposed by Denham Harman is the most widely accepted, and observations from both preclinical and clinical studies have substantiated the hypothesis [2].

Free radical theory proposes that excess generation of free radicals causes oxidative damage to biomolecules, and a progressive and irreversible accumulation of products of oxidation, decreasing the cellular levels or activities of antioxidant systems. This, in combination with the increased inflammatory responses, apoptosis, altered cell signaling, and defective tissue renewal, contributes to impaired physiological function, increased incidence of disease, and a decrease in lifespan [2].

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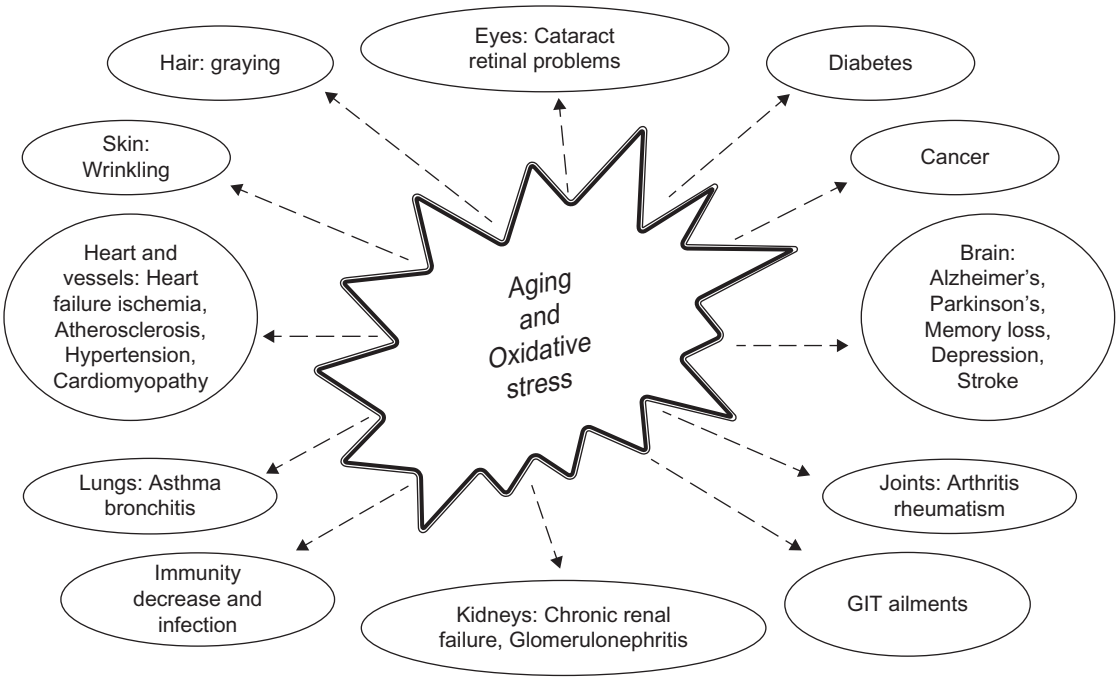

FIGURE 16.1 Common diseases seen with aging and due to, or aggravated by, oxidative stress.

The balance between reactive oxygen species (ROS) production, cellular antioxidant defenses, activation of stress-related signaling pathways, and the production of various gene products, as well as the effect of aging on these processes, determines whether a cell exposed to an increase in ROS will be destined for survival or death. Mitochondrial DNA is considered the most vulnerable candidate for oxidative damage, as the mitochondria are constantly exposed to high oxygen pressure, and the genetic mechanisms that protect the DNA from damage are lacking or deficit in mitochondria. DNA repair enzymes, the third-line defense against oxidative stress, decline with age [2].

Numerous studies have shown that oxidative DNA damage accumulates in the brain, muscle, liver, and kidney, and in long-lived stem cells. This cumulative DNA damage is the likely cause of the decline in gene expression and loss of functional capacity observed with increasing age. Oxidative stress is also implicated in the etiopathogenesis of many age-related diseases and clinical complications, such as atherosclerosis, diabetes mellitus, muscular dystrophy, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [2].

Tissue repair and regeneration are essential for longevity in complex animals, and often depend on the proliferation of unspecialized cells known as stem or progenitor cells. In many tissues, especially the muscle, the regenerative capacity of stem cells decreases with age, and these changes are thought to trigger age-related symptoms and diseases. Additionally, due to failure or decrease in the repair and regeneration mechanisms, the damaged tissues will not undergo repair and regeneration effectively, and the resulting accumulation of defective cells will contribute towards the pathogenic process [2].

16.3 AYURVEDA AND AGING

Ayurveda ("science of life") is the traditional system of Indian medicine, and dates back to 5000 years BC. It is an integral part of the Indian culture and materia medica and remains an influential system of medicine, especially in the Indian subcontinent. The concept and treatment principles of Ayurveda are different from those of modern medicine. While modern medicine is evidence-based and makes use of a distinct, well-defined chemical entity for treatment, emphasis in Ayurveda is mainly on disease prevention and promotion of good health, by following a proper lifestyle and adopting measures that rejuvenate the cells of the body. Additionally, the disease-preventive and health-promotive approach of Ayurveda takes into consideration the whole body, mind, and spirit when dealing with the maintenance of health promotions [1,3].

In Ayurveda, rejuvenation of the cells is addressed in the *Rasayana Shastra*. In colloquial terms, Rasayana means "the path of juice," or "juice-incorporate" (*Rasa* = juice and *Ayana* = path), or *Elixir vitae*. In Ayurveda, consumption of specific Rasayanas at the appropriate time and in

the recommended pattern is supposed to increase the *Rasa* in the cell and body, which in modern terms may be categorized as rejuvenating the system. Rasayana drugs are supposed to possess five different actions: (1) vayasthapana (delaying the process of aging), (2) aayuskara (increase in the lifespan), (3) balakara (having anabolic properties to strengthen the body), (4) medha balakara (improvement of cognitive ability), (5) roga-apaharana (gaining immunity and curing from diseases) [4].

According to *Charaka Samhita*, a Rasayana is a drug that promotes intelligence, memory, freedom from disease, longevity, strength of the senses, and a great increase in the strength of the digestive system [3]. Administration of Rasayana is supposed to nourish blood, lymph, muscles, adipose tissue, bones, bone marrow, and semen. On account of this, it prevents any kind of degenerative changes and illness, increases lifespan, and arrests the signs of aging. Studies suggest that Rasayana therapy acts by modulating the neuroendocrine immune system, in turn rejuvenating the complete functional dynamics of the organs by delaying aging and enhancing intelligence, memory, strength, youth, luster, sweetness of voice, and vigor [5,6]. Rasayana is reported to be started at or before the age of 40 years and to be selected only after considering factors such as age, constitution, and compatibility of the individual, following clinical investigation [7].

Rasayana is deemed beneficial for nearly all diseases, with a special emphasis on the disorders of aging, where the body is deprived of adequate nutrition and vigor by means of optimization or homeostasis. It is used either to rejuvenate the general health of the body or to aid the body in attaining its maximum functional potential [3]. Rasayana is beneficial to people irrespective of their age, sex, or ethnicity. Rasayana treatment defers old age and, when taken in good health, optimizes all aspects of the physiology and maintains youthfulness and vigor, as well as vitality of the body. This has a major role in increasing vitality and keeping diseases at bay. The health benefits of consuming Rasayana drugs are cumulative with time and regularity of intake, and are devoid of side effects even when taken indefinitely [3].

16.4 TYPES OF RASAYANA DRUGS AND SOME COMPOSITIONS

The Rasayana drugs or preparations used to achieve the benefits are customarily a complex mixture of medicinal plants, with miniscule amounts of minerals, pearls, corals, gems, and *Shilajit* (mineral exudates) included [3]. Many plants have been extensively used as "Rasayana drugs" in Ayurveda for the management of neurodegenerative diseases, and as rejuvenators, immunomodulators, aphrodisiacs, and nutritional supplements [3]. Some of the most commonly used plants are *Emblica officinalis* (Indian gooseberry, *Amla*), *Tinospora cordifolia* (*Guduchi*), *Asparagus racemosus* (*Shatavari*), *Withania somnifera* (*Ashwagandha*), *Terminalia chebula* (*Haritaki*), *Terminalia bellirica* (*Bibhitaki*), *Boerhavia diffusa* (*Punarnava*), *Aloe vera* (*Kumari*), *Bacopa monnieri* (*Mandukaparni*), *Glycyrrhiza glabra* (*Yesthamaddhu*), and *Picrorhiza kurroa* (*Katuki*) [3].

Several recipes for Rasayana are presented in Ayurveda and, depending on the composition of the plants and their ratio, they may be organ-/tissue-specific (brain, heart, reproductive organs, etc.) or for general/whole body use (Table 16.1) [3]. Some of the famous Rasayana formulations include the *Triphala*, *Chyawanprash*, *Aamalaki Rasayana*, *Amrita Rasayana*, *Brahm Rasayana*, *Ashwagandha Rasayana*, *Narasimha Rasayana*, *Amritaprasham*, *Anwala churna*, *Brahmi Rasayana*, and *Amalkadi Ghrita* [3]. In the following section the validated pharmacological observations on some of the Ayurvedic Rasayana drugs are addressed, while Table 16.2 lists the composition of Rasayana drugs [8–14].

16.4.1 Amalakayas Rasayana

Amalakayas Rasayana is composed principally of the fruits of *Emblica officinalis* Gaertn. or *Phyllanthus emblica* Linn. (colloquially known as the Indian gooseberry in English, and amla or Amlaki in most Indian language), and is an important Rasayana drug. Amalakayas Rasayana also contains *Tinospora cordifolia*, *Terminalia chebula*, *Pluchea lanceolata*, *Alpenia galangal*, *Leptadenia reticulate*, *Asparagus racemosus*, *Centella asciatica*, *Desmodium gangiticum*, *Boerhavia diffusa*, and lauha bashama, and is reported to have vayasthapana (antiaging), medhya (enhancing intellectual power), and balakara (body-strengthening) properties [8]. Scientific studies have shown that Amlaki Rasayana possesses free radical scavenging effects [15], gastroprotective effects [16], and adaptogenic activity [17]. Experimental studies have also shown that feeding the common fruit fly (*Drosophila melanogaster*) a diet that contains Amlaki Rasayana afforded longevity, proper development, and increased fecundity and stress tolerance [18].

16.4.2 Chyavanaprasha

Chyavanaprasha, named after its inventor Rishi (Sage) Chyavana, is one of the oldest and most popular Ayurvedic preparations. It is the foremost of all herbal rejuvenating tonics, and the most commonly used Rasayana drug in India and abroad. It is a tridoshic Rasayana, and due to its numerous nutritional properties is regarded as "the elixir of life" [3]. The first documented evidence of this formulation is observed in the Ayurvedic text *Charaka Samhita*. According to the chikitsasthana section of the *Charaka Samhita*, Chyawanprash

TABLE 16.1 Organ-specific Effect of Rasayana Plants and Drugs in the Prevention of Aging [3]

Scientific name(s)	Sanskrit name	Action
Phyllanthus emblica or Emblica officinalis	Amalaki	Eye, heart, respiratory system, immunomodulator
Terminalia chebula	Haritaki	GI tract, cardioprotective, immunomodulator
Tinospora cordifolia	Amrutha	Immunomodulator
Boerhavia diffusa	Punarnava	Kidney, heart, hemopoietic stimulator
Bacopa monnieri	Mandookaparni	Brain
Convolvulus pluricaulis	Shankhapushpi	Brain
Asparagus recemosus	Shathavari	Reproductive system
Glycyrrhiza glabra	Yashmadhu	Brain
Withania somnifera	Ashwagandha	Brain and nervous system, immunomodulator
Picrorhiza kurroa	Katuki	Skin, gastrointestinal system
Piper longum	Pippali	GI tract, respiratory system
Acorus calamus	Vacha	GI tract, respiratory system
Embelia ribes	Vidanga	Worm infestation, children
Semecarpus anacardium	Bhallataka	Skin
RASAYANAS DRUGS		
Amruthaprasham	Respiratory system, hemopoeitic	
Amalakayas Rasayana	Digestive system, intellectual power, immunomodulator	
Narasimha Rasayana	Hair, skin, speech, intellectual power	
Triphala	GI tract, immunomodulator, hemopoietic, eyes, hair	
Anwala Churma	Digestive system, immunomodulator, brain tonic	
Brahmi Rasayana	Brain	
Amlakadi Gritha	Brain, immunomodulator	
Chyavanaprash	Respiratory system, immunomodulator	
Brahma Rasayana	Brain	

is used to treat cough, dyspnea, consumption, voice problems, and cardiac conditions [3].

Animal studies have shown that Chyavanaprasha possesses radioprotective effects and protected mice against radiation-induced sickness and mortality [10]; decreased carbon tetrachloride-induced liver damage in rats [19]; and reduced tumor growth (ascites and solid tumor volume) while concomitantly increasing the lifespan of tumor-bearing mice [20]. Additionally, recent studies have shown that administration of Chyavanaprasha was effective in reducing cisplatin-induced acute nephrotoxicity in mice [21], enhancing radiation-induced DNA repair [22], and increasing antioxidant enzymes in mice [22]. Clinical studies have also shown that Chyavanaprasha is an anabolic agent, and benefits people of all ages and health status [3]. Consumption of 20 g of Chyawanprash twice a day for 2 months by bidi smokers decreased their coughing, increased their appetite, and helped them gain body weight [23].

16.4.3 Brahma Rasayana

In Ayurveda, Brahma Rasayana is a brain-specific geriatric tonic. Its regular consumption is supposed to improve resilience to mentally demanding chores, promote mental clarity, improve memory and cognition, and reduce the symptoms of aging (such as wrinkling of skin and graying of hair) [3,24]. Administration of Brahma Rasayana to cancer patients undergoing chemo- or radiotherapy caused a rapid increase in the levels of lymphocytes and neutrophils, while concomitantly reducing the total number of consecutive days of leukopenia, neutropenia, and lymphopenia [24]. Experiments have also shown it to be effective in reducing cisplatin-induced acute nephrotoxicity in mice [21].

Experimental studies have shown that Brahma Rasayana is myeloprotective, and increases the total leukocyte count; the percentage of polymorphonuclear cells, bone marrow cellularity, and α-esterase positive cells; and the number of spleen colonies in mice exposed to ionizing radiation. It also enhanced the levels of interferon-gamma (IFN-γ), interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF) in the serum of both normal and irradiated mice. These results suggest that the proliferation of stem cells induced by Brahma Rasayana in irradiated mice may be related to stimulation of cytokine production [25,26].

Brahma Rasayana is reported to scavenge hydroxyl, superoxide, nitric oxide, and lipid peroxides uninitiated in rat liver homogenate *in vitro*. It also inhibited generation of lipid peroxides by the Fe^{2+}-ascorbate and Fe^{3+}-ADP-ascorbate system with rat liver homogenate *in vitro*. Preclinical studies with laboratory mice have shown that Brahma Rasayana was effective against ethyl methanesulfonate- and methyl methanesulfonate (MMS)-induced genotoxicity [27]. Additionally, administering Brahma Rasayana to mice after their exposure to radiation resulted in faster cellular DNA repair, as revealed from the increased cellular repair index and

TABLE 16.2 Composition of Various Rasayana Drugs

Rasayana	Composition	References
Amalakayas Rasayana	*Emblica officinalis Gaertn* or *Phyllanthus emblica*, *Tinospora cordifolia*, *Terminalia chebula*, *Pluchea lanceolata*, *Alpenia galangal*, *Leptadenia reticulate*, *Asparagus racemosus*, *Centella asciatica*, *Desmodium gangiticum*, *Boerhavia diffusa*, and *lauha bashama*	[8]
Amruthaprasham	*Holstemma annulare*, *Vigna vexilata*, *Phaseolus adenanthus*, *Glycyrrhiza glabra*, *Zingiber officinale*, *Asparagus recemosus*, *Boerhaavia diffusa*, *Sida retusa*, *Clerodendrum serratum*, *Macuna pruriens*, *Hedychium spicatium*, *Phylanthus niruri*, *Piper longum*, *Vitis viniferra*, *Embelica officinalis*, *Purerira tuberose*, *Saccharum officinalum*, *Piper nigrum*, *Cinnamomum zeylanica*, *Elettaria cardamomum*, *Garcinia morella*, and *Mesua ferrea*	[9]
Ashwaganda Rasayana	*Withania sominifera*, *Purerira tuberosa*, *Hemidesmus indicus*, *Ciminum cuminum*, *Aloe barbidensis*, *Vitis vinifera*, *Elettaria cardamomum*, *Zingiber officinale*, *Piper nigrum*, and *Piper longum*	[9]
Brahma Rasayana	*Embelica officinalis*, *Terminalia chebula*, *Urarira pitca*, *Desmodium gangeticum*, *Gmelina arborea*, *Solanum nigrum*, *Tribulus terrestris*, *Aegle marmelos*, *Premna tomentosa*, *Stereospermum suaveolens*, *Oroxylon indicum*, *Sida rhombilfolia*, *Boerhaavia diffusa*, *Ricinus communis*, *Vigna vexilata*, *Phaseolus adenanthus*, *Asperagus recemosus*, *Holostemma annulare*, *Leptadenia reticulata*, *Desmostachya bipinnata*, *Saccharum officinalum*, *Oryza malampuzhensis*, *Cinnamomum iners*, *Elettaria cardamomum*, *Cyperus rotundus*, *Curcuma longa*, *Piper longum*, *Aquilaria agallocha*, *Santalum album*, *Centella asciatica*, *Mesua ferrea*, *Clitoria ternate*, *Acorus calamus*, *Scirpus crossus*, *Glycyrrhiza glabra*, and *Embelia ribes*	[9]
Chyavanaprasha Chyawanprash chyavanaprasha, chyavanaprash chyawanaprash	*Emblica officinalis*, *Bambusa arundinacea*, *Agele marmelos*, *Clerodendrum phlomidis*, *Oroxylum indicum*, *Gmelina arborea*, *Stereospermum suaveolens*, *Sida cordifolia*, *Desmodium gangeticum*, *Uraria picta*, *Teramnus labialis*, *Piper longum*, *Tribulus terrestris*, *Solanum indicum*, *Solanum xanthocarpum*, *Pistacia integerrima*, *Phaseolus trilobus*, *Phyllanthus niruri*, *Vitis vinifera*, *Leptadenia reticulata*, *Inula racemosa*, *Aquilaria agallocha*, *Tinospora cordifolia*, *Terminalia chebula*, *Ellettaria cardamomum*, *Cinnamom cassia*, *Cinnamom iners*, *Habenaria intermedia*, *Microstylis walichii*, *Microstylis museifera*, *Mesua ferra*, *Hedychium spicatum*, *Cyperus rotundus*, *Boerhaavia diffusa*, *Polygonatum verticillatum*, *Nymphaea alba*, *Santalum album*, *Pueraria tuberosa*, *Adhatoda vasica*, *Roscoea alpina*, *Martynia diandra*, and *Sesamum indicum*	[10]
Narasimha Rasayana	*Acacia catechu*, *Plumbago xylanica*, *Xylia dolabriformis*, *Pterocarpus marsupium*, *Embelia ribes*, *Semicarpus anacardium*, *Eclipta alba*, *Terminalia chebula*, *Embelica officinalis*, and *Terminalia belerica*	[9]
Triphala	*Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*	[10]
Anwala churna	*Emblica officinalis*	[11]
Amlakadi Gritha	*Emblica officinalis*	[12]
Brahmi Rasayana	*Bacopa monnieri*	[13,14]

decrease in the formation of micronuclei [22]. Together, these observations indicate that Brahma Rasayana accelerated the recovery of the hemopoitic system, increased antioxidant enzymes, and decreased the levels of serum lipid peroxidation and DNA mutagenesis [22,24,27].

Oral administration of Brahma Rasayana (50 mg/dose per animal) inhibited the PMA-induced superoxide generation in mice peritoneal macrophages. A dose-dependent inhibition of the nitrite production in peritoneal macrophages of mice was also observed at doses of 10 and 50 mg/dose per animal, where 25.2% and 37.8% inhibition was observed, respectively [28]. Brahma Rasayana increased the levels of superoxide dismutase and catalase, and tissue and serum levels of reduced glutathione, with concomitant decrease in the lipid peroxides in the liver [29].

Studies have also shown that Brahma Rasayana enhances the *in vivo* antioxidant status in cold-stressed [30] and heat-stressed [31] chickens. Recently, Guruprasad and colleagues [32] observed that feeding older mice with Brahma Rasayana for 8 consecutive weeks did not induce mutagenesis. Additionally, it was observed that Brahma Rasayana marginally increased the sperm count and increased the number of mitotic cells, validating its rejuvenating effect. Feeding Brahma Rasayana to mice protected them against radiotoxic effects, reduced the loss of organ weight (spleen liver and kidney) and body weight, and decreased the levels of serum and liver lipid peroxides, alkaline phosphatase, and alanine amino transaminase [6].

Recent studies by Sharma and co-workers [33] have shown that Brahma Rasayana was effective as an anabolic

agent in geriatric populations. The investigators suggested a dose of 10 g of the Rasayana twice daily with milk for 45 days, and followed up every 15th day. At the end of the study, it was observed that the volunteers who took the Rasayana regularly had increased their body weight, muscle strength, foot thrust, grip power, visual analog scale, mental and physical drive, and exertional capacity. Significant results were also seen in appetite, sleep, bowel habits, and Hbgm%, clearly indicating that Brahma Rasayana possesses anabolic effects and is safe to be given to geriatric patients [33].

16.4.4 Ashwagandha Rasayana

Ashwagandha Rasayana, which contains *Withania somnifera* (Ashwagandha), is a widely acclaimed Ayurvedic drug for people of all ages. It has a revitalizing action on the nerves, bone marrow, and reproductive organs. Regular consumption is believed to retard senescence, rectify abnormalities of the sense organs and hypotrophy of muscles, rejuvenate the reproductive organs, and increase fertility. It is also useful in stress, weakness, nervous exhaustion, sexual debility, geriatric problems, memory loss, muscular weakness, insomnia, and cough. Furthermore, it is supposed to inhibit aging and catalyze the anabolic processes of the body [3].

Preclinical studies have shown that Ashwagandha Rasayana possesses radioprotective effects, reduces radiation-induced emaciation and decrease in organ weight, and decreases the radiation-induced increase in serum alanine amino transaminase levels and lipid peroxidation [6]. Innumerable studies have shown that *Withania somnifera* increases longevity by promoting physical and mental health, and rejuvenates the body in debilitated conditions. Ashwagandha is also useful in epilepsy, stress, and neurodegenerative diseases (such as Parkinson's and Alzheimer's disorders, tardive dyskinesia, and cerebral ischemia), and contributes to general well-being [34].

16.4.5 Narasimha Rasayana

Narsimha Rasayana, consisting of more than 10 medicinal plants, is supposed to be potent in reversing aging, improving the immune system, and increasing sexual vigor and potency [3]. It contains *Semicarpus anacardium*, *Eclipta alba*, *Terminalia chebula*, *Embelica officinalis*, and *Terminalia belerica*, which are Rayasana plants possessing myriad anti-aging and pharmacological properties. Preclinical studies have shown that feeding mice with 50 mg/kg body weight of Narasimha Rasayana 5 days prior to radiation, and subsequently for a month, arrested radiation-induced deleterious effects. When compared to the radiation-alone cohorts, administering Narsimha Rasayana before exposure to radiation increased the body weight and organ weights of the mice, and decreased the levels of serum and tissue lipid peroxides, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) [6].

16.4.6 Triphala

Triphala (in Sanskrit, *tri* = three and *phala* = fruits) is another important Ayurvedic medicinal preparation comprising three fruits: *Phyllanthus emblica* or *Emblica officinalis*, *Terminalia chebula*, and *Terminalia belerica* [35]. It is an antioxidant-rich herbal formulation, and possesses diverse beneficial properties. In Ayurvedic practice, Triphala is used to treat gastrointestinal disorders such as dyspepsia, malabsorption, constipation, and ulcerative colitis; it is also a colon cleanser and tonifier. Triphala is also useful in treating ailments such as anemia, asthma, cough, fever, jaundice, leucorrhea, pyorrhea, and obesity [3].

Triphala is one of the most well-studied Ayurvedic formulations, and experiments have shown it to possess antibacterial, antifungal, free radical scavenging, antioxidant, anti-inflammatory, laxative, antiarthritic, anticataleptic, hypolipidemic, antihyperlipidimic, hepatoprotective, anti-stress, antidiabetic, antimutagenic, anticancer, chemopreventive, chemoprotective, radioprotective, and immunomodulatory properties [35]. All these properties may have contributed to the observed anti-aging effects.

16.4.7 Amritaprasham

According to Ayurvedic practitioners, regular intake of Amritaprasham in the early morning is supposed to improve strength and stamina, and retard aging [3]. It is also reported to be useful in the treatment of chronic fever, cough, bronchial asthma, and burning sensations; in seminal abnormalities such as azoospermia or oligospermia; and in erectile dysfunction and menstrual disorders. It is indicated in urinary disorders, hemorrhoids, gastrointestinal disorders, epistaxis, anorexia, thirst, vomiting, and loss of consciousness. It is supposed to improve strength and provide hemopoietic stimulatory action [3]. Amritaprasham is also reported to possess radioprotective effects and to ameliorate the ill effects of radiation [6].

16.4.8 Anwala Churna

Anwala churna, consisting of dried *Emblica officinalis* Gaertn. powder, is a potent Ayurvedic preparation with myriad pharmacological properties. Amla is a potent rejuvenator and is useful in stalling degenerative and senescence processes, promoting longevity, enhancing digestion, treating constipation, reducing fever, purifying the blood, reducing cough, alleviating asthma,

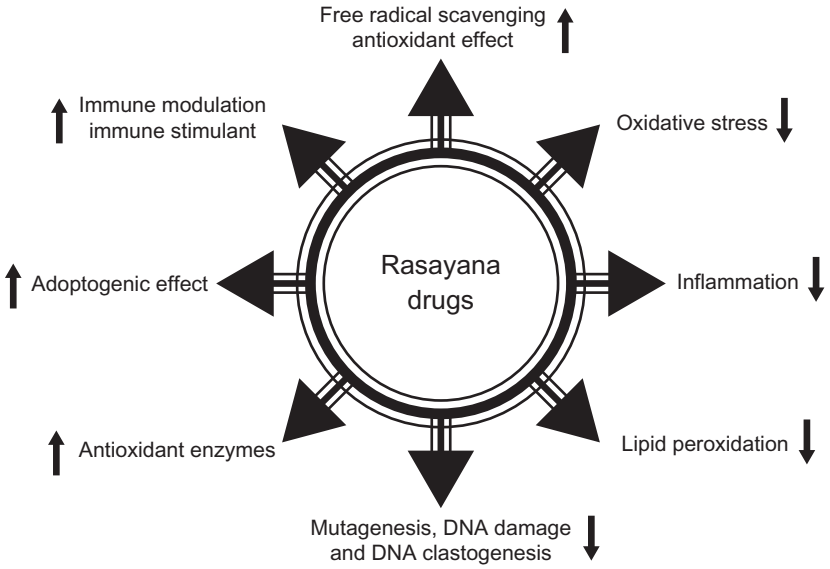

FIGURE 16.2 Biochemical targets responsible for anti-aging and other beneficial uses.

strengthening the heart, benefiting the eyes, stimulating hair growth, enlivening the body, and enhancing intellect. Studies have shown that oral administration of Anwala churna (50, 100, and 200 mg/kg) to both young and aged mice for 15 consecutive days caused a dose-dependent improvement in memory, reversed the scopolamine- and diazepam-induced amnesia, and reduced brain cholinesterase activity and total cholesterol levels [11].

16.4.9 Amalkadi Ghrita

Amalkadi Ghrita, made from *Emblica officinalis*, *Glycyrrhiza glabra*, and cow's ghee (clarified butter fat), is an important Ayurvedic formulation useful in the treatment of liver disorders. It also has pharmacological action on the CNS. Preclinical studies have shown that Amalkadi Ghrita (100 and 300 mg/kg, p.o.) possesses hepatoprotective activity against carbon tetrachloride (CCl4)-induced hepatic damage in rats, and the results were comparable to that of silymarin used as a positive control. When compared to the controls, administering Amalkadi Ghrita reduced the levels of serum AST (aspartate aminotransferase), ALT, ALP, ACP (serum acid phosphatase), and bilirubin. Histopathological studies further confirmed the protective effects, as Amalkadi Ghrita-treated cohorts exhibited almost normal architecture [12].

16.4.10 Brahmi Rasayana

Brahmi Rayasana, consisting of Brahmi (*Bacopa monniera*), is a important medhya Rayasana (neurotonic). In Ayurveda, regular consumption of this Rayasana is supposed to improve memory, learning ability, and concentration. It is also prescribed in mental disorders, epilepsy, mania, hysteria, memory loss, and depression [13]. Preclinical studies have shown that Brahmi Rasayana possesses anti-inflammatory effects comparable to those of indomethacin, and mediates the effect possibly by interfering with the action and/or synthesis of prostaglandins, and probably by stabilizing the lysosomal membranes [14]. Studies have also shown that Brahmi Rasayana possesses sedative effect, prolonged the hypnotic action of pentobarbitone, and caused a variable blockade of conditioned avoidance response. It also offered protection against electroshock seizures and chemoconvulsions. Furthermore, it was effective in antagonizing haloperidol-induced catalepsy, suggesting involvement of the GABA-ergic system in mediating the beneficial effects [13].

16.5 MECHANISMS RESPONSIBLE FOR THE BENEFICIAL EFFECTS

The exact mechanism of action responsible for the beneficial effects of these Rasayana drugs is unknown. As these formulations contain many plants with diverse pharmacological properties, it is logical to expect that myriad protective mechanisms are concomitantly operating. Some of the studied and reported mechanisms are explained in the following sections (Figure 16.2, Table 16.3 [36]).

16.5.1 Free Radical Scavenging

Excess generation of free radicals like the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), nitric

TABLE 16.3 Plants With Various Pharmacological Properties and Which Are an Integral Part of Rasayana Drugs [36]

Pharmacological properties	Plants
Free radical scavenging	*Emblica officinalis*, *Withania somnifera*, *Terminalia chebula*, *Terminalia bellerica*, *Asparagus racemosus*, *Tinospora cordifolia*, *Ocimum sanctum*, *Curcuma longa*, *Zingiber officinale*, *Agele marmelos*, *Oroxylum indicum*, *Sida cordifolia*, *Tribulus terrestris*, *Phyllanthus niruri*, *Vitis vinifera*, *Ellettaria cardamomum*, *Cinnamom cassia*, *Cyperus rotundus*, *Boerhaavia diffusa*, *Santalum album*, *Adhatoda vasica*, *Sesamum indicum*, *Cinnamomum zeylanica*, *Glycyrrhiza glabra*, *Centella asciatica*, *Acorus calamus*, *Glycyrrhiza glabra*, *Embelia ribes*, *Hemidesmus indicus*, *Ciminum cuminum* and *Aloe barbidensis*
Antioxidant	*Asparagus racemosus*, *Ocimum sanctum*, *Podophyllum hexandrum*, *Tinospora cordifolia*, *Hippophae rhamnoides*, *Zingiber officinalis*, *Centella asiatica*, *Syzygium cumini*, *Ligusticum wallichii* and *Vitis vinifera*
Anti-inflammatory	*Glycyrrhiza glabra*, *Allium sativum*, *Aloe vera*, *Tinospora cordifolia*, *Hippophae rhamnoides*, *Curcuma longa*, *Centella asiatica*, *Syzygium cumini*, *Ocimum sanctum*, *Moringa oleifera*, *Zingiber officinale* and *Eleutherococcus senticosus*
Antimutagenic and prevention of DNA damage	Triphala, Chyavanaprasha, *Ocimum sanctum*, *Curcuma longa*, *Zingiber officinale*, *Agele marmelos*, *Phyllanthus niruri*, *Mentha piperita*, *Tinospora cordifolia*, *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*
Immunomodulatory and adaptogenic activities	*Emblica officinalis*, *Withania somnifera*, *Viscum album*, *Ocimum sanctum* and *Tinospora cordifolia*

oxide (NO), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2), causes damage to cell structures, including lipids and membranes, proteins, and DNA. Scientific studies have shown that phytochemicals and antioxidant molecules are effective in nullifying the free radical-induced damage and delaying the pathogenic process. Studies also suggest that Brahma Rayasana scavenged Fe^{2+}-ascorbate and Fe^{3+}-ADP-ascorbate-induced lipid peroxidation, and scavenged the hydroxyl, superoxide and nitric oxide generated *in vitro*. It also inhibited phormol myristate acetate (PMA)-induced superoxide generation in mice peritoneal macrophages, and nitrite production in peritoneal macrophages [28]. Triphala and Chyavanaprash have also been observed to scavenge nitric oxide *in vitro* [14]. Rasayana drugs are composite herbal formulations, and many plants that are an integral part of Rasayana preparations possess free radical scavenging and antioxidant effects [6,9,37,38].

16.5.2 Inhibition of Lipid Peroxidation

Polyunsaturated fatty acids (PUFAs) are vulnerable to peroxidative attack, and the damage it inflicts on the cell membranes leads to various pathogenic processes. *In vitro* studies have shown that both alcoholic and aqueous extracts of Brahma Rasayana inhibit enzyme-induced and non-enzyme-induced microsomal lipid peroxidation in a concentration-dependent manner [26–29]. Brahma Rasayana treatment decreased the radiation-induced increase in the serum lipid peroxidation in cancer treatment [24], and serum and liver lipid peroxidation in the chickens subjected to heat stress [30]. Triphala is also observed to decrease radiation-induced lipid peroxidation *in vitro* [37] and in normal animals [39].

16.5.3 Increase in Antioxidant Enzymes

The antioxidant enzymes SOD (superoxide dismutase), GPx (glutathione peroxidase), and catalase cooperate or work synergistically to protect cells against oxidative stress and prevent or delay pathological changes. Studies have shown that Triphala and Brahma Rayasana increase the levels of glutathione and antioxidant enzymes and protect against oxidative stress [29,39,40]. The administration of aqueous extracts of medicinal plants like *Aegle marmelos*, *Terminalia chebula*, and *Emblica officinalis*, which are an integral part of many Rasayana formulations, have been reported to be effective in modulating the oxidative stress and enhancing the antioxidant status in rodents [39,41,42].

16.5.4 Antimutagenic Activities

Mutagenesis is an important step in the process of carcinogenesis, and its prevention is of great importance in preventing cancer. There is increasing evidence that many phytochemicals, plants, and their compound formulations can act as inhibitors of mutagenesis and carcinogenesis [36,38,43,44]. Kaur and colleagues [43] have reported that aqueous, chloroform, and acetone extracts of Triphala were observed to possess antimutagenic effects against both direct and indirect mutagens in the Ames histidine reversion assay. The extracts inhibited the mutagenicity induced by both direct- and indirect-acting mutagens, but the inhibition was greater for S9-dependent mutagens [43]. The acetone and chloroform extracts were observed to be better than the aqueous extracts in TA98 and TA100 tester strains of *S. typhimurium*. Maximum inhibition was observed for the acetone extract [43].

Triphala and its individual constituents are also reported to prevent γ-radiation-induced DNA strand break formation in the plasmid DNA (pBR322) *in vitro* [45]. Feeding of Triphala was also observed to have inhibited the radiation-induced DNA strand breaks in leukocytes and splenocytes of mice exposed to whole body irradiation of 7.5 Gy [40]. Studies by Yadav *et al*. [23] have shown that consumption of Chyawanprash by bidi smokers decreased the genotoxic risk caused by tobacco mutagens when compared with bidi smokers alone; consumption of Chyawanprash decreased the mitotic index, chromosomal aberrations, sister chromatid exchanges, and satellite associations.

16.5.5 Anti-inflammatory Effects

Anti-inflammatory drugs, especially non-steroidal forms, have great importance in modern medical practice. Studies have shown that Rasayana drugs and some of their constituent plants are potent inhibitors of inflammation, as shown by reduction in paw edema induced by carrageenan, and alleviation of various experimentally induced inflammatory reactions [14,46] in rheumatoid arthritis [46]. Brahmi Rasayana is also reported to possess anti-inflammatory effects comparable to those of indomethacin [14]. Triphala is shown to be effective in preventing Freund's adjuvant-induced arthritis and inflammation in mice. The effect was observed to be better than that of indomethacin, and the levels of lysosomal enzymes, tissue marker enzymes, glycoproteins, and paw thickness were significantly altered in the Triphala group to near normal conditions [47].

16.5.6 Hemopoietic Stimulation

Scientific studies indicate that the basal hematopoiesis is maintained throughout life but is weakened in advanced age to cope with hematological stress. Exposure to ionizing radiation decreases hematopoiesis in mammals, and is a good model to study the effect of drugs in both protecting and stimulating hematopoiesis when subjected to hematological stress [35,48]. Preclinical studies suggest that the Rasayana drugs possess hemopoietic stimulatory function against the cytotoxic effects of anticancer agents. Triphala, Brahma Rayasana, Narasimha Rayasana, Ashwaganda Rayasana, Amruthaprasham, and Chayawanprash have been observed to attenuate radiation-induced damage to the hemopoietic system [6,9]. The plants *Acanthopanax senticosus*, *Embelica officinalis*, *Ocimum sanctum*, *Withania somenifera*, *Tinospora cordifolia*, and *Boerhaavia diffusa* provide total body radiation protection by stimulating hematopoiesis [48].

16.5.7 Immune Modulation

The immune system, which is the primary defense mechanism of the body against various pathogens and cancer, grows weaker with age. Immune response can be modulated by immune modulators, and studies indicate that certain phytochemicals possess immunostimulatory (immunopotentiation, or strengthening of immune reaction) effects. Clinical studies have shown that administration of Brahma Rasayana increased the activity of lymphocytes, and an increase in serum granulocyte macrophage colony stimulating factor (GM-CSF) was observed [24]. Triphala has been reported to possess immunomodulatory activity and to stimulate neutrophil function in immunized rats and stress-induced suppression of neutrophil function [49].

16.5.8 Adaptogenic and Anti-stress Properties

An adaptogen increases the powers of resistance against physical, chemical, or biological noxious agents, and has a normalizing influence on the body. A number of plants possess adaptogenic activity due to diverse classes of chemical compounds. The plant adaptogens reduce the reactivity of host defense systems and decrease the damaging effects of various stressors due to the increased basal level of mediators involved in the stress response [31]. Oral administration of Triphala is reported to significantly prevent the noise [50] and cold stress [51] -induced behavioral and biochemical abnormalities in albino rats. Studies have also shown that Brahma Rasayana enhances the *in vivo* antioxidant status in cold-stressed and heat-stressed chickens [30,31]. Studies have shown that the Rasayana plants *Withania somnifera*, *Emblica officinalis*, *Asparagus racemosus*, and *Tinospora cordifolia* also possess adaptogenic effects and contributed to the observed effects (Table 16.3) [5,38,52,53].

16.6 CONCLUSIONS

Scientific studies carried out over the past decade have shown conclusively that the Ayurvedic Rasayana drugs Amalaki Rasayana, Chyavanprasha, Triphala, Brahma Rasayana, Ashwagandha Rasayana, Brahmi Rasayana, Narasimha Rasayana, Amalkadi Ghrita, Anwala churna, and Amritaprasham are effective in various ailments and stress. The mechanism of action of herbal drugs differs in many respects from that of synthetic drugs, and can be characterized as a polyvalent action and interpreted as additive or, in some cases, potentiating. A combination of factors, including free radical scavenging, prevention of lipid peroxidation, inhibition of DNA damage, and

protection and rapid regeneration of bone marrow stem cells increase/restoration of antioxidants, would have also contributed to the beneficial effects.

Most of the published observations for the various pharmacological properties of the Rasayana drugs have been with experimental animals, and help in justifying their applicability to humans. Additionally, these Rasayana drugs have been consumed by inhabitants of the Indian subcontinent since time immemorial, and the non-toxic nature of these drugs gives an immense advantage in initiating human studies. For optimal use and application, detailed studies in healthy human volunteers are required to understand the maximum permissible dose and toxic effects of each of these Rasayana drugs. The results of these experiments will be of immense use for future clinical trials aimed at both the treatment and prevention of aging and associated ailments.

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CHAPTER

17

Can Phytochemicals be Effective in Preventing Ethanol-Induced Hepatotoxicity in the Geriatric Population? An Evidence-Based Revisit

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17.1 INTRODUCTION

Globally, long-term heavy alcohol use is one of the most important causes of illness and death from liver disease. Reports indicate that alcoholic liver disease is the second most common reason for liver transplantation [1]. Chronic ethanol ingestion is reported to induce free radicals, stimulate hepatic oxygen consumption, and cause fatty liver, hepatomegaly, inflammation, fibrosis, and cirrhosis. Classically, the alcoholic liver injury and sequential pathological features comprise fatty liver, alcoholic hepatitis, and alcoholic cirrhosis. Fatty liver is present in more than 90% of chronic alcoholics, while about 10–20% of heavy drinkers progress to alcoholic hepatitis and cirrhosis, indicating that factors such as genetic background, nutrition, viral infection (HBV), and chronic intake/exposure to paracetamol, aflatoxins, heavy metals, and xenobiotics interact to influence progression of liver disease [1].

Ethanol is metabolized by multiple metabolic pathways, and several molecular phenomena are involved in the pathogenesis of alcoholic liver disease. Ethanol is primarily metabolized by alcohol dehydrogenase and aldehyde dehydrogenase enzyme systems generating acetaldehyde and acetate as products [2]. Acetaldehyde forms adducts with vital biomolecule DNA and proteins, which are responsible for the impaired structure and function of the liver. Another metabolic pathway which plays significant role in alcohol toxicity is the cytochrome P450 (CYP) system (CYP2E1) of liver microsomes. Induction of CYP2E1 by ethanol, subsequent generation of reactive oxygen species (ROS) by CYP2E1 and the mitochondrial electron transport chain, impairment of antioxidant mechanisms, and the resultant oxidative stress trigger inflammatory responses and necrosis and apoptosis of the hepatocytes. The by-products generated are more dangerous than the alcohol itself, and contribute to alcohol-induced liver damage [2]

With respect to the deleterious effects of ethanol consumption, scientific studies have shown that the elderly (individuals >65 years) population is at a higher risk than younger populations [3]. The process of senescence causes marked changes in the physiology of many organs, including the liver, and this contributes immensely to the observed hepatotoxic effects of ethanol in the elderly [3,4]. Liver volume and blood flow decline

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with age in humans, and this contributes to diminished clearance of drugs that exhibit first-pass kinetic profiles [3,4]. This, along with the accumulation of dense bodies and loss of smooth-surfaced endoplasmic reticulum, causes a decline in the amounts and specific activities and rates of induction of several microsomal monooxygenases (cytochrome P450 isoform CYP3A) and a concomitant decrease in the levels of the antioxidant enzyme superoxide dismutase contributes to the overall damage [3–8].

17.2 PHYTOCHEMICALS IN PROTECTION AGAINST ALCOHOL-INDUCED HEPATOTOXICITY

Conventional treatment modalities for liver diseases suffer from side effects, and the search is on for plant-based traditional therapeutic regimens that are not only useful in treating the complications of alcoholic toxicity but also have immense value in prevention. Traditional medicine systems such as Ayurveda, homeopathy, Unani, Siddha, and Chinese medicine have been using plants and their phytochemicals for the treatment of liver ailments for many years. The phytochemicals (which are natural constituents of fruits and vegetables) that offer protection against liver ailments can be incorporated into our daily diet to prevent any liver damage resulting from exposure to xenobiotics such as alcohol and drugs. Phytochemicals possess antioxidant, anti-inflammatory, antimutagenic, and antifibrotic effects. This chapter gives an account of the hepatoprotective effects of some of the important phytochemicals: β-carotene, betaine, curcumin, ellagic acid, epigallocatechin-3-gallate, ferulic acid, hydroxystilbenes, lutein, morin, mesozeaxanthin, quercetin, and ursolic acid (Figure 17.1).

17.2.1 Beta-Carotene

Beta-carotene (Figure 17.1), the well-studied and the most important of all carotenoids, is a precursor for vitamin A. It is found predominantly in carrots, mango, maize, lentils, dark green leaves, amaranth, and spinach. β-Carotene is reported to be a potent antioxidant and to protect the liver against the toxic effects of various drugs and xenobiotics [9]. Studies with rats have shown that supplementation with β-carotene in the diet prevented an ethanol-induced increase in serum aminotransferases and inhibited depletion of the antioxidant molecule glutathione (GSH) in the liver [10]. Additionally, *in vitro* studies with the hepatocytes isolated from ethanol-fed rats have also shown that β-carotene improved the cell viability, and increased catalase activity and levels of glutathione [11]. Mechanistic studies performed with hepatocytes isolated from ethanol-fed rats have also shown that β-carotene ameliorated oxidative stress, enhanced antioxidant activity, and also decreased both the expression of CYP2E1, and apoptosis [12].

17.2.2 Lutein

Lutein (Figure 17.1), a xanthophyll and naturally occurring carotenoid present in increased concentrations in green leafy vegetables and some fruits (for example, avocado, kiwi, etc.), has strong free-radical and antioxidant effects. Animal studies have shown that it also possesses hepatoprotective effects against xenobiotics such as paracetamol, carbon tetrachloride, and alcohol [13]. Lutein has been shown to reduce the elevated serum levels of aminotransferases, alkaline phosphatase, and bilirubin, and to decrease the levels of lipid peroxidation, conjugated diene, and hydroperoxides, in the livers of rats treated with ethanol [13]. Lutein treatment to ethanol-administered rats also reversed the histopathological abnormalities and reduced the levels of hydroxyproline, an indicator of fibrosis [13].

17.2.3 Meso-Zeaxanthin

Zeaxanthin (Figure 17.1) is one of the most common carotenoid alcohols found in nature, and the 3R,3′S stereoisomeric form is referred to as meso-zeaxanthin. This is the pigment that gives paprika (made from bell peppers), corn, saffron, and many other plants their characteristic color. Spinach, goji berry, kale, turnip greens, collard greens, romaine lettuce, broccoli, zucchini, kiwi fruit, corn, garden peas, Swiss chard, and Brussels sprouts are good sources of meso-zeaxanthin. It is an antioxidant, and this property contributes to its myriad beneficial effects [13–15]. Firdous *et al*. [13] have shown that treatment with meso-zeaxanthin reduces ethanol-induced toxicity. Mechanistic studies have shown that, in the liver of ethanol-administered rats, meso-zeaxanthin reduced the serum levels of aminotransferases, alkaline phosphatase, and bilirubin; and the levels of lipid peroxidation, conjugated diene, and hydroperoxides. It also reduced the levels of hydroxyproline and reversed histopathological abnormalities [13].

17.2.4 Betaine

Betaine (trimethyl glycine) (Figure 17.1) is a metabolite formed in the body from choline. It is a natural constituent of beets, broccoli, grains, shellfish, spinach, and marine algae. Animal studies have shown that betaine is effective in reducing ethanol-induced hepatotoxicity [16]. When compared to ethanol-alone treated cohorts, co-treatment with betaine resulted in increased levels of vitamin A and GSH in the liver, decreased malondialdehyde levels in liver tissue, and reduced serum levels

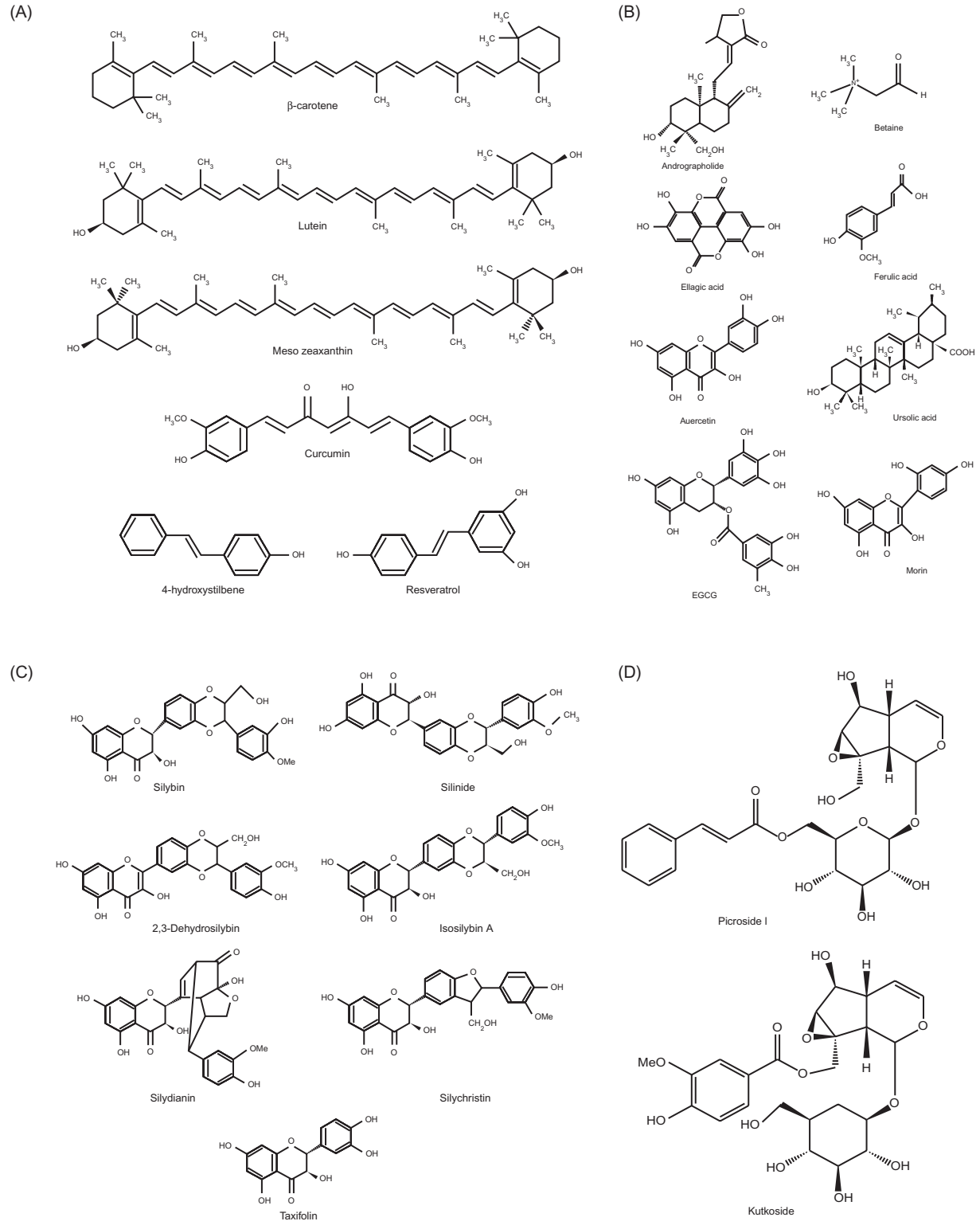

FIGURE 17.1 Phytochemicals possessing protective effects against ethanol-induced liver damage.

of aminotransferases [16]. Betaine reduced the elevated hepatic levels of lipids, homocysteine, endoplasmic reticulum stress response, and apoptosis [17]. Betaine attenuated alcoholic liver disease by mitigating oxidative stress, increasing synthesis of S-adenosyl methionine and GSH, and decreasing the hepatic homocysteine level. It also triggers a cascade of events that lead to mobilization of triglycerides from liver and concomitantly reduces the endoplasmic reticulum stress responses [18]. Studies with guinea pigs have also shown that betaine prevents ethanol-induced increases in lipid peroxides and triglycerides in the liver, aminotransferase levels in serum, and halts the decrease in the levels of GSH in the liver [19].

17.2.5 Curcumin

Curcumin (Figure 17.1), the principal curcuminoid of the popular Indian spice turmeric, is arguably one of the highly investigated phytochemicals. Scientific studies have shown that curcumin possesses antitumor, antioxidant, antiarthritic, antiamyloid, anti-ischemic, and anti-inflammatory properties [20,21]. With regard to the protective effect of curcumin against alcohol-induced hepatoxicity, studies have shown that curcumin mitigated oxidative stress and prevented liver cell damage in experimental animals [22–24]. *In vitro* studies with liver slice culture have shown that curcumin decreased lipid peroxidation, reduced the release of lactate dehydrogenase (LDH), and attenuated the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and GSH-Px (glutathione peroxidase) [22]. Studies with rat hepatocytes have also shown that curcumin decreased the ethanol-induced increase in malondialdehyde, decreased the levels of LDH and aspartate aminotransferase (AST), increased GSH levels, and induced heme-oxygenase in the liver cells [23]. Curcumin decreased the hepatic levels of prostaglandins, and serum levels of AST and alkaline phosphatase, in rats subjected to ethanol-induced hepatotoxicity [25]. Molecular studies have also shown that administration of curcumin to rats with alcohol-induced liver disease prevented the activation of NF-κB and suppressed the expression of cytokines, chemokines, cyclooxygenase (COX)-2, and iNOS in Kupffer cells of the liver [26].

17.2.6 Ferulic Acid

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) (Figure 17.1), a phenolic compound found in the cell wall of plants, is a potent free-radical scavenger and antioxidant. With regard to its hepatoprotective effect, ferulic acid has been shown to decrease the elevated serum levels of the liver marker enzymes AST, ALT, alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) in rats subjected to ethanol-induced hepatotoxicity [27]. Ferulic acid ameliorated oxidative stress and improved the antioxidant status in ethanol-fed rats [28].

17.2.7 Ellagic Acid

Ellagic acid (Figure 17.1), a polyphenol found in fruits and vegetables including blackberries, raspberries, strawberries, cranberries, walnuts, pecans, pomegranates, wolfberry and other plant foods, is one of the well-studied phytochemicals. It possesses antioxidant, anti-mutagenic and anticancer properties. Administering ellagic acid to rats with ethanol-induced hepatotoxicity was shown to ameliorate the increased serum levels of aminotransferases, lipid peroxides, and hydroperoxides and also to reduce the elevated hepatic contents of cholesterol, free fatty acids, triglycerides, and phospholipids [29]. Ellagic acid mitigated the alcohol-induced toxicity in rats by improving the body weight, restoring antioxidant status, modulating micronutrients, and attenuating the lipid levels in blood [30]. Studies have also shown that ellagic acid decreases ethanol-induced hepatotoxicity by modulating ethanol-induced alterations in the expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases [31]. Additionally, ellagic acid is also reported to possess antifibrotic effects and thus to be of benefit in preventing alcoholic liver disease [31,32].

17.2.8 Epigallocatechin-3-Gallate

Epigallocatechin gallate (EGCG) (Figure 17.1), the ester of epigallocatechin and gallic acid, belongs to the catechin class of phytochemicals. It is the most abundant phytochemical in green tea and is a potent free-radical scavenger and antioxidant. With regard to the protective effect of EGCG on ethanol-induced hepatotoxicity, studies have shown that EGCG prevents liver cell injury and release of enzymes to blood [33,34], mitigates oxidative stress [34,35], promotes mobilization of fat from the liver [36], and reduces expression of pro-inflammatory molecules [34]. Administration of EGCG effectively prevented/reversed the histological changes and increased serum levels of aminotransferases in ethanol-fed mice with an overload of iron [33]. Dietary EGCG prevented fatty liver by enhancing the activities of the enzymes carnitine palmitoyl-transferase 1 and phospho-acetyl CoA carboxylase, thus promoting β oxidation of fatty acids [36]. EGCG supplementation reduced elevated expressions of CD14, tumor necrosis factor (TNF)-α, COX-2, and iNOS in the liver of rats subjected to ethanol toxicity [34]. *In vitro* studies with Chang liver cells (normal hepatocyte cell line) also demonstrated that EGCG ameliorated ethanol-induced reduction in the growth of liver cells, leakage of LDH from cells, and reduction in GSH, lipid peroxidation, and apoptosis [35].

17.2.9 Quercetin

Quercetin (2-[3,4-dihydroxyphenyl]-3,5,7-trihydroxy-4H-chromen-4-one) (Figure 17.1) is a flavonoid found ubiquitously in fruits, vegetables, leaves, and grains. It is the aglycone form of a number of other flavonoid glycosides (such as rutin and quercitrin) found in citrus fruit, buckwheat, and onions. Quercetin is shown to possess myriad pharmacological effects, including free-radical scavenging, antioxidant, and anti-inflammatory properties. With regard to hepatoprotective effects, *in vitro* studies have shown that quercetin ameliorated ethanol-induced liver cell injury, lipid peroxidation, depletion of GSH, and release of LDH and AST from liver cells, and upregulated hemeoxygenase-1 via the mitogen-activated protein kinase (MAPK)/Nrf2 pathways in human hepatocytes [37,38]. *In vivo* studies with rats have also shown that quercetin prevented and reversed ethanol-induced hepatotoxicity by reducing the elevated serum levels of AST, alanine aminotransferase (ALT), alcohol dehydrogenase (ADH), GGT, triglycerides (TG), interleukins IL-1β, IL-1, IL-6, IL-8, and TNF-α; reducing the levels of malondialdehyde and increasing the levels of GSH in the liver; and increasing IL-10 in plasma. Treatment with quercetin following ethanol intoxication also reversed the increases in serum levels of aminotransferases and liver tissue levels of lipid peroxides and hydroperoxides, and restored the levels of GSH, SOD, GSH-Px, and glutathione reductase (GR) in rats [39].

17.2.10 Morin

Morin (Figure 17.1), a pentahydroxyflavone, is an important phytochemical in many plants belonging to the Moraceae family (*M. alba*). Morin possesses antiviral, antibacterial, and antioxidant effects. It inhibits Δ^5-lipoxygenase, iodothyronine deiodinase, lens aldose reductase, and ionophore-induced arachidonic acid release and metabolism. With regard to the hepatoprotective action of morin, studies have observed that administration of morin to alcohol-intoxicated rats for 30 days showed significant decreases in lipid peroxidation and restoration of the antioxidants vitamin C, vitamin E, and GSH in liver, and amelioration of the cellular architecture of liver [40].

17.2.11 Hydroxystilbenes and Resveratrol

Hydroxystilbenes (Figure 17.1) and their derivative resveratrol (3,5,4′-trihydroxy-trans-stilbene) (Figure 17.1) are natural phenols and phytoalexins produced by several plants, including grapes, raspberries, blueberries, peanuts, and mulberries. They have been shown to possess antioxidant, anticarcinogenic, antidiabetic, anti-inflammatory, cardioprotective, hepatoprotective, and neuroprotective effects. Preclinical studies have shown that resveratrol reduced lipid peroxidation and restored levels of the antioxidant enzymes SOD, CAT, and GSH-Px in the liver of rats treated with ethanol [41]. Studies in mice subjected to ethanol-induced toxicity have also shown that administering 4-hydroxystilbenes and resveratrol were effective in reversing ethanol-induced liver cell injury and in inhibiting the oxidation of PUFA [42]. Resveratrol treatment caused reduction in lipid synthesis, increased rates of fatty acid oxidation, and prevented alcoholic liver steatosis in mice [43]. Resveratrol is a potent activator of sirtuin 1 (SIRT1) and adenosine monophosphate (AMP)-activated kinase (AMPK), two critical signaling molecules regulating the pathways of hepatic lipid metabolism. It increased SIRT1 expression, stimulated AMPK activity, suppressed sterol regulatory element binding protein 1 (SREBP-1), and activated peroxisome proliferator-activated receptor γ co-activator α (PGC-1α) in the liver of ethanol-fed mice [43].

17.2.12 Ursolic Acid

Ursolic acid (Figure 17.1) is a pentacyclic triterpene acid present in many plants, including apples, bilberries, cranberries, elderflower, peppermint, lavender, oregano, thyme, hawthorn, and prunes. Ursolic acid has many pharmacological effects, including antioxidant, anti-inflammatory, antibacterial, and antifungal properties. Administration of ursolic acid to ethanol-fed rats ameliorated hepatotoxicity by reversing the histological changes, decreasing lipid peroxidation, and increasing the circulatory antioxidants GSH, ascorbic acid, and alpha-tocopherol [44]. When compared to the ethanol-treated cohorts, administration of ursolic acid reduced the serum levels of ALT, AST, and bilirubin, and restored the serum albumin level [44].

17.2.13 Andrographolide and Arabinogalactan Proteins of *Andrographis paniculata* Nees

Andrographis paniculata Ness, commonly known as the "King of Bitters," is an important medicinal plant in both Ayurvedic and traditional Chinese medicine for treating various ailments, including those of the liver. Preclinical studies by Singha *et al*. [45] have shown that andrographolide (Figure 17.1), a labdane diterpenoid and arabinogalactan protein of *Andrographis paniculata*, possesses hepatoprotective effects [45]. The authors observed that intraperitoneal administration of the phytochemicals (62.5, 125, 250, and 500 mg/kg body weight for 7 consecutive days) before administering ethanol was effective in ameliorating both hepato- and renotoxicity in mice. Biochemical parameters evaluated suggested a reduction in the levels of AST, ALT, ACP, ALP, and LP levels in the liver and kidneys, indicative of its benefit.

17.2.14 Picroliv

Picroliv, the ethanolic fraction of the herb *Picrorhiza kurroa* which grows at an altitude of 3000–5000 meters above sea level in the Himalayan ranges of India, Pakistan, and Nepal, is a potent hepatoprotective agent. Chemical studies indicate that the ethanolic extract contains 50–60% of a mixture of two iridoid glycohepatosides, picroside-I and kutkoside (Figure 17.1), in a ratio of 1:1.5, and preclinical studies have shown that picroliv possesses potent hepatoprotective effects against various hepatotoxins, including alcohol [46]. Animal studies have shown that oral administration of picroliv was effective in ameliorating the ethanol-induced chronic hepatotoxicity in rats [46,47]. Studies with cultured rat hepatocytes have also shown that picroliv was effective in protecting against the cytotoxic effects of ethanol, and in reducing the levels of the alcohol-metabolizing enzymes aldehyde dehydrogenase and acetaldehyde dehydrogenase [47]. Animal studies have also shown that, when compared to the alcohol-alone cohorts, coadministering picroliv restored the altered levels of lipid, glycogen, and protein in the liver; decreased the levels of AST, ALT, and ALP in serum; and increased bile volume, bile salts, and bile acids [47]. Together, all these observations clearly indicate the usefulness of picroliv against ethanol-induced hepatotoxicity and in improving bile-functioning.

17.2.15 Silymarin

Silymarin, isolated from the milk thistle (*Silybum marianum*), is arguably the most commonly used medication for various liver diseases. It is a mixture of flavonolignans, consisting of silibinin A and B, isosilybin A and B, silychristin, and silydianin (Figure 17.1). With regard to hepatoprotective effects, silymarin is shown to possess protective effects in both acute and chronic models of ethanol toxicity [48–50]. In acute toxicity studies, administration of silymarin is shown to ameliorate the alcohol-induced increase in the level of ALT in the serum, to reduce the levels of hepatic lipid peroxidation and production of TNF, to restore the levels of GSH, and to reduce hepatic microvesicular steatosis [48]. Additionally, in the chronic model of ethanol intoxication studies, administration of silymarin is shown to decrease the levels of serum aminotransferases and GGT [49,50]. Silymarin was also shown to ameliorate ethanol-induced oxidative stress in the liver of baboons, and to mediate these effects by decreasing the ethanol-induced increase in lipids, reducing the increase in mRNA for alpha-1 (I) procollagen, and decreasing the type I collagen in the liver [50].

In vitro studies have also shown that silymarin or its constituent silibin scavenged ethanol-derived hydroxyl and hydroxyethyl radicals [51,52] to inhibit the induction of CYP2E1, generation of ROS, and *in vitro* proliferation of the hepatocellular carcinoma cells [53]. Studies with cultured hepatocytes have shown that silybin counteracted the ethanol-mediated decrease in incorporation of glycerol into phospholipids and increased the incorporation of glycerol into neutral lipids [54]. Silphos, a complex of silybin and lecithin, also offered protection to fetal liver and prevented elevation of GGT in maternal and fetal liver [55]. Additionally, phytochemicals such as silipide, a complex of silybin and lecithin, have also shown to be of use in preventing ethanol-induced toxicity [56]. However, clinical observations with silymarin have been inconclusive and contradictory [57–59].

17.3 MECHANISMS

Scientific studies have shown that the phytochemicals β-carotene, betaine, curcumin, ellagic acid, epigallocatechin-3-gallate, ferulic acid, hydroxystilbenes, lutein, morin, meso-zeaxanthin, quercetin, and ursolic acid present in plants offer protection against ethanol-induced hepatotoxicity by multiple mechanisms (Figure 17.2). Ethanol-induced generation of free radicals with depletion of antioxidants and oxidative damage to vital biomolecules is the primary molecular phenomenon involved in pathogenesis of alcoholic liver disease. The universal mechanism of hepatoprotection by most phytochemicals is amelioration of ethanol-induced oxidative stress in the liver by inhibition of lipid peroxidation and attenuation of hepatic antioxidant defense systems [11–13,18,24,27,38,39,44]. Phytochemicals also possess anti-inflammatory effects [26,34,43,60], suppress the expression of CYP2E1, prevent apoptosis by inhibiting caspases [12], act as lipotropic factors by increasing mobilization of fat from liver, and inhibit lipid synthesis in the liver [31,36,43]. All these properties contribute to the observed hepatoprotective effects.

17.4 CONCLUSIONS

Numerous preclinical *in vivo* and *in vitro* studies have demonstrated the hepatoprotective actions of phytochemicals. Phytochemicals exert their protective effect against alcohol-induced liver damage by antioxidant, anti-inflammatory, antimutagenic, and lipotropic actions, all of which are of significant use in elderly people. Phytochemicals are beneficial not only in treating the hepatotoxic complications of alcohol but also in preventing adverse effects of alcohol on the liver. There is a need for clinical studies with phytochemicals in order to validate their clinical usefulness and make their use acceptable in modern medicine.

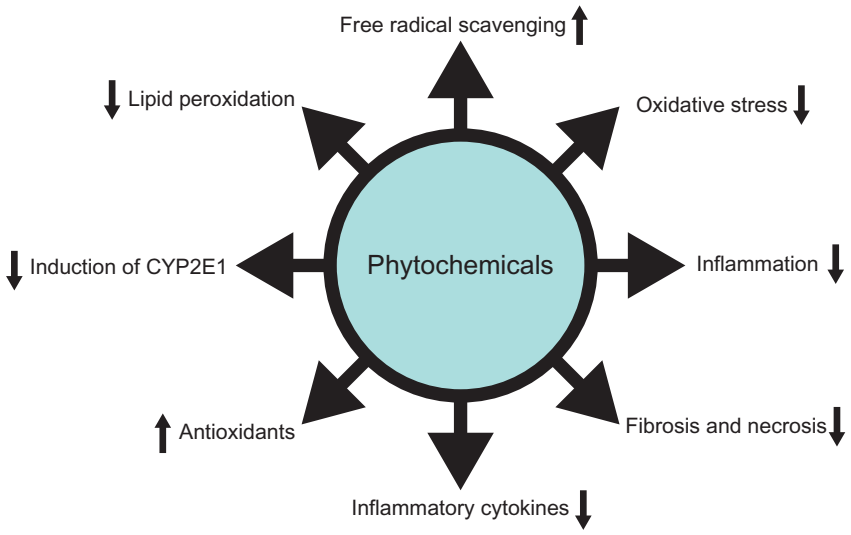

FIGURE 17.2 Mechanisms responsible for the hepatoprotective effects of various phytochemicals (↑, increase; ↓, decrease).

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CHAPTER

18

Chamomile: A Herbal Agent for Treatment of Diseases of the Elderly

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18.1 INTRODUCTION

Many human disorders are associated with aging. It is assumed that between 2015 and 2050, the proportion of the world's older adults will increase and approximately double from 11% to 22%. In absolute terms, this is an expected increase from 605 million to 2 billion people over the age of 60. Older people face physical and mental health challenges which need to be understood and resolved [1]. In general, elderly health and social care is needed in preventing disease and managing chronic illnesses. The aging population has become more vulnerable to various types of diseases, such as lifestyle disorders and age-associated challenges. A substantial change in metabolism and physiology during aging also contribute to age-associated diseases such as Alzheimer's disease, arthritis, arthrosclerosis, cataract, diabetes, dementia, insomnia, benign prostatic disease, obesity, and perhaps cancer. To address these aging disorders, plants offer a most acceptable modality because of their effectiveness, abundance, and minimal side effects, establishing their importance in human life [2–7]. Phytotherapies have been recognized as integral to both traditional and non-traditional forms of medicine dating back at least 5000 years [7–9]. This non-traditional practice of medicine has been placed in the category of complementary and alternative medicine (CAM) [10]. The term CAM refers to therapies provided in addition to mainstream medicine and that are expected to provide symptomatic relief and to improve quality of life. CAM may be a desirable addition and balance to technologically sophisticated disease care [11]. CAM therapies are often unsupported by scientific data, and some cases may produce adverse effects due to inherent toxicities [11–14]. Nonetheless, the majority of CAM therapies are beneficial to patients [15,16]. Another important reason for the popularity of CAM therapies is that they are usually much less expensive than conventional medical treatments [17]. It is reported that CAM are more popular among the elderly, and approximately 41% of seniors reported the use of CAM to their primary care physicians. Different CAM therapies now in practice are the use of medicinal herbs, chiropractic treatments, massage therapy, and acupuncture. According to a study by Astin *et al*. [18], 80% of seniors reported receiving substantial benefit from the use of various CAM therapies [18].

18.2 THE PLANT – CHAMOMILE

Chamomile is a native of the Old World, is widely distributed around the globe, and is grown extensively in countries such as Germany, Hungary, France, Russia, Yugoslavia, Brazil, and India. Chamomile is also reported in North Africa, North and South America, Australia, and New Zealand [19,20]. It is a member of the daisy

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family (Asteraceae or Compositae) and is traditionally known for its healing applications. It is widely represented by two well-known varieties, namely German chamomile (*Matricaria chamomilla*) and Roman chamomile (*Chamaemelum nobile*) [21]. The history of chamomile dates back even further, at least to the time of the ancient Egyptians, when it was dedicated to their gods for cure for the "ague" (acute fever). Since then chamomile has been recognized as a wonderful plant and a universal cure for almost all common ailments of human beings. Because of its broad curative properties, it is also known as "doctor plant." Today, chamomile, in the form of tea, is highly popular in the Western world [22]. According to one estimate, more than a million cups of chamomile tea are consumed worldwide on a daily basis [23]. As a popular remedy, chamomile has been recognized as the "European counterpart of ginseng" and is, according to Varro Tyler, a renowned German clinician and expert in herbal medicine, *"alles zutraut"* – capable of anything [24,25].

18.3 PHYTOCHEMICALS IN CHAMOMILE

Wide varieties of phytochemicals are reported from chamomile flowers, and are mostly used in cosmetics or in medicinal preparations [25]. Chamomile contains 0.24–1.9% volatile oil, which turns dark yellow after storage. Approximately 120 secondary metabolites are reported, including 28 terpenoids and 36 flavonoids [22,26]. The major components of the essential oil extracted from German chamomile flowers are the terpenoids α-bisabolol and its oxide azulenes, including chamazulene and acetylene derivatives. Chamazulene and bisabolol are very unstable and are best preserved in an alcoholic tincture. Roman chamomile contains less chamazulene than German chamomile, and is mainly constituted from esters of angelic acid and tiglic acid. It also contains farnesene, α-pinene, and sesquiterpene lactones of the germacranolide type, mainly nobilin and 3-epinobilin. α-Bisabolol, bisabolol oxides A and B and chamazulene or azulenesse, farnesene and spiroether quiterpene lactones, glycosides, hydroxycoumarins, flavanoids (apigenin, luteolin, patuletin, and quercetin), coumarins (herniarin and umbelliferone), terpenoids, and mucilage are considered to be the major bioactive ingredients [27,28]. Apigenin and its glycosides are the most promising compounds having important biological activities. Free apigenin is present in 4–6% only, with the rest being present in its glycoside form [29–31]. Some of the flavonoids identified in *Matricaria chamomilla*, such as apigenin, luteolin, and apiin, are also present in Roman chamomile, as are phenolic carboxylic acids (caffeic, ferulic), coumarins, and thiophene derivatives.

18.4 USE OF CHAMOMILE BASED ON TRADITIONAL PRACTICE

Traditionally, various preparations of chamomile are known to be beneficial against 123 different human ailments, including abdominal bloating, abrasions, abscesses, acne, anorexia, anxiety, arthritis, asthma, back pain, bedsores, bladder disorders, bruises, burns, canker sores, carpal tunnel syndrome, catarrh, chicken pox, constipation, contact dermatitis, convulsions, cough, Crohn's disease, croup, cystitis, delirium tremens (DTs), diaper rash, diverticulitis, dry skin, dysmenorrhea (painful menstruation), ear infections, eye disorders (blocked tear ducts), eye infections, fatty liver, fever, flu-like symptoms, frostbite, gallstones, gingivitis, glomerulonephritis, gout, gum irritation, hay fever, headaches, heartburn, heat rash, hemorrhoids, hepatic disorders, hives, hypoglycemia (low blood sugar), hysteria, impetigo, inflammatory conditions, insect bites, insomnia, intestinal cramps, irregular menstrual cycles, irritable bowel syndrome, kidney disorders, leg ulcers, liver disorders, low back pain, malaria, mastitis (breast inflammation), menopause, menstrual cramps, menstrual disorders, morning sickness, morphine withdrawal, motion sickness, muscle strength, nasal inflammation, nausea, nervous stomach, neuralgia (nerve pain), nightmares, oral hygiene (mouthwash), osteoporosis, parasites/worms, peptic ulcers, perineal trauma, poison ivy, psoriasis, post-natal depression rash (heat), respiratory inflammation, restlessness, rheumatism, Roehmheld's syndrome, sciatica, sea sickness, seizure disorders, sinusitis, stomach cramps, sunburn, sunstroke, teething pain (mouth rinse), tension, tics, toothache, travel sickness, tuberculosis, ulcerative colitis, ulcers, uterine disorders, vaginal infections, viral infection (flu-like symptoms), viral infection (polio), vomiting, and vomiting/nausea during pregnancy. They also possess abortifacient, antibacterial, anticoagulant, antifungal, anti-inflammatory, antioxidant, antipruritic, antispasmodic, antiseptic, aromatic, blood purification, diaphoretic, diuretic (increasing urination), fistula healing, uterine stimulant, and uterine tonic properties [32–40 and references therein]. Preparation of chamomile as used in its traditional form is described in Table 18.1 [41–51].

18.5 USE OF CHAMOMILE BASED ON SCIENTIFIC EVIDENCE

18.5.1 Allergy

Allergic manifestations are common in old age. A methanolic extract of chamomile exhibited an inhibitory effect on anaphylaxis induced by compound 48/80. It has been found to be effective in controlling pruritis

TABLE 18.1 Traditional Chamomile Preparations and Their Common Uses

Preparation	Details/use/indications	References
Aqueous extracts/tea	Aqueous chamomile extract with a specific concentration (w/v) is referred to as tea. Chamomile tea is good for relaxation and general health complications.	[41]
Aromatherapy and massage oil	Chamomile essential oil derived from flowers is applied to relieve anxiety, general depression and depression associated with menopause, irritability, insomnia, hysteria, and hypersensitivity. To prepare massage oil, 85 g of dried flowers are mixed with 700 ml of olive or sweet almond or other suitable vegetable oil, placed in a glass jar, and kept in the sun for 2 weeks or more. This oil can be used for body massage, facials, masks, compresses, body wraps, baths, and hair care.	[42,43]
Bath additive	50 g dried flowers are added per 10 liters of water. The water is used for a bath, and is recommended for soothing ano-genital inflammation. Use of soap during the bath is not advisable, as it will coat the skin and not allow the chamomile to penetrate.	[44]
Cosmetic preparations	Roman chamomile is used widely in cosmetic preparations. It has a soothing and softening effect on the skin, and has been used for hair preparations (shampoos and rinses) to lighten and condition. Water-soluble fractions of the essential oil dissolved in the condensed steam from the distillation process are utilized in cosmetic preparations that involve a water base, such as soaps, shampoos, and creams. This is known as "floral water" or "distillation water" of chamomile.	[45–47]
Dry flower powder	Powder is prepared by grinding dry flowers; 2–4 g powder is recommended for use three times, for general well-being.	[48,49]
Gargle	3–10 g of dry chamomile flowers are added to 100 ml boiling water and steeped, covered, for 5–10 minutes. The tea infusion is used as a wash or gargle for inflammation of the mucous membranes of the mouth and throat; an alternative mixture is a 5 ml tincture poured into 100 ml warm water and gargled three or more times daily.	[45]
Herbal beers and lotion	The whole chamomile plant is used mainly for making herbal beer, and also for lotion. It is beneficial for external application in toothache, earache, neuralgia, etc. Approximately 28–30 g of the dried herb is infused in 500 ml of boiling water and allowed to cool. The herb has also been employed in hot fomentations in cases of local and intestinal inflammation	[44]
Homeopathic medicine	Chamomile has applications in the homeopathic system of medicine. It is provided in conditions such as acidity, anger, asthma (with a psychological basis), colic, congestion, convulsions, cramp, diarrhea, dysmenorrhea, dyspepsia, earache, fainting, fever, flatulence, gout, headache, hernia, hysteria, influenza, irritability, jaundice, mastitis, miscarriage, mumps, neuralgia, night sweats, numbness, over-sensitivity due to abuse of coffee and narcotics, pain, peritonitis, pregnancy problems, rheumatism, sciatica, teething, ulcers, whooping cough, etc.	[50]
Inhalation	100 ml boiling water are poured over 3–10 g dried flowers and left to steep, covered, for 5–10 minutes, or 15 ml tincture is poured into 0.5 l boiled water, 1–3 times daily. Steam vapor is inhaled for inflammation of the upper respiratory tract.	[46–48]
Oral infusion	150 ml of boiling water is poured over approximately 3 g dried flowers and steeped, covered, for 5–10 minutes. However, the official Swiss tea infusion dose for the same indication is 900 mg of dried chamomile flowers.	[51]
Poultice	10 parts chamomile flowers and 5 poppy capsules are added to 100 parts of distilled water. Bags may be loosely stuffed with flowers and steeped well in boiling water before being applied as a foment. The antiseptic power of chamomile is stated to be 120 times stronger than that of sea water.	[44]
Standardized extract	A standardized chamomile extract has specific and measurable levels of ingredients or active constituents which make the product beneficial for health promotion. Standardized extracts are available over the counter claiming a level of 1.2% apigenin.	[45–48]
Tincture	A chamomile tincture is prepared by adding one part of chamomile dry flowers to five parts of 45% ethanol (w/v). The tincture may also be prepared as one part chamomile flowers in four parts of water with 12% grain alcohol. The tincture is advised for summer diarrhea. Chamomile is also used with purgatives to prevent cramping. The suggested dose of this preparation is 6–12 drops from 1 to 3 times a day. The extract, in doses of 10–15 grains, is combined with myrrh and preparations of iron. It can also be used as a tonic.	[44,45]
Vapor bath	A vapor bath is used to treat lower abdominal problems, including menstrual cramps and various irritations of the anus and genitals. A hot chamomile infusion prepared from one cup of dry flowers and 1 quart water (where 1 US quart = 946.35 ml) is used to create a vapor bath in a shallow basin. The patient then sits over the steam, wrapped with a blanket to create a tent. Strict precautions are required to avoid burns. Alternatively, a cloth or towel soaked with the hot preparation can be applied to the affected area.	[48,49]
Wine	Chamomile wine is recommended for relieving an upset stomach and is prepared by adding one handful of dried flowers to one bottle of white wine and allowing it to steep for 7–10 days. After filtration, it is stored; a 1-tablespoonful dose is administered for upset stomach.	[48,49]

by inhibiting mast cell degranulation [50]. Excessive inflammation is an important factor in the pathogenesis of common diseases of the elderly, such as rheumatoid arthritis, atherosclerosis, and diabetes. In a study, aqueous chamomile extract and isolated polyphenolic compounds (namely, apigenin, quercetin, and salicylic acid) were incubated with THP1 macrophages, and levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) were measured. At concentrations of 10 μM, both apigenin and quercetin significantly reduced IL-6 levels ($P < 0.05$). Apigenin at 10 μM and quercetin at 25 μM significantly reduced TNF-α levels ($P < 0.05$). These findings endorse the anti-inflammatory activity of chamomile [52]. In a human study, a herbal beverage composed of chamomile, meadowsweet, and willow bark (CMW) was developed and tested for its anti-inflammatory effect in a cohort of healthy adults ($n = 20$) during a 4-week intervention. Subjects were randomized to either the treatment or the placebo group. The three herbs under study were delivered in a berry extract matrix used as a control in the experiment. The objective of the study was to assess the herbs' effects on systemic inflammation and joint function by examining circulating cytokines and mechanical joint flexibility. Blood serum was analyzed for cytokines IL-1β, IL-6, and TNF-α. There was an average decrease of 21.7% IL-1β in the treatment group, whereas the decrease observed in the placebo group was 3%. Quartile analysis based on baseline production of TNF-α demonstrated a decrease in the treatment group's IL-6 levels. This group showed improvements in mechanical joint function, and reduced pain upon movement of joints specific to the knee and lower back [51].

18.5.2 Cancer

Most evaluations of tumor growth inhibition by chamomile involve studies on apigenin, one of the bioactive constituents present in the extract. Studies on preclinical models of skin, prostate, breast, and ovarian cancer have shown promising growth-inhibitory effects [53–59]. In a recently conducted study, chamomile extracts were shown to cause minimal growth-inhibitory effects on normal cells, but significant reductions in cell viability in various human cancer cell lines. Chamomile exposure induced apoptosis in cancer cells but not in normal cells at similar doses [60]. In another study, the anticancer effect of apigenin on *Helicobacter pylori*-induced atrophic gastritis and gastric cancer progression in Mongolian gerbils was assessed. Mongolian gerbils aged 5–8 weeks were inoculated with *Helicobacter pylori* for 4 weeks without (atrophic gastritis group) or with (gastric cancer group) N′-methyl-N′-nitro-N-nitrosoguanidine (MNNG) in their drinking water, and were then rested for 2 weeks. During the 7th-32nd (atrophic gastritis group) or the 7th-52nd (gastric cancer group) weeks, the animals were provided with various doses (0–60 mg/kg body weight per day) of apigenin. At the end of the 32nd (atrophic gastritis group) or the 52nd (atrophic gastritis group) week, histological changes of *Helicobacter pylori* colonization, neutrophil and monocyte infiltrations, and atrophic gastritis in both atrophic gastritis and gastric cancer Mongolian gerbils were examined. Apigenin treatments (30–60 mg/kg body weight per day) effectively decreased atrophic gastritis (atrophic gastritis group) and dysplasia/gastric cancer (gastric cancer group) rates in Mongolian gerbils. Apigenin treatment (60 mg/kg body weight per day) significantly decreased *Helicobacter pylori* colonization and *Helicobacter pylori*-induced histological changes of neutrophil and monocyte infiltrations and atrophic gastritis in both atrophic gastritis and gastric cancer [61]. Further investigations through well-designed clinical studies of chamomile are warranted in evaluating the potential usefulness of this herbal remedy in the management of cancer patients.

18.5.3 Common Cold

The common cold (acute viral nasopharyngitis) is the most common human disease. It is a mild viral infectious disease of the upper respiratory system. The common cold usually lasts not more than 3–5 days, with residual coughing and catarrh lasting approximately 3 weeks. Typically the common cold is not life-threatening, although its complications (such as pneumonia) can lead to death if not properly treated [62]. Studies indicate that inhalation of steam with chamomile extract has been helpful in common cold symptoms; however, further research is needed to confirm these findings.

18.5.4 Cardiovascular Conditions

It has been suggested that regular use of flavonoids consumed in food may reduce the risk of death from coronary heart disease in elderly men. A study assessed the flavonoid intake of 805 men aged 65–84 years who were followed up for 5 years [63]. Flavonoid intake (analyzed in tertiles) was significantly inversely associated with mortality from coronary heart disease (P for trend = 0.015) and showed an inverse relation with incidence of myocardial infarction, which was of borderline significance (P for trend = 0.08). The relative risk of coronary heart disease mortality in the highest versus the lowest tertile of flavonoid intake was 0.42 (95% CI 0.20–0.88), suggesting that flavonoid intake is inversely related to coronary heart disease mortality. In another study, on 12 patients with cardiac disease who underwent cardiac catheterization, hemodynamic measurements obtained prior to and 30 minutes after the

oral ingestion of chamomile tea exhibited a small but significant increase in the mean brachial artery pressure [64]. No other significant hemodynamic changes were observed after chamomile consumption. Of the 12 patients, 10 fell into a deep sleep shortly after drinking the beverage. A large, well-designed, randomized controlled trial is needed to assess the potential value of chamomile in improving cardiac health.

18.5.5 Colic/Diarrhea

Two clinical trials have evaluated the efficacy of chamomile for the treatment of colic in children. Chamomile tea was combined with other herbs (vervain, licorice, fennel, balm mint) for administration. In a prospective randomized, double-blind, placebo-controlled study, 68 healthy term infants aged 2–8 weeks who had colic received either herbal tea or placebo (glucose, flavoring). Each infant was offered treatment with every bout of colic, of up to 150 ml/dose, no more than three times a day. After 7 days of treatment, parents reported that the tea eliminated colic in 57% of the infants, whereas the placebo was helpful in only 26% ($P < 0.01$). No adverse effects with regard to the number of night-time awakenings were noted in either group [65]. In another randomized, double-blind, placebo-controlled trial, a standardized herbal preparation containing chamomile (71.1 mg/kg per day), fennel (65.7 mg/kg per day), and balmmint (38.8 mg/kg per day) was compared with a placebo in the management of colic in infants. Daily administration of the herbal preparation for 1 week reduced crying time among breastfed infants ($n = 41$) compared with a placebo ($n = 47$, $P < 0.005$). Significant results were attained within 4 days of treatment, and no side effects were observed with this dosage. Another study examined the effects of a chamomile extract and apple pectin preparation in 79 children (age 0.5–5.5 years) with acute, non-complicated diarrhea. The children received either the chamomile/pectin preparation ($n = 39$) or placebo ($n = 40$) for 3 days. Diarrhea ended sooner in children treated with chamomile and pectin (85%) than in the placebo group (58%). The duration of the diarrhea was also significantly shortened by administration of the chamomile and pectin treatment. These results provide evidence that chamomile can be used safely to treat infant colic disorders [66].

18.5.6 Diabetes

The effects of chamomile hot water extract and its major components on the prevention of hyperglycemia and the protection against or improvement of diabetic complications in diabetes mellitus were investigated. Hot water extract, esculetin, and quercetin have been found to exhibit moderate inhibition of sucrose, with IC50 values of 0.9 mg/ml and 72 μM, respectively. In a sucrose-loading test, administration of esculetin (50 mg/kg body weight) fully suppressed hyperglycemia after 15 and 30 minutes, but the extract (500 mg/kg body weight) and quercetin (50 mg/kg body weight) were less effective. On the other hand, a long-term feed test (21 days) using a streptozotocin-induced rat diabetes model revealed that the same doses of extract and quercetin showed significant suppression of blood glucose levels. It was also found that these extracts increased liver glycogen levels. Moreover, chamomile extract showed potent inhibition against aldose reductase, with an IC50 value of 16.9 μg/ml, and its components (umbelliferone, esculetin, luteolin, and quercetin) significantly inhibited accumulation of sorbitol in human erythrocytes. These results clearly suggest that daily consumption of chamomile tea with meals could contribute to the prevention of the progress of hyperglycemia and diabetic complications [67].

18.5.7 Dermatitis

Extracts and decoctions made from chamomile are often recommended for treatment of a number of skin diseases and conditions, such as inflammation, wounds, and itching. A systematic review explored the evidence base of the dermatological effects of chamomile. While numerous beneficial effects of chamomile have been suggested, no studies have so far been able to substantiate these claims. The absence of evidence is primarily caused by the design and quality of the studies identified [68]. Peristomal skin complications interfere with stoma appliance use and negatively affect patient quality of life. A study compared the effect of a German chamomile solution to topical steroids on peristomal skin lesions in colostomy patients; persons seeking care for the treatment of a peristomal skin lesion were assigned to a treatment regimen of once-a-day hydrocortisone 1% ointment ($m = 36$) or a twice-a-day chamomile compress ($n = 36$) application. Treatments were assigned by matching patient demographic, history, and skin condition variables. Lesions were assessed every 3 days for a maximum of 28 days. Lesions healed significantly faster in the chamomile than in the hydrocortisone group (mean time to healing 8.89 ± 4.89 and 14.53 ± 7.6 days, respectively; $P = 0.001$). Stoma patient symptoms (pain and itching) also resolved more expediently in the chamomile than in the hydrocortisone group. Because corticosteroids are non-specific anti-inflammatory agents, herbal extract use can prevent the side effects of long-term topical corticosteroid use. The results of this study suggest that German chamomile can be recommended to relieve itching and inflammation, and that twice-daily application facilitates healing of peristomal skin lesions [69].

18.5.8 Eczema

The use of chamomile by topical application for diseases of the skin has been authorized by the German Commission E. Topical applications of chamomile have been shown to be moderately effective in the treatment of atopic eczema [70]. Chamomile was found to be about 60% as effective as 0.25% hydrocortisone cream [71]. Roman chamomile of the Manzana type (Kamillosan®) may ease discomfort associated with eczema when applied as a cream containing chamomile extract. The Manzana type of chamomile is rich in active ingredients, and does not exhibit chamomile-related allergenic potential [72]. In a partially double-blind, randomized study performed as a half-side comparison, Kamillosan® cream was compared with 0.5% hydrocortisone cream, and a placebo consisting only of vehicle cream, in patients suffering from medium-degree atopic eczema [73]. After 2 weeks of treatment, Kamillosan® cream showed a slight superiority over 0.5% hydrocortisone and a marginal difference as compared to placebo. Further research is needed to evaluate the usefulness of topical chamomile in managing eczema.

18.5.9 Fungal Infections

Fungal infections are very common in old age, and often seen because of deprived immunity and the dying skin cells in various parts of the body providing a good substrate for growth. Antifungal activity of *Matricaria chamomilla* flower essential oil was evaluated against *Aspergillus niger*, with the emphasis on the plant's mode of action, by electron microscopy. In total, 21 compounds were identified in the plant oil using gas chromatography/mass spectrometry, accounting for 93% of the oil composition. The main compounds identified were α-bisabolol (57%), trans-trans-farnesol (16%), cis-beta-farnesene (7%), guaiazulene (4%), α-cubebene (3%), α-bisabolol oxide A (2%), and chamazulene (2%). In the bioassay, *Aspergillus niger* was cultured on potato dextrose broth medium in six-well microplates in the presence of serial two-fold concentrations of plant oil (15.62–1000 μg/ml) for 96 hours at 28°C. Based on the results obtained, *Aspergillus niger* growth was inhibited dose-dependently, with a maximum of approximately 93% at the highest oil concentration. A marked retardation in conidial production by the fungus was noticed in relation to the inhibition of hyphal growth. The major changes observed by transmission electron microscopy were: disruption of cytoplasmic membranes and intracellular organelles; detachment of plasma membrane from the cell wall; cytoplasm depletion; and complete disorganization of hyphal compartments. In scanning electron microscopy, swelling and deformation of hyphal tips, formation of short branches, and collapse of entire hyphae were noted. These findings indicate the potential of *Matricaria chamomilla* L. essential oil in preventing fungal contamination and subsequent deterioration of stored food and other susceptible materials [74].

18.5.10 Gastrointestinal Conditions

Chamomile is used traditionally for numerous gastrointestinal conditions, including digestive disorders, spasm or colic, upset stomach, flatulence (gas), ulcers, and gastrointestinal irritation. Chamomile is especially helpful in dispelling gas, soothing the stomach, and relaxing the muscles that move food through the intestines. Chamomile tea is often recommended to treat nausea and vomiting, in the form of one to two teaspoons of dried or fresh chamomile leaves steeped in one cup of hot water for 5–10 minutes, sweetened as needed with honey, and consumed in the morning and after dinner. The protective effect of a commercial preparation (STW 5, Iberogast®), containing the extracts of bitter candy tuft, lemon balm leaf, chamomile flower, caraway fruit, peppermint leaf, liquorice root, angelica root, milk thistle fruit, and greater celandine herb, against the development of gastric ulcers has been reported [75]. STW 5 extracts produced a dose-dependent anti-ulcerogenic effect associated with a reduced acid output, an increase in mucin secretion, an increase in prostaglandin E(2) release, and a decrease in leukotrienes. The results obtained demonstrated that STW 5 not only lowered gastric acidity as effectively as a commercial antacid, but was also more effective in inhibiting secondary hyperacidity [76].

18.5.11 Hemorrhoids and Hemorrhagic Cystitis

It has been reported that the combination of chamomile baths, chamomile bladder washes, and antibiotics is superior to antibiotics alone for hemorrhagic cystitis. Anti-inflammatory agents that have been reported to be useful for the treatment of cystitis include chamomile, ginger, marshmallow, and echinacea. Treatment is taken in the form of hot chamomile compresses applied over the bladder, or a chamomile hip or sitz bath [77]. Tincture of chamomile has also been used to improve hemorrhoids. Tincture of Roman chamomile may reduce inflammation associated with hemorrhoids [78].

18.5.12 Health Promotion

It has been claimed that consumption of chamomile tea boosts the immune system and helps fight infections associated with the common cold. The health promoting benefits of chamomile were assessed in a study which involved 14 volunteers who each drank five cups of the herbal tea daily for 2 consecutive weeks. Daily urine samples were taken and tested throughout the study,

both before and after drinking chamomile tea. Drinking chamomile was associated with a significant increase in urinary levels of hippurate and glycine, which have been linked with increased antibacterial activity. Levels of both hippurate and glycine remained elevated for up to 2 weeks after the study participants stopped drinking the tea, indicating that the compounds may remain active for quite some time [79]. Additional studies are needed before a more definitive link between chamomile and its alleged health benefits can be established.

18.5.13 Inflammatory Conditions

Inflammation is associated with many gastrointestinal disorders, such as esophageal reflux, diverticular disease, and inflammatory bowel disease. Studies in preclinical models suggest that chamomile inhibits *Helicobacter pylori*, the bacteria that can contribute to stomach ulcers [80]. However, few studies in humans have evaluated chamomile's effects, and there is no evidence that it speeds the healing of gastric ulcers. Chamomile is believed to be helpful in reducing smooth muscle spasms associated with various gastrointestinal inflammatory disorders. Chamomile is often used to treat mild skin irritations, including sunburn, rashes, sores, and even oral and eye inflammations [81–83], but its value in treating these conditions has not been shown with evidence-based research.

18.5.14 Mucositis

Mouth ulcers are a common condition with a variety of etiologies. Stomatitis is a major dose-limiting toxicity in bolus 5-fluorouracil-based (5-FU) chemotherapy regimens. A double-blind, placebo-controlled clinical trial including 164 patients was conducted [84]. Patients were entered into the study at the time of their first cycle of 5-FU-based chemotherapy. All patients received oral cryotherapy for 30 minutes with each dose of 5-FU. In addition, each patient was randomized to receive a chamomile or placebo mouthwash three times daily for 14 days. There was no suggestion of any stomatitis difference between patients randomized to either protocol arm. There was also no suggestion of toxicity. Subset analysis did reveal unsuspected differential effects between males and females that could not be explained by reasons other than chance. This suggested that a chamomile mouthwash might ameliorate this toxicity, and a prospective trial was developed to test chamomile in this situation. Data obtained from this clinical trial did not support the pre-study hypothesis that chamomile could decrease 5-FU-induced stomatitis. The results of other similar studies are conflicting, and it remains unclear whether chamomile is helpful in this situation.

18.5.15 Microbial Infections

Antibacterial properties of chamomile essential oil and its ingredients like α-terpineol and β-pinene were determined using a microdilution method against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) bacteria. Furthermore, their antibacterial activity was tested against 10 bacterial species. Hexadecanoic acid (15%) was recognized as the main constituent, together with tau-cadinol (7%), in both aerial parts and roots of *Anthemis*. The oils from the aerial parts showed good activity against Gram-positive bacteria. These results suggest that the plants could potentially be used in food manufacture and cosmetology as preservative agents, or in medicine as new antibiotics [85–87].

18.5.16 Osteoporosis

Osteoporosis is a metabolic bone disease resulting from low bone mass (osteopenia) due to excessive bone resorption. Sufferers are prone to bone fractures from relatively minor trauma. Agents which include selective estrogen receptor modulators (SERMs), biphosphonates, or calcitonin are frequently used to prevent bone loss. To prevent bone loss that occurs with increasing age, chamomile extract was evaluated for its ability to stimulate the differentiation and mineralization of osteoblastic cells. Chamomile extract was shown to stimulate osteoblastic cell differentiation and to exhibit an anti-estrogenic effect, suggesting an estrogen receptor-related mechanism [88]. However, further studies are needed before it can be considered for clinical use.

18.5.17 Parkinson's Disease

In Parkinson's disease, dopamine receptor agonists provide a viable alternative to levodopa therapy that is associated with fewer motor complications and dyskinesia; however, its long-term use causes profound adverse effects in some patients. These adverse effects include amplification of non-motor symptoms already experienced by Parkinson's disease patients. Nausea from dopamine agonists generally lessens with time and may be responsive to both antiemetic therapy and complementary remedies such as ginger, peppermint, and chamomile [89]. Unfortunately, compulsive behaviors, as well as peripheral edema caused by dopamine agonists, are poorly responsive to pharmacological therapy and require a reduction or discontinuation of agonist therapy.

18.5.18 Sleep Aid/Sedation

Traditionally, chamomile preparations such as tea and essential oil aromatherapy have been used to treat

insomnia and to induce sedation (calming effects). A study was conducted on a randomly selected sample of adults ($n=997$; 60.9% women) from the province of Quebec, where a total of 18.5% of participants reported having used natural products as sleep aids in the past 12 months, with chamomile being the most popular product. Participants who exclusively used natural products as sleep aids (10.3% of the sample) were predominantly females, younger, and had a higher educational level than those using prescribed medications. Natural products users reported engaging in more health-promoting behaviors than the non-users of sleep aids and, despite the presence of sub-threshold insomnia symptoms (mean Insomnia Severity Index score = 9.33), tended to perceive themselves as healthier when compared with prescribed medication users and non-users of sleep aids [90]. In another human trial it was observed that chamomile could provide modest benefits in daytime functioning and mixed benefits on sleep diary measures, relative to placebo, in adults with chronic primary insomnia [91]. However, further studies in selected insomnia patients would be needed to investigate these conclusions.

Chamomile is widely regarded as a mild tranquilizer and sleep-inducer. Sedative effects may be due to the flavonoid apigenin, which binds to benzodiazepine (BDZ) receptors in the brain. Studies in preclinical models have shown anticonvulsant and CNS-depressant effects [92]. Clinical trials are notable for their absence, although 10 cardiac patients are reported to have immediately fallen into a deep sleep lasting for 90 minutes after drinking chamomile tea [93]. Although randomized, placebo-controlled studies have been conducted to evaluate a few compounds, rigorous scientific data supporting a beneficial effect have not been found for the majority of available herbal or dietary supplements. Studies are limited by small numbers of participants and, in some instances, inadequate design, lack of statistical analysis, and sparse use of objective measurements. Few or no scientific data are available to assess the efficacy of most products as hypnotics. Investigations on the hypnotic activities of chamomile and passiflora extracts using the sleep-disturbed preclinical model demonstrated a significant decrease in sleep latency observed with chamomile extract at a dose of 300 mg/kg, while passiflora extract showed no effects on sleep latency even at a dose of 3000 mg/kg. No significant effects were observed with either of the herbal extracts on total times of wakefulness, non-rapid eye movement (non-REM) sleep, and REM sleep [94]. Flumazenil, a benzodiazepine receptor antagonist, at a dose of 3 mg/kg showed a significant antagonistic effect on the shortening in sleep latency induced by chamomile extract. No significant effects were observed with chamomile and passiflora extracts on delta activity during non-REM sleep. In conclusion, chamomile extracts exhibit benzodiazepine-like hypnotic activity [95]. In another study, inhalation of the vapor of chamomile oil reduced a stress-induced increase in plasma adrenocorticotropic hormone (ACTH) levels. Diazepam, co-administered with the chamomile oil vapor, further reduced ACTH levels, while flumazenile, a BDZ antagonist, blocked the effect of chamomile oil vapor on ACTH. According to Paladini *et al*. [96], the separation index (ratio between the maximal anxiolytic dose and the minimal sedative dose) for diazepam is 3 while for apigenin it is 10. Compounds other than apigenin present in extracts of chamomile can also bind BDZ and gamma-aminobutyric acid (GABA) receptors in the brain, and are thought to be responsible for some of the sedative effect; however, many of these compounds are as yet unidentified.

18.5.19 Sore Throat

The efficacy of lubrication of the endotracheal tube cuff with chamomile before intubation on postoperative sore throat and hoarseness was determined in a randomized double-blind study [97]. The study involved 161 patients whose American Society of Anesthesiologists physical status was I or II and who were undergoing elective surgical, orthopedic, gynecological, or urological surgeries. The patients were divided in two groups: the study group received 10 puffs of chamomile extract (Kamillosan® M spray, total 370 mg of chamomile extract) at the site of the cuff of the endotracheal tube for lubrication, while the control group did not receive any lubrication before intubations. Standard general anesthesia with tracheal intubations was given in both groups. Of the 81 patients in the chamomile group, 41 (50.6%) reported no postoperative sore throat in the post-anesthesia care unit, compared with 45 of 80 patients (56.3%) in the control group. Postoperative sore throat and hoarseness both in the post-anesthesia care unit and at 24 hours postoperation were not statistically different. Lubrication of the endotracheal tube cuff with chamomile extract spray before intubation cannot prevent postoperative sore throat and hoarseness. Similar results were obtained in another double-blind study [98].

18.5.20 Vaginitis

Vaginal inflammation is common in women of all ages. Vaginitis is associated with itching, vagional discharge, or pain with urination. Atrophic vaginitis most commonly occurs in menopausal and postmenopausal women, and its occurrence is often associated with reduced levels of estrogen. A chamomile douche may improve symptoms of vaginitis, with few side effects. Because infection (including sexually transmitted

diseases), poor hygiene, or nutritional deficiencies can cause vaginitis, medical attention should be sought by people with this condition [99]. There are insufficient research data to allow conclusions concerning the possible potential benefits of chamomile for this condition.

18.5.21 Wound Healing

The efficacy of topical use of chamomile in enhancing wound healing was evaluated in a double-blind trial on 14 patients who underwent dermabrasion of tattoos. The effects on drying and epithelialization were observed, and chamomile was judged to be statistically efficacious in producing wound-drying and in speeding epithelialization [100]. Another study evaluated wound-healing activity in animals, using excision, incision, and production of dead space wounds. The test animals were treated with an aqueous extract of *Matricaria recutita* (120 mg/kg per day) mixed in their drinking water, whereas control animals were given plain drinking water. Healing was assessed by the rate of wound contraction, period of epithelialization, wound-breaking strength, granulation tissue weight, and hydroxyproline content. Antimicrobial activity of the extract against various microorganisms was also assessed. The test group, on day 15, exhibited a greater reduction in the wound area when compared with the controls (61% versus 48%), faster epithelialization, and a significantly higher wound-breaking strength ($P < 0.002$). In addition, wet and dry granulation tissue weight and hydroxyproline content were significantly higher. The increased rate of wound contraction, together with increased wound-breaking strength, hydroxyproline content, and histological observations, support the use of *Matricaria recutita* in wound management [101]. In a linear incision-healing experiment, application of chamomile extract registered a significant healing ability [102]. Further chamomile stimulated re-epithelialization and formation of collagen fibers in a lesion [103]. However, further studies are needed before it can be considered for clinical use.

18.5.22 Quality of Life in Cancer Patients

Essential oils obtained from Roman chamomile are the basic ingredients of aromatherapy. A recently conducted research study on cancer patients has assessed the positive effect of aromatherapy/massage on psychological morbidity, distress, or quality of life [104–108]. The results suggested that the most consistent beneficial effect of massage or aromatherapy massage was on anxiety, with four of the trials (a total of 207 patients) reporting a reduction in anxiety. It is unclear whether aromatherapy, when added to massage therapy, provided additional benefit in reducing anxiety. Of three trials that examined depression in cancer patients, only one reported significant differences. Two trials reported a reduction in nausea, and three reported a reduction in pain. Clinical trials of aromatherapy in cancer patients have shown no statistically significant differences between treated and untreated patients [105]. Another pilot study investigated the effects of aromatherapy massage on anxiety and self-esteem experience in Korean elderly women. A quasi-experimental control group, pretest–post-test design comprised 36 elderly females: 16 in the experimental group and 20 in the control group. Aromatherapy massages using lavender, chamomile, rosemary, and lemon were given to the experimental group only. Each massage session lasted for 20 minutes, and was performed three times per week for two 3-week periods with an intervening 1-week break. The intervention produced significant differences in levels of anxiety and self-esteem and no significant differences in blood pressure or pulse rate, between the two groups. These results suggest that aromatherapy massage exerts positive effects on anxiety and self-esteem [106]. However, more objective clinical measures should be applied in a future study with a randomized placebo-controlled design.

18.6 ADVERSE EFFECTS, ALLERGIC REACTIONS, AND SAFETY ISSUES WITH CHAMOMILE

A relatively low percentage of people are sensitive to chamomile and develop allergic reactions. People sensitive to ragweed and chrysanthemums or other members of the Compositae family are more prone to developing contact allergies to chamomile, especially if they take other drugs that help to trigger the sensitization. A large-scale clinical trial was conducted in Hamburg, Germany, between 1985 and 1991 to study the development of contact dermatitis secondary to exposure to a mixture of components derived from the Compositae family. Twelve species of the Compositae family, including German chamomile, were selected and tested individually when the mixture induced allergic reactions. During the study, 3851 individuals were tested using a patch with the plant extract. Of these patients, 118 (3.1%) experienced an allergic reaction. Further tests revealed that feverfew elicited the most allergic reactions (70.1% of patients), followed by chrysanthemums (63.6%) and tansy (60.8%). Chamomile fell in the middle range (56.5%) [109,110]. In another study, it was shown that eye-washing with chamomile tea in hay fever patients who have conjunctivitis exacerbates the eye inflammation, whereas no worsening of eye inflammation was noted when chamomile tea was ingested orally [111]. There are rare reports of persons who experienced

an anaphylactic reaction to ingestion of chamomile tea [112,113]. All such patients suffered from hay fever, and one of them had bronchial asthma caused by a variety of pollens. One patient additionally ingested aspirin, which may have triggered the anaphylactic shock. Chamomile is listed on the Food and Drug Administration's GRAS (generally recognized as safe) list. It has been reported that chamomile can cause severe allergic reactions in persons who are allergic to ragweed; however, there are no convincing data to show that chamomile is more allergenic than other known allergenic plants [114]. It is possible that some reports of allergic reactions to chamomile may be due to contamination of chamomile by "dog chamomile," a highly allergenic and bad-tasting plant of similar appearance. It is notable that coumarin, a natural blood thinner, is present in chamomile, and it has been reported that a patient taking therapeutic doses of warfarin and also ingesting chamomile experienced excessive "blood thinning," which led to internal bleeding [115]. Evidence of cross-reactivity of chamomile with other drugs is not well documented, and further study of this issue is needed prior to reaching conclusions. Safety in young children, pregnant or nursing women, or those with liver or kidney disease has not been established, although there have not been any credible reports of toxicity caused by this common beverage tea.

18.7 CONCLUSIONS

Chamomile has been used as an herbal medication since ancient times, is still popular today, and probably will continue to be used in the future. Establishing whether or not CAM therapies are beneficial to patients will require research and generation of scientific evidence. Without such evidence, it will remain unclear whether these untested and unproven medical treatments are truly beneficial to patients. Clearly, some patients report improvements, but it remains uncertain whether the improvements can be attributed to medication effect, spontaneous disease remission, or placebo effect. Scientists play an important role in conducting studies and in helping the public understand the benefits of CAM therapies. Very few CAM therapies have undergone rigorous scientific testing. Clinicians should await reports of well-conducted scientific research, including clinical studies of various herbs that clearly demonstrate the efficacy of CAM, before recommending such treatments to their patients. Chamomile, a herb that has been familiar to practitioners and in common use for centuries in traditional herbal medicine, has recently been receiving attention from the scientific community with regard to more clearly defining its potential usefulness as a therapeutic entity for a variety of maladies.

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PART III

NUTRITIONAL APPROACHES TO THERAPY IN CLINICAL MEDICINE IN OLD AGE

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CHAPTER

19

Effects of Omega-3 on Neurodegenerative Diseases and Stroke

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19.1 OMEGA-3 PUFA AS A DIET SUPPLEMENT

This chapter focuses on use of omega-3 polyunsaturated fatty acids (PUFAs) as a supplement that has the potential to prevent neurodegenerative diseases. This section looks at their structure, function in the body, and role in the aging brain.

19.1.1 Introduction

Recent studies support the hypothesis that, apart from providing enough nutrients to meet metabolic requirements, diet has the potential to modulate several functions in the body. Concepts in nutrition regarding supplemented diets are expanding, and medical research has increasingly been regarding food constituents as a potent medical treatment. Studies currently emphasize the use of specific food types to promote well-being and better health, and reduce the risk of disease [1]. These concepts are particularly important in light of the increasing cost of health care, increased life expectancy, and the desire of older people to improve the quality of their later years. From bolstering immunity to preventing cancer, specific components of diet might one day seize a share of the pharmaceutical industry as well as reduce the cost of health worldwide by prevention of diseases with accessible food products.

Among the most promising molecules in functional food science we can highlight the PUFAa, notably the omega-3 fatty acids (ω-3 PUFAs). Research on molecular and physiological areas has led to scientific evidence demonstrating preventive and therapeutic effects in conditions such as trauma; cardiovascular, immune, cognitive, and psychiatric disorders; and neurodegenerative diseases. This chapter will emphasize the benefits of omega-3 PUFA on the brain and neurodegenerative diseases.

19.1.2 Structure of PUFAs

Two families of PUFAs exist in nature: omega-3 and omega-6 (ω-6), which are structurally classified by (1) the number of carbons present in the hydrocarbon chain (≥18 carbons); (2) position of the double bond; and (3) the proximity of the first double bond to the methyl (omega) terminal of the fatty acid acyl chain [2].

Alpha-linolenic acid (ALA) and linoleic acid (LA) constitute the precursors of the ω-3 and ω-6 PUFA families, respectively. They are considered essential fatty acids since they cannot be synthesized *de novo* by vertebrates, which lack the natural desaturase enzymes Δ-15 and Δ-12. Therefore, they are necessarily acquired through diet. ALA is present in many vegetables, grains, and oils, such as flaxseed (*Linum usitatissimun*, 22.8%), flaxseed oil (53%), chia seed (*Salvia hispanic*, 17.6%), and canola oil (*Brassica campestris*, 9.1%). LA is abundant in several oils, such as sunflower seed (*Helianthus annuus*), corn (*Zea mays*), and soybean (*Glycine max*).

After intake, approximately 60–85% of the ALA or LA content is directed toward β-oxidation by dint of significant utilization by tissues for energy (e.g., heart and muscle), or is recycled by tissue to be used as a carbon source for production of other fatty acids, amino acids, and sterols (e.g., brain and liver) [3]. The remaining percentage is converted through a series of chain

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elongation and desaturation processes originating in ω-3 and ω-6 PUFA metabolites represented by docosahexaenoic acid (DHA) and arachidonic acid (AA), respectively. These reactions are catalyzed by an enzymatic system that comprises fatty acyl-CoA synthetases as well as Δ-6 and Δ-5 desaturases and respective elongases [4] (Figure 19.1). Evidence from studies *in vivo* and *in vitro* indicate that these two fatty-acid families not only share these same enzymes, but also compete for them [5]. All of these reactions occur in the endoplasmic reticulum. However, recently it has been accepted that 24:6ω-3 PUFA and 24:5ω-6 chains are transferred to the peroxisome, where they undergo the last step of β-oxidation [6]. Both liver and brain have the enzymes necessary for these transformations, but the liver has a greater capacity to perform them.

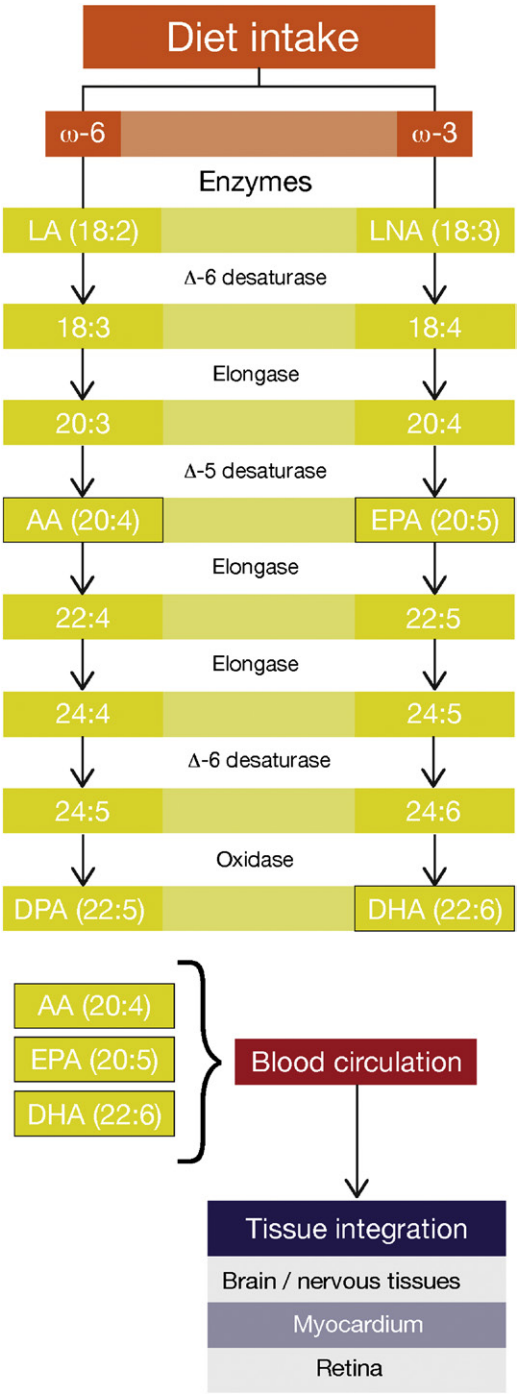

FIGURE 19.1 General overview of omega-3 and omega-6 fatty acid elongation and desaturation pathways.

This transformation process from ALA to DHA (ω-3 PUFA family) and from LA to AA (ω-6 family) has been studied in different species, including mice, hamsters, monkeys, rabbits, and guinea pigs (for review, see Brenna *et al.* [7]). However, studies demonstrate that ALA is usually rapidly converted into other ω-3 PUFAs, namely eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), with the conversion from ALA into DHA being less significant. The conversion of ALA to DHA in infants may be more efficient than in adults [8].

Although some lower-order animals have enzymes to convert ω-6 fatty acids to ω-3 PUFAs [3], mammals do not possess the enzymatic machinery necessary for this intergroup conversion [2]. This means that ω-3 PUFA molecules remain within this family of fatty acids until ultimately eliminated. The same process happens with ω-6, and this, combined with the low intake of PUFAs in the Western diet, further decreases the amount of omega-3 PUFA in the body. Fortunately, however, several studies have shown that humans who were supplemented directly with DHA rapidly incorporated it into the membranes of erythrocytes [9]. This is good news for preventive medicine because DHA and EPA can have innumerable beneficial effects on the body, as will be seen below.

19.1.3 From Intake to Function: Omega-3 PUFA and the Brain

We have covered the major sources of PUFAs among vegetables, grains, and oils above, and know that these are rich in ALA (an ω-3 PUFA) and LA (ω-6 PUFA). However, the major representatives of the ω-3 PUFA family – that is, DHA and EPA – are primarily found in organisms that live in cold water. These organisms are especially dependent on the physicochemical properties of the fatty acids. This is due to the high number of double bonds in the molecule, which ascertain that biological structures retain the fluidity necessary for life processes even at low temperatures. Significant amounts of DHA and EPA are found in fish such as salmon, mackerel, herring, and tuna. Microalgae (e.g., *Schizochytrium*) and Antarctic krill (*Euphausia superba*) also have these PUFAs, but in lesser amounts. These compounds, after various technological steps of processing, can be marketed as nutritional supplement capsules. In general, the

fish-oil capsules contain about 18% EPA and 12% DHA, which means that each triacylglycerol molecule contains one ω-3 PUFA [10].

The central nervous system (CNS) is the system with the second largest concentration of lipids, only exceeded by adipose tissue. The adult brain contains approximately 50–60% of its dry weight as lipid, and approximately 35% of the lipids are PUFAs. In the blood, fatty acids can be transported both in esterified form in triacylglycerols and in non-esterified form bound to plasma proteins such as albumin [11]. The PUFAs, particularly AA and DHA, are inserted into membrane phospholipids, and account for 21–36% of the fatty acids in the membrane composition. AA is distributed in relatively large amounts in most tissues [12]. One of the most important cascades of inflammation is known to derive from AA, resulting in formation of eisosanoids – namely, thromboxanes, prostacyclin, prostaglandins, and leukotrienes. DHA, however, is more specific in its tissue distribution. Neuronal tissues, such as the brain and retina, and a few tissues outside the CNS, namely testis, are especially high in amounts of DHA (Table 19.1) [13–19], hence its relevance to the CNS. Within the cellular membranes DHA is preferentially found in phosphatidylserine and phosphatidylethanolamine forms, whereas AA constitutes phosphatidylethanolamine and phosphatidylinositol [12].

The cell membrane composition is paramount to PUFAs' function in the body. Studies have shown that DHA can alter the basic properties of the cell membrane, including the order of the fatty acid chains, membrane fluidity, elastic compressibility, ion permeability, fusion, flip–flop movement, and protein functions. Recently, *in vitro* studies demonstrated that DHA also influences the organization of lipid raft domains [20]. It is worth emphasizing how phospholipids are essential for neuronal and synaptic structures, specifically. They play an important role in the signal transduction response to dopamine, serotonin, acetylcholine, and glutamate [1]. Therefore, supplementation of the diet with DHA can potentially protect the brain tissue against schizophrenia, depression, hyperactivity, stroke, and Alzheimer's and Parkinson's diseases via maintenance of neural membranes, correction of abnormal signal transduction processes, and restoration of neural membrane integrity.

The major mechanism associated with the release of PUFAs from neural membrane glycerophospholipids involves phospholipase A_2 (PLA_2). This group of enzymes hydrolyzes the phospholipids at the central (sn-2) acyl bond and releases the fatty acid into the cytosol [21]. Inside the cytosol, these products serve as intracellular second messengers themselves. While AA is metabolized into the potent inflammatory mediators named eicosanoids in the inflammatory cascade mentioned above, EPA can also act as substrate for both cyclooxygenase-2 (COX-2) and lipoxygenase-5 and give rise to eicosanoids, albeit with a slightly different structure from those formed from AA and 10- to 100-fold less potency [22]. Recent studies have also identified a novel group of mediators with anti-inflammatory properties again produced by COX-2. Those derived from EPA were dubbed E-series resolvins, whereas the DHA-derived mediators are named D-series resolvins, docosatrienes, and neuroprotectins. This is the rationale behind the anti-inflammatory properties of ω-3 PUFAs.

Clinical assessment and animal studies demonstrated that dietary supplementation with ω-3 PUFAs had a beneficial impact in a wide range of human diseases in

TABLE 19.1 Analysis of Fatty Acid Concentration (g/100 g total fatty acids)

	ω-6 family		ω-3 family			
Tissue	**LA**	**AA**	**ALA**	**EPA**	**DHA**	**Reference**
Adipose tissue	11.85 ± 2.86	0.25 ± 0.09	0.58 ± 0.20	0.02 ± 0.04	0.07 ± 0.06	[14]
Bone (femoral head)	8.9 ± 1.8	9.1 ± 2.5	–	<0.1	0.8 ± 0.6	[15]
Cheek	8.2 ± 2.9	2.4 ± 0.7	0.6 ± 0.7	0.27 ± 0.25	1.3 ± 0.6	[16]
Cerebral cortex	0.65 ± 0.06	8.56 ± 0.36	–	0.08 ± 0.03	12.49 ± 0.97	[13]
Erythrocyte	11.33 ± 0.41	14.90 ± 0.24	0.16 ± 0.03	0.47 ± 0.04	2.58 ± 0.21	[17]
Heart	9.1 ± 2.8	9.1 ± 3.6	0.3 ± 0.2	0.18 ± 0.10	1.5 ± 0.8	[16]
Liver	14.6 ± 4.1	12.2 ± 1.9	–	0.8 ± 1.1	2.4 ± 1.2	[15]
Muscle	24.9 ± 4.6	12.0 ± 1.8	–	0.7 ± 0.3	2.3 ± 1.2	[15]
Rectal tissue	9.53 ± 1.15	5.86 ± 0.47	0.97 ± 0.24	0.29 ± 0.04	0.97 ± 0.19	[17]
Retina	–	10.4 ± 1.0	–	–	22.3 ± 1.7	[18]
Sperm	3.2	2.5	–	0.77	13.8	[19]

which unresolved inflammation is suspected as a key component in disease pathogenesis [23]. Several possible mechanisms to explain ω-3 PUFAs' benefits on inflammation are widely discussed, including: (1) via substrate competition, preventing conversion of AA into pro-inflammatory eicosanoids; (2) by serving as an alternative substrate to produce less potent 5-series leukotrienes and 3-series prostaglandins and thromboxanes; and (3) by facilitating the resolution phase with the formation of by-products such as resolvins and neuroprotectins [24].

Advanced studies performed in different animal models are useful in discovering or deciphering other mechanisms through which PUFAs induce their effects in the brain. Another proposed mechanism for the neuroprotection action of DHA is antioxidative activity. There is evidence that DHA increases glutathione reductase activity, thus decreasing the accumulation of oxidized proteins and levels of lipid peroxide and reactive oxygen species (ROS) [25]. Additionally, DHA is hydrolyzed to plasmalogens by plasmalogen-selective phospholipase A_2. Plasmalogens have antioxidant properties, and deficits in DHA-containing plasmalogens may not only affect the integrity of neural membranes but also alter the activities of metabolic reactions such as superoxide dismutase (SOD) and nitric oxide synthase (NOS) [26].

Finally, another type of mechanism through which ω-3 PUFAs may alter cellular signaling is by acting directly as ligands at nuclear receptors, including peroxisome proliferator-activated receptors (PPARs) or retinoid X receptor. These nuclear transcription factors bind lipid ligands to regulate gene expression, thereby mediating biological functions ranging from lipid metabolism and homeostasis to cell differentiation and cell death [27].

In summary, the four chief putative mechanisms through which ω-3 PUFAs exert their beneficial effects on the central nervous system are: (1) altering membrane properties, such as fluidity and protein function; (2) anti-inflammatory effects via competition with AA in the eicosanoid cascade; (3) antioxidative activity via glutathione reductase; and (4) direct binding to nuclear receptors, with consequent regulation of gene expression.

19.2 THE AGING BRAIN AND ITS RELATION TO OMEGA-3 PUFA

The way we conceptualize aging is on the verge of important changes. Researchers at present have not fathomed whether the process of aging is a straightforward phenomenon of passage of time, or whether it reflects pathological conditions that could be delayed. Probably there is a combination of various situations, including a normal "pure" aging process, pathological neurodegeneration, and a susceptibility of certain brain tissues to react to a variety of insults, the latter of which would result in the pattern of cerebral aging that we know [28]. The medial temporal and the frontostriatal systems are commonly affected by the two processes, which leads to the theory that these two systems are in fact more prone to injury than other circuits in the brain. The hippocampus is exceedingly sensitive to stress, aging, and neurodegeneration [29,30].

The expression "brain aging" means the inherent and inevitable processes that change neuronal structure and impair brain function, whereas the term "neurodegeneration" is used when referring to pathological processes that, despite coinciding with an aged brain, have an outside cause. Naturally, there is an overlap between the two definitions, and so far it has not been possible to separate them completely. This dichotomy has led to the concept of "normal aging" – that is, aging when disease is excluded.

The modern Western diet in the past 100 years has changed significantly with the decreased availability of ω-3 PUFA [31,32]. This ω-3 PUFA deficiency is worsened during aging because the aging brain seems to lose DHA, which is the most abundant ω-3 PUFA in the brain. There is evidence that links deficiency in dietary ω-3 PUFA with the emergence of neurological disorders [33,34]. Additionally, the total lipids in the brain increase during the first 20 years of life and then suffer a moderate decline, but from 80 years onwards there is a rapid decline [35,36]. It is important to note that the enzymes involved in DHA formation are also decreased in elderly people, causing deficits in neuronal membranes and reinforcing the fact that these fatty acids have to be provided from the diet [37,38].

Brain aging features brain atrophy (due to decreased volume occupied by neurons, particularly evident in cortical layer III), alteration in dendrites, decrease in the number of synapses, and amyloid deposition [39]. It also has marked alteration in the function of cholinergic receptors and a decrease in dopaminergic neurons. However, there is a myriad of other mechanisms involved in the regulation of cerebral function. An increased concentration of DHA in the brain optimizes synaptic transmission and aids in the maintenance of synaptic homeostasis, affecting several of the pathways involved in aging. Major pathways comprise response to stress, neurotransmission, neuroprotection, neurogenesis, and anti-inflammation processes. For this reason, it is thought that ω-3 PUFAs could provide beneficial effects on the process of aging [35].

Regarding stress and aging, the theory known as the glucocorticoid hypothesis of stress and aging [29,30,40,41] claims that cellular changes in the hippocampus as well as the behavioral alterations that occur during aging might be exacerbated by stressful events during life. Prolonged exposure to stress could lead to

inflammation, increased risk of age-related brain disorders [42,43], and worsening of age-related cognitive deficits [44–46]. Taking these into account, it goes without saying that a combination of stress and ω-3 PUFA deficit could render the CNS even more vulnerable to cell aging. There is evidence suggesting that ω-3 PUFAs are involved in individual sensitivity and response to stress [47]. Moreover, cross-sectional studies have shown inverse relationships between the risk of psychological distress and ω-3 PUFA concentration in the plasma of Cree Indian [48] and Canadian Inuit populations [49].

Concerning neurotransmission, deregulation of the glutamatergic synapse in the hippocampus and prefrontal cortex prompts age-related decline of cognition, as suggested by some studies [50,51]. Aging impairs glutamate in three steps: presynaptic release, receptor binding, and elimination of it by astroglial transporters [35]. Furthermore, age has effects on both LTP (long-term potentiation) and LTD (long-term depression) [52–56], which are defined as a persistent increase or decrease in the strength of glutamate neurotransmission, respectively. These are regarded as the major cellular mechanism underlying learning and memory in the hippocampus [57,58]. ω-3 PUFAs have a favorable influence on the function of glutamatergic synapses, and also act on storage of the neurotransmitter, the dynamics of its release [59,60], expression of receptors and transporters, presynaptic exocytosis, and some components contributing to synaptic plasticity [61]. Other studies have linked the antioxidant action of EPA with restoration of LTP and memory in old rats by reducing the age-related activation of microglia and the associated increase in interleukin (IL)-1β in the hippocampus [62–64].

In terms of neuroprotection, astrogliosis or astroglial hypertrophy is deemed a hallmark of brain aging, and many studies have shown increased glial fibrillary acidic protein (GFAP, a marker specific to astroglial cells) expression or immunoreactivity in the brain of aged rodents and humans [65–68]. Astrocytes play a major role in regulating the homeostasis of the glutamatergic synapse. They do this by maintaining an optimal concentration of glutamate both within and surrounding neurons by transporting glutamate and by regulating the volume of the extracellular content [69,70]. Disruption of this optimal glutamatergic environment can cause the brain injury that is associated with aging [71–73]. These cytokines attract and activate astrocytes, which in turn take up glutamate and release growth factors in order to restore the synaptic function and repair neurons. Astrogliosis ensues if these insults are ongoing or overly intense [74,75] and aggravates neuronal damage, progressively exacerbating the dysfunctions associated with aging [76].

Astrocytes have a high concentration of DHA in their membrane, which correlates with the amount of ω-3 PUFA in the diet [77]. Hence, via the aforementioned mechanisms, DHA can influence the regulation of brain homeostasis [35]. ω-3 PUFAs can aid astrocytes in neuroprotection by acting on gap junctions and on the morphological plasticity that is involved in their ability to regulate synaptic transmission and protect neurons. Also, free DHA (as opposed to membrane DHA) has a role in the transport systems, decreasing glutamate transport in cultured astrocytes [78].

As for neurogenesis, three areas of the brain continuously generate new neurons throughout life; namely, the dentate gyrus of the hippocampus, the subventricular zone, and the olfactory epithelium. The adult hippocampus contains neural stem cells (NSCs) that contribute to neural repair by generating daughter cells which become neurons or glia. However, neurogenesis is markedly reduced in the aging brain [79]. Several studies have concluded that ω-3 PUFA supplementation diets have a beneficial effect on adult neurogenesis. A recent study concluded that neurogenesis was increased in transgenic *Fat1* mice that can synthesize large amounts of DHA, and that the animals also had improved spatial learning [80]. *In vitro* proliferation of NSCs is increased when the cells are grown in medium supplemented with DHA [81,82]. It also improves neuronal differentiation even in cells isolated from older animals [83,84].

Finally, there are several human-based studies showing that an increased ω-3 PUFA intake can protect cognitive performance or slow a decline in aging [85–90]. Recent examples include a randomized controlled trial on 45 healthy elderly people, which concluded that krill oil rich in ω-3 PUFA incorporated in phosphatidylcholine was especially beneficial in cognitive function in the elderly [91], and another study which concluded that lower erythrocyte DHA levels were associated with smaller brain volumes and cognitive impairment in the elderly free of clinical dementia [92].

In conclusion, ω-3 PUFAs have effects on response to stress, neurotransmission, neuroprotection, neurogenesis, and anti-inflammation processes, which are major pathways in brain aging. Supplementation with ω-3 PUFA is very promising with regard to age-related conditions, as will be described in the following sections.

19.3 OMEGA-3 PUFA, NEURODEGENERATIVE DISEASES, AND STROKE

This section attempts to summarize studies that investigated the effects of ω-3 PUFAs on two of the most prevalent neurodegenerative diseases (Parkinson's disease and Alzheimer's disease) and the most prevalent vascular brain disease (stroke).

19.3.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting over 6 million people worldwide, making it the most common neurodegenerative disease after Alzheimer's disease [93]. The annual incidence rate of PD in the United States at ages 65 and older is about 160 per 100,000 persons. Each year in the United States about 60,000 new cases are diagnosed [94], and over 20,000 deaths are attributed to PD [95].

PD is characterized by a progressive loss of neuromelanin-containing dopaminergic neurons in the substantia nigra (SN) and consequent depletion of dopamine (DA) in the striatum. Common parkinsonian symptoms are resting tremor, bradykinesia, rigidity, and loss of postural reflexes [96], which only appear after 50–60% of neuronal loss of the SN [97]. The exact mechanisms underlying the process of the massive death of dopaminergic nigrostriatal neurons are unclear, but oxidative stress, inflammation, and mitochondrial dysfunction may play a large role [98].

Currently, the available treatments for PD reduce patients' symptoms considerably and improve quality of life for over 5–8 years. However, at the end of this period most patients develop neuropsychiatric and motor complications (fluctuations and dyskinesia) [99]. This observation highlights the need for neuroprotection therapies that produce lasting benefits by favorably influencing the disease's etiology or subsequent deficits as dopaminergic neuronal loss.

Various food groups and specific nutrients have been investigated as factors related to a high or low risk of PD. According to population-based studies, the prevalence of PD is generally lower in East Asian regions (e.g., China, Taiwan, and Japan) than in Western regions (e.g., Europe and the United States) [100]. Based on this endemic distribution, more attention has recently been given to dietary habits among East Asian populations. A prospective study of dietary intakes was assessed with semiquantitative food frequency questionnaires in more than 130,000 people. The principal analysis identified two dietary patterns: prudent and Western. The prudent dietary pattern, characterized by high intakes of fruit, vegetables, and fish, was inversely associated with PD risk, but the Western pattern was not [101].

Since high fat consumption could be a modifiable risk factor, some authors evaluated the impact of a 2-month high intake in calories from fat on brain in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. As expected, the MPTP treatment produced nigral dopaminergic degeneration, as evidenced by the loss of striatal dopamine and the decreased number of nigral tyrosine hydroxylase (TH) and dopamine transporter-expressing neurons. Notwithstanding, exposure to a high-fat diet exacerbated the effects of MPTP on striatal TH and dopamine levels, indicating that diet-induced obesity is associated with a reduced capacity of nigral dopaminergic terminals to cope with MPTP-induced neurotoxicity [102].

Although post mortem gas chromatographic analysis of brain fatty acid profiles has revealed no significant differences in ω-3 PUFA concentrations in brain cortex between PD patients and age-matched controls [13], specific studies have reported the therapeutic benefit of ω-3 PUFA in a PD animal model. For instance, Samadi *et al.* [103] investigated the action of DHA on levodopa-induced dyskinesias (LIDs) in parkinsonian MPTP-treated monkeys. This experiment explored the effect of DHA in two paradigms. First, a group of MPTP monkeys was primed with levodopa for several months before introducing DHA. A second group of MPTP monkeys was exposed to DHA before levodopa therapy. DHA administration reduced LIDs in both paradigms, indicating that DHA can reduce the severity or delay the development of LIDs in a non-human primate model of Parkinson's disease [103].

As already mentioned, by influencing biophysical properties of membranes, ω-3 PUFAs can modulate neurochemistry, signal transduction, and gene expression [104]. These fatty acids also suppress the formation of inflammatory eicosanoids, by competing with AA for COX-2 [22]. Based on these pleiotropic effects of ω-3 PUFA, Bousquet *et al.* [105] exposed mice to either a control or a high ω-3 PUFA diet from 2 to 12 months of age and then treated them with MPTP. To probe for a protective effect of ω-3 PUFAs, these authors focused the investigations on six important markers of dopaminergic activity, three assessing the nigral cell population (TH immunostaining, Nurr1, and DAT mRNA) and three measuring striatal axonal innervation (catecholamine concentrations, DAT autoradiography, and TH immunostaining). The results showed that the deleterious effect of MPTP was completely blunted in animals fed the high ω-3 PUFA diet, suggesting an important protective effect of this fatty acid [105].

As inflammatory response in CNS has been considered one of the causes of PD, in recent years animal experiments showed that immune cells, including microglia and astrocytes, are activated in the course of PD, and that the use of anti-inflammatory drugs can ameliorate neuronal and behavioral deficits caused by the loss of dopaminergic neurons [106]. Following these lines, authors hypothesized that ω-3 PUFAs could trigger microglia inhibition and provide an effective strategy against neurotoxic effects. Recently, this theory has ratified and elucidated that ω-3 PUFA inhibited lipopolysaccharide-induced activation of nuclear factor-κB (NF-κB), an important transcription factor involved in microglial activation [23].

In recent years, researchers have tried their best to explain the possible mechanisms through which ω-3

PUFAs may act on the symptoms of PD. The relationship between DHA and lipid peroxidation/antioxidant enzyme activities in the brain of experimental Parkinson's model was studied by Ozsoy *et al.* [107]. DHA, given by gavage for 4 weeks, significantly diminished the amount of cell death in the MPTP + DHA group as compared to the MPTP group. Nevertheless, this fatty acid was not able to modify the glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) activities. These authors posited that the DHA treatment exerts neuroprotective actions on an MPTP experimental mouse model of PD by an alternative pathway, since it did not alter brain lipid oxidation [107].

On the other hand, 10 months of high ω-3 PUFA treatment that preceded MPTP exposure induced an increase in cortical brain-derived neurotrophic factor (BDNF) protein immunostaining, striatal BDNF mRNA expression, and striatal TrkB mRNA levels, as well as a slight increase in striatal BDNF protein. These data combined confirm that ω-3 PUFA is involved in a complex regulation of BDNF, which may account in part for its neuroprotective effect in an animal model of PD [108].

In summary, the neuroprotective effect of ω-3 PUFA should not be interpreted as a single phenomenon, but rather as a part of a wide spectrum of protective activities. Although the biological/molecular mechanisms that associate the effect of ω-3 PUFA intake with PD are not completely understood at present, it is evident that nutritional protective strategies are valuable tools and should be adopted. They could potentially provide sufficient symptomatic control and mainly maintain a satisfying quality of life, or alleviate the symptoms of this disease.

19.3.2 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to progressive dementia, the initial stages of which feature a profound inability to form new memories. This condition affects 5–10% of the population over 65 years of age. Other symptoms besides decline in memory include impairment of cognitive abilities, so that permanent care for the patient is necessary. These symptoms are likely derived from malfunctioning and loss of synaptic connections and neurons, and from alterations in neurotransmitter systems (e.g., acetylcholine) in the hippocampus and cerebral cortex [109–114].

Among the brain changes believed to contribute to the development of AD are the accumulation of β-amyloid (Aβ) protein outside neurons in the hippocampus and in the association cortices, and the accumulation of tau protein inside neurons. The extracellular deposits of Aβ peptide and intracellular deposits of microtubule-associated tau protein form the so-called senile plaques and neurofibribillary tangles, respectively [111–114]. The deleterious process begins with abnormal processing of the transmembrane Aβ precursor protein (APP). The APP sequential cleavage by α- and β-secretases results in a non-amyloidogenic pathway characterized by a release of a neurotrophic APP ectodomain called sAPPα. However, the β- and γ-secretases are also responsible for proteolysis of extracellular domains, which results in an amyloidogenic pathway that leads to Aβ family production. Aβ42, the most insoluble of these peptides, has a propensity for self-aggregation into fibrils that compose the senile plaques. Regarding the tau protein, it is a normally occurring protein that becomes abnormally hyperphosphorylated and forms neurofibrillary tangles that can rupture neurons [111,112].

The brain contains approximately 100 trillion synapses, which are specialized connections that enable communication between neurons and are fundamental to establishing the brain's circuits. This complex circuitry is what generates emotions, movements, motor skills, and memory. In AD, the number of synapses declines with the death of neurons, and consequently the synaptic function is hampered. Thus, the molecular basis for memory loss and cognitive impairment in AD may be synaptic dysfunction caused by accumulation of Aβ peptide outside these synapses [111–113]. Moreover, blockade of transport of nutrients to the neurons caused by intracellular accumulation of tau protein is believed to prompt cell death. Finally, genetic mutation for the amyloid precursor protein on chromosome 21, the gene for the presenilin-1 protein on chromosome 14, and the gene for the presenilin-2 protein on chromosome 1, can also cause AD. The AD caused by these genetic mutations is known as "familial" AD [113].

In terms of etiology and risk assessment, it is essential to identify the several biological factors involved in it. Apolipoprotein E (ApoE) is the most important genetic risk factor for the development of AD. ApoE has three forms: ε2, ε3, and ε4. Everyone inherits one form of the ApoE gene from each parent. Those who inherit one ε4 gene have an increased risk of developing AD. Furthermore, age, gender, education level, social activities, familial history, cardiovascular disease, traumatic brain injury, and nutrition constitute major epigenetic factors for AD [112,113].

Taking nutrition into consideration, several epidemiological studies have reported that reduced levels of ω-3 PUFAs, such as EPA and DHA intake or fish consumption, is associated with a higher risk for AD. Moreover, an adequate dietary intake of ω-3 PUFA consumption can prevent cognitive impairment associated with the brain aging, irrespective of differences in age, gender, education, smoking, or other risk factors. Therefore, nutrition could be a potent tool for delaying the onset of AD or even delaying its progression [33,35–38,111,115,116].

In order to investigate cognition-enhancing effects of ω-3 PUFA, some studies were conducted in healthy

elderly, elderly with mild cognitive decline, and patients with dementia or AD [35,117]. In studies with healthy elderly, ω-3 PUFAs aided in slowing down cognitive decline [118,119]. In patients with mild age-related cognitive decline, a slight beneficial ω-3 PUFA effect has been found with amelioration in performance on episodic memory, but not working memory [120]. However, in studies with patients who had been consistently diagnosed with dementia and supplemented with these fatty acids, only the milder cases exhibited a significant reduction in cognitive decline [121,122].

Nevertheless, the ω-3 PUFA intake of the subjects in most of these studies depended on eating fish or taking PUFA supplements, and cannot be isolated from confounding factors on cognitive function, such as socioeconomic status and health habits. Also, ω-3 PUFA effects cannot be distinguished from the effects elicited by other nutrients [35].

As for animal models, they generally investigate the ω-3 PUFA effect on normal aging and on neurodegeneration models. Results are obtained from animals that are deficient in ω-3 PUFA and animals that are supplemented with DHA, EPA, or fish oil. It is important to highlight that there are different models of deficiency: first-generation deficiency and second-/third-generation deficiency. However, the conclusions of these experimental studies in general pointed to the idea that ω-3 PUFA deficiency impairs memory and that ω-3 PUFA supplementation can restore age-related cognitive impairment [64,123,124].

The neuroprotective role of ω-3 PUFAs in the aging brain may be in promoting neuronal survival and synaptic function by normalizing membrane rigidity, modulating the APP amyloidogenic process, activating pathways whose signals prompt neuronal survival, promoting neurogenesis/synaptogenesis, increasing the release of neurotransmitters, and avoiding the deleterious oxidative and inflammatory cascades.

The double bonds in PUFAs have great influence on the physical properties of cellular membranes, being indispensable for membrane ionic permeability and the function of membrane receptors, which in turn are primordial for synaptic transmission [125,126]. Therefore, diets deficient in ω-3 PUFAs may alter the perfect function of biological membranes. Furthermore, age and neurodegenerative diseases likely reduce membrane unsaturation and consequently cause inhibition of membrane-bound enzymes, ion-channels, and receptors [36,127]. Supplementation with ω-3 PUFAs, or their dietary intake from natural sources, may restore the changes caused by inadequate dietary and/or neurodegenerative processes [36,128].

Omega-3 PUFAs, particularly DHA, exert protective effects against neurotoxicity induced by Aβ peptide, limiting the production and accumulation of this peptide from the APP. This process results in elevated secretion of non-amyloidogenic APPα [38,115]. In addition to that, the neuroprotectin D1, a bioactive mediator derived from DHA, has been reported to decrease Aβ peptide production, reinforcing the protective effect of DHA [129].

The protection from memory deficits in AD model rats that is provided by ω-3 PUFAs may be related to the role of these fatty acids in promoting neuronal survival. The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (TOR) signaling pathway, a critical pathway in cell survival, is tightly affected by ω-3 PUFA status in membranes, so DHA can promote neuronal survival by stimulating the PI3K/Akt pathway [116,126]. The neurotrophin BDNF has been shown to be important in modulating synaptic plasticity and cognition function through the PI3K/Akt pathway [126], and DHA appears to increase this neurotrophin production in the frontal cortex and hippocampus of aged mice. Thus, researchers have been suggesting that the protective role of ω-3 PUFAs in cognition deficits from AD in rodent brains could be related to the effects of these fatty acids in increasing the BDNF levels [38].

A connection has been shown between age-associated decline in synaptic plasticity and memory loss [52]. Several studies have been investigating whether restoration of PUFA levels in the brain could rescue the age-related impairment in memory formation. In this investigation, the authors compared the effects of AA, EPA, and DHA and concluded that all fatty acids presented neurotrophic effects, but DHA had the strongest effect on stimulating the neurons in aged rats. Also, the decrease in neurogenesis observed in the hippocampus of aged rats was attenuated by treatment with fish oil (rich in EPA and DHA) [117]; moreover, fish oil supplementation was able to revert decreases in the GluR2 subunit of N-methyl-D-aspartate receptor (NMDA) and the subunit NR2B of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor that are the result of aging. These results involving NMDA and AMPA, glutamatergic receptors important for the process of learning and memory, are reinforced by studies showing that EPA and DHA improve memory impairment and LTP in aged rats [35,64].

The influence of aging on memory formation can result from a disruption of homeostasis of glutamatergic hippocampal synapses partly due to decreased presynaptic glutamate release [130]. It has been shown that the hippocampus of aged rats and mice exhibited decreased expression of VGlut-1 and VGlut-2 associated with a loss of glutamate release capacity in this structure. The ω-3 PUFAs alter the efficacy of glutamatergic synapses by affecting the expression and function of glutamate receptors and transporters and also affecting the function of the SNARE protein complex at

the active zone, which is fundamental in the exocytosis process [61,131].

Regarding the neuron–astrocyte crosstalk at the glutamatergic synapse, astrocytes are crucial for enabling the brain to adapt to aging. Some events caused by stress, such as hormonal alterations or release of pro-inflammatory cytokines by microglia, can activate astrocytes, which contribute a neuroprotective action via restoration of the synaptic function. On the other hand, the continual recurrence of these events during life disrupts astrocyte physiology, a process known as astrogliosis [35]. Astrogliosis contributes to the impairment of synaptic transmission and the loss of memory in the aging process. Excess of glutamate may release an excessive amount of AA from the cell membrane, especially when the brain is poor in ω-3 PUFAs. The exaggerated AA release may initiate a pro-inflammatory cascade of events [31,132]. Adequate brain DHA incorporation promoting a balanced AA/DHA ratio in the synaptic membranes may cause high DHA release from PLA2 action. Also, DHA in membrane stimulates mechanisms underlying the morphological plasticity of the astrocytes. Therefore, dietary supplementation with ω-3 PUFAs throughout life could also have a role in synaptic and astroglial physiology.

Addressing the importance of the inflammatory process in AD, investigation of the beneficial impact of DHA showed that this fatty acid did not alter PLA2 activation, but neuroprotectin D1, a metabolite, has been reported to decrease Aβ peptide production as well as apoptosis induced *in vitro* by Aβ oligomers [37].

The Aβ oligomers are responsible for increased oxidative stress in AD. The antioxidant role of fish oil or DHA has been established in conditions such as encephalopathy [133], in corpus striatum [134] as well as in the cortex and hippocampus of an AD model rat [135].

There is compelling evidence suggesting that adequate lipid status in the membranes of neurons and astrocytes is fundamental in the beneficial effects of ω-3 PUFAs in preventing neuronal damage and cell death in elderly experimental animals and in AD animal models. Among the ω-3 PUFAs, DHA has a potential neuroprotective action against cognitive impairment and dementia.

The reasonable cost, safety, and multipurpose aspects of ω-3 PUFA supplements indicate their value as a good and affordable option for care for age-related diseases such as AD. Nevertheless, we cannot minimize problems concerning the reproducibility of animal models, and we cannot disregard the interference of many factors in the final analysis of samples obtained from population studies. Thus, the promise of ω-3 PUFAs in preventing memory deficit in the elderly, or even in preventing/delaying neurodegenerative disorders, needs to be supported by moving forward our current knowledge of the mechanisms of these substances.

19.3.3 Stroke

Stroke is the second most common cause of death worldwide, accounting for 10% of all deaths, and is a major global cause of disability. Given the high incidence of stroke globally, stroke prevention is of major interest. Healthcare costs related to stroke continue to increase, and also to escalate as a result of morbidities that ensue [136].

Stroke is a sudden loss of neurological function caused by lack of blood flow to the parenchyma. It can be broadly categorized into hemorrhagic or ischemic stroke. The former occurs due to blood leak from a vessel, and can be either an intracerebral hemorrhage or a subarachnoid hemorrhage [137]. The latter occurs due to lack of blood supply, without a leak, and thus inadequate amounts of oxygen and nutrients to a part of the brain, usually due to thrombosis, embolism, or systemic hypoperfusion. Approximately 20% of strokes are hemorrhagic and 80% are ischemic. Each of these two categories can be further divided into subtypes that have their own pathogeneses, with different clinical presentations, courses, and outcomes, and with specific treatment approaches.

Prevention is attainable, since the literature suggests modifiable risk factors for stroke. Hypertension is universally reported to be the main risk factor for stroke in general, whereas low HDL cholesterol and diabetes mellitus are risk factors for ischemic stroke. The associations of total or LDL cholesterol and triglycerides with the risk of total or ischemic stroke are weak or absent [138]. However, an increase has been observed in the number of cerebrovascular events in developing countries that correlates with food and lifestyle changes arising from industrialization and urbanization [139].

There has been plenty of research on dietary patterns and on diet components, and this book is an updated source of information. Because hypertension is the major risk factor for stroke, special attention should be paid to types of food that increase blood pressure – such as increased salt or sodium intake, decreased potassium intake, excess weight, and excess alcohol consumption [140]. A diet that is low in sodium and high in potassium is recommended to reduce blood pressure, as indicated in the Dietary Guidelines for Americans from the US Department of Health and Human Services and Department of Agriculture.

Adherence to a Mediterranean-style diet pattern has also been associated with lower all-cause mortality in individuals with cardiovascular disease [141]. Some suggest that high ω-3 PUFAs and low ω-6 PUFAs , with their favorable profile and anti-inflammatory and plaque-stabilizing effects, may be the protective mediators of the Mediterranean diet [142].

The rationale behind stroke prevention with ω-3 PUFA supplementation comes from the multiple beneficial

effects that were mentioned towards the beginning of this chapter. ω-3 PUFAs affect a myriad of molecular pathways, including alteration of physical and chemical properties of cellular membranes, direct interaction with and modulation of membrane channels and proteins, regulation of gene expression via nuclear receptors and transcription factors, changes in eicosanoid profiles, and antioxidative effects.

The pathogenesis of stroke is closely related to cardiovascular health. Therefore, the beneficial effects of ω-3 PUFAs on the cardiovascular system have a direct effect on this pathological condition. ω-3 PUFA consumption improves vascular and cardiac hemodynamics, triglycerides, and, possibly, endothelial function, autonomic control, inflammation, thrombosis, and arrhythmia [143]. Interestingly, according to a prospective population-based study, an increased concentration in serum of ω-3 PUFA, especially DHA, may protect against atrial fibrillation [144–146]. Atrial fibrillation alone is a strong risk factor for an embolus and consequent cerebral infarction due to embolism. Therefore, a higher concentration of ω-3 PUFAs may be of benefit for the prevention of stroke via its decrease in the risk of embolization.

Moreover, a study indicated that incorporation of DHA in the brain might protect against post-ischemic inflammation and injury. The authors of the study suggest that an increased DHA intake could provide protection against acute immune response and brain damage in the acute setting post-ischemic stroke [147]. Furthermore, increased intake of ω-3 PUFAs, especially EPA, leads to a decrease in clotting and an increase in bleeding time in humans. EPA competes with AA for COX-2, leading to an increase in the thromboxane A3/thromboxane A2 ratio [148]. Changes in this ratio appear to reduce platelet aggregation and vasoconstriction, and thus cause prolonged bleeding.

Regarding the stroke outcome associated with ω-3 PUFAs, the medical literature is abundant with observational studies and prospective studies on ω-3 PUFAs and stroke, including randomized controlled clinical trials. In a 2011 meta-analysis of 15 prospective studies with 9360 stroke events among 383,838 participants [149], fish consumption was noted to be weakly inversely associated with the risk of stroke. However, another meta-analysis, published in 2012, included eight prospective studies with 5238 stroke events among 242,076 participants. Although this meta-analysis showed no overall association between ω-3 PUFA intake and stroke, it suggested that women might benefit from a higher intake of these PUFAs [150].

Another meta-analysis from 2012 looked at both primary and secondary prevention studies – i.e., participants with or without cardiovascular disease at baseline [151]. There were prospective cohort studies and randomized controlled trials reporting on associations of fish consumption and ω-3 PUFAs (based on dietary questionnaires), ω-3 PUFA biomarkers, or supplementations, with cerebrovascular disease. Even though observational data indicated moderate inverse associations of fish and long-chain ω-3 PUFA consumption with risk of cerebrovascular events, there was no evidence for similar inverse associations with cerebrovascular disease for long-chain ω-3 PUFAs measured as circulating biomarkers in observational studies, or supplements in primary and secondary prevention trials based on both primary and secondary prevention studies.

All these studies involved different methods and populations. Thus it is difficult to make comparisons among them or to reach conclusions that could be extrapolated to the population. These studies assess the ω-3 PUFAs in subjects via various methods: ω-3 PUFAs in the plasma, fish oil supplementation, or fish consumption frequency. For this reason, a number of points should be taken into consideration when investigating the relationship between ω-3 PUFAs and stroke.

First, it is paramount to look at the duration and the dose of ω-3 PUFA intake. Perhaps an association between ω-3 PUFA and prevention of stroke would only be evident if the study were performed over a longer period – more than 5 years, for instance. It could be hypothesized that a healthy diet might take longer to have a beneficial effect, making it different from a drug trial. This hypothesis could explain why the studies from countries that maintain frequent fish consumption throughout life tend to show positive results when compared to US studies. Moreover, it is possible that an individual might need a dosage larger than 1 g for the ω-3 PUFA to have an effect. There can also be gender differences. A higher ω-3 PUFA or fish intake was related to a lower stroke risk in women in one study, while for men an inverse association could not be demonstrated [152].

Secondly, special attention should be given to the type of fish and the method of preparation [153]. While all fish contain EPA and DHA, the quantities vary among and within species depending on their diet and whether the fish are wild or farm-raised. Fatty fish such as salmon, herring, and mackerel have significantly higher EPA and DHA contents than lean fish such as cod and haddock. In the United States, most fish that are served fried tend to be from lean species, which do not have great PUFA content [154]. Steaming and stir-frying are the predominant methods of cooking fish in China, while deep-frying may be more common in Europe and North America. A cross-sectional study showed that while African-American subjects consumed more total fish than white subjects, most of the additional consumption was in the form of fried fish. In addition, participants living in the Stroke Belt and Stroke Buckle in the US were more likely to consume more servings of fried fish than those living elsewhere [153]. The act of frying fish has been

associated with loss of natural fatty acids in the fish and their replacement with cooking oil [155].

Finally, to date there have not been convincing data from randomized trials that can establish that fish oil consumption would change the risk of stroke. On the one hand, observational evidence suggests that fish consumption may reduce the risk of ischemic stroke; on the other hand, there is no sufficient evidence from randomized trials that fish consumption or fish oil supplementation either increases or decreases the risk of stroke. Data regarding dietary fats and stroke have led to conflicting results. Nevertheless, when combined with robust global evidence from observational studies, the documented effects on risk factors in short-term trials, and experimental and mechanistic evidence, it is clear that ω-3 PUFAs are bioactive nutrients that play an important role in cardiovascular health, in particular for reducing the risk of cardiac mortality [143].

In conclusion, because of these conflicting findings, no firm recommendations are yet warranted regarding the effect of ω-3 PUFA or fish intake on stroke risk. This is due to research method disparities that lead to confusing conclusions. More research is necessary to demonstrate the correct method of assessing the beneficial effects of ω-3 PUFAs on stroke.

19.4 CONCLUSIONS

It is known that the Western diet features a high ω-6 to ω-3 PUFA ratio, which is responsible for the plummeting of DHA levels in the neuronal membranes. Several studies, from epidemiological to animal model-based research, have confirmed that ω-3 PUFA intake improves the incorporation of these fatty acids, especially DHA, to cellular membranes. It is also acknowledged that there is a firm relationship between diet and the process of senescence.

The data compiled in this chapter point to an overt involvement of ω-3 PUFAs in brain mechanisms that are capable of preventing or delaying the onset of brain diseases. Therefore, an adequate concentration of these fatty acids can optimize the physiological functions of the synaptic transmission and cognitive processes, thereby reducing secretion of cytokines by glial cells throughout life. Among these fatty acids DHA stands out, as it can modulate exacerbated glial reactions, reducing neuroinflammatory and oxidant processes that constitute the background of various neuropathologies. This provides a strong neuroprotective effect against myriad conditions. Parkinson's disease, Alzheimer's disease, and stroke are among the pathological conditions whose incidence grows with aging, and which benefit from a change in the nutritional pattern so as to increase the overall amount of ω-3 PUFAs.

More studies are necessary to further elucidate the mechanisms that underline the nutritional therapies, such as their specific targets in the brain and the specificity of the impacted cognitive traits. The take-home message is that there is compelling evidence that ω-3 PUFAs are beneficial to health, that they have major neuroprotective actions in the central nervous system, and that the Western diet renders the population prone to earlier and more severe neurodegenerative diseases. The consumption of these fatty acids could potentially benefit the quality of life of the elderly with regard to both normal aging and neurodegenerative diseases.

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CHAPTER

20

Selenium Binding Protein 1: A Moonlighting Protein

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20.1 INTRODUCTION

The element selenium (Se) was first discovered by the Swedish chemist Jöns Jacob Berzelius in 1817. Selenium was considered to be a poisonous substance until 1973, when its role as an essential micronutrient was first recognized as important to human health [1]. As a trace nutrient, selenium is required by humans in very small amounts [2] and excessive selenium intake can cause selenosis [3]. Selenium deficiency is associated with susceptibility to several diseases, including early identified Keshan's disease (an endemic cardiomyopathy) and Kashin-Beck disease (a deforming arthritis) [4]. Selenium deficiency can increase the virulence of infections [3] such as coxsackie B virus [5], influenza [6], and HIV [7]. Selenium deficiency is also linked to cancers, cardiovascular and neurological diseases, etc. [3]. The inverse relationship between selenium intake and cancer incidence has been observed frequently in clinical and epidemical studies [8,9]. To uncover the mechanism behind this beneficial effect against cancer or other diseases, several of the associated proteins or molecules, including selenoproteins and selenium binding proteins (SBPs), have been investigated [10–13].

Selenocysteine has been referred to as the "21st amino acid" [14]. It is coded by UGA, which functions primarily as a stop codon but can be translated into an amino acid in the presence of the selenocysteine insertion sequence (SECIS) in the 3′ UTR of mammals [15]. The proteins containing selenocysteine(s) are called selenoproteins. To date, 25 selenoproteins have been identified in humans [16]. These proteins play a role in several important physiological functions: in thyroid hormone metabolism, in immunity, in intracellular redox state, etc. [3]. Low selenium intake affects the expression of these selenoproteins [17], especially those with enzymatic functions. For example, Beckett *et al*. [18] demonstrated that the activity of the enzymatic selenoprotein type 2 iodothyronine deiodinase (D2) decreases due to selenium deficiency.

Different from selenoproteins, which incorporate selenocysteine co-translationally, SBPs bind selenium via their structural configurations. Three categories of selenium-containing proteins are summarized in Figure 20.1. The first is selenoprotein with selenocysteine(s) as a component. The second category is made up of the non-specific SBPs, typically those proteins rich in methionine or cysteine that can bind selenium non-specifically. The third category is made up of the specific SBPs, including SBP1, 14-kDa fatty acid binding protein [19,20], etc.

The beneficial effects of selenium are typically attributed to selenoproteins, whereas the roles of SBPs are often neglected. For example, SBPs are good candidates as selenium transporter(s) due to their selenium binding properties [21], although their role in selenium metabolism is not yet fully elucidated. This chapter systematically reviews the possible functions of SBPs, especially SBP1, which has been shown to participate in many important physiological pathways.

20.2 SELENIUM BINDING PROTEIN 1

20.2.1 Characteristics of SBP1

In 1989, Bansal *et al*. [19] analyzed liver lysates taken from BALB/c mice injected with ^{75}Se and found a novel

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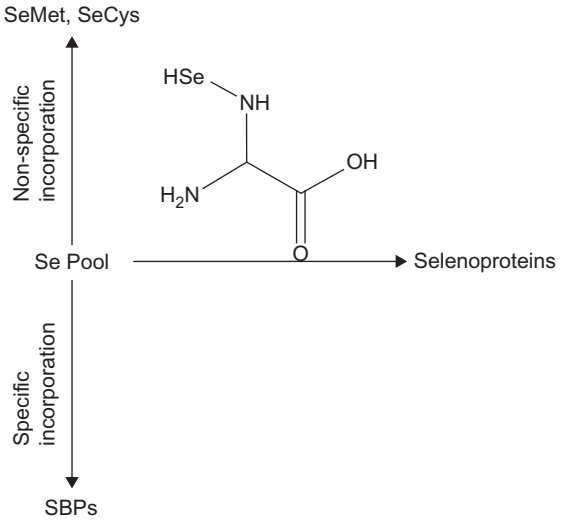

FIGURE 20.1 Categories of selenium-containing proteins. Three categories of selenium containing proteins are illustrated: selenoproteins, non-specific selenium binding proteins, and specific selenium binding proteins.

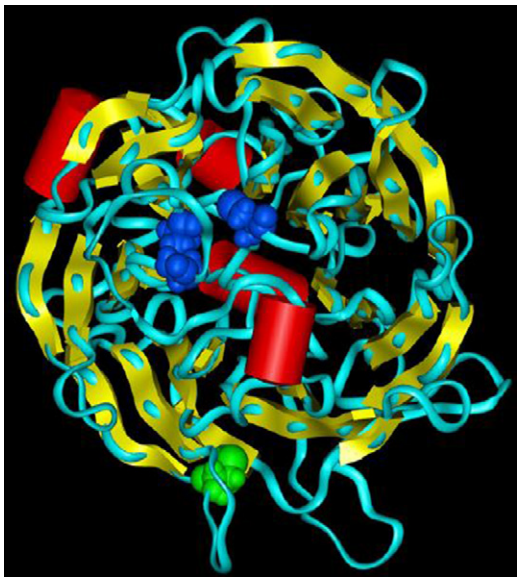

FIGURE 20.2 Predicted human SBP1 structure. The β-strand and α-helix regions are shown. Four cysteines involved in two disulfide bridges are shown, while the Cys50-predicted selenium binding site is also shown. Reproduced from Raucci *et al*. [26], *Biochimica et Biophysica Acta* 2011; 1814(4):513–522, with permission.

56-kDa protein using electrophoresis [19]. This protein was later named Selenium Binding Protein 1 (SBP1) [22]. In 1997, human SBP1 was cloned [23]. SBP1, also known as SBP56, is a cytosolic protein that is heavily tyrosine phosphorylated *in vivo* (tyr12 and tyr335) [24]. The human gene *SELENBP1* is located at chromosome 1q21–22, and the corresponding cDNA is 1668 bp in length. The translated complete protein is 472 amino acids long [23]. SBP1 protein has different isoelectric points, suggesting possible modifications [24,25]. A search for SBP1 variants in the NCBI website (www.ncbi.nlm.nih.gov/) resulted in finding four isoforms of SBP1. The molecular weight of canonical human SBP1 isoform 1 is 52.25 kDa and the estimated isoelectric point is 6.13 [23]. The predicted structure of SBP1 is an alpha-beta protein with two disulfide bonds (see Figure 20.2). The cysteine-57 located domain is predicted to bind selenium [26].

SBP1 is highly expressed in liver, kidney, and lung, with lower levels found in heart and intestine. SBP1 is barely detectable in brain, thymus, muscle, spleen, skin, mammary tissues, testis, and ovary [23,27]. SBP1 is localized in the cytosol, membranes [28], and nucleus [25,29–31]. Hydropathy analysis has excluded SBP1 as a transmembrane protein [32].

20.2.2 Selenium and SBP1

SBP1 is thought to interact with selenium covalently via a selenosulfide bond (perselenide) [31]. Little is known about the role of SBP1 in selenium metabolism. SBP1 has been shown to participate in the late stage of Golgi protein transport downstream of the Rab family [28], a set of proteins involved with membrane traffic. Selenium is not required during this process [28]. Based on its selenium binding property, SBP1 is believed to participate in selenium delivery; however, to date there is no confirming evidence that SBP1 delivers selenium. Due to its cellular location, SBP1's selenium delivery function is probably limited between intracellular compartments. In fact, selenium itself can increase SBP1 expression [33–35]. Whether the induced expression is to meet the need for selenium delivery or only for selenium detoxification is not yet understood.

20.2.3 Downregulation of SBP1 in Tumorigenesis

SBP1 may play an important role in selenium's anticancer effect [36]. SBP1 expression decreases in tumor cells of various origins, including prostate [37], stomach [38], ovary [34,39], lung [25], bronchial epithelial carcinogenic process [40], colon and rectum [30,41], liver [29], esophageal adenocarcinoma [35], etc. The suppression of SBP1 expression in cancer cells is caused neither by genomic deletion [25] nor by loss of heterozygosity [35]. Hypermethylation of the promoter region of SBP1 gene may explain its suppression in colon and esophageal cancer, but not in other types of cancers [35,41,42]. SBP1 is downregulated reversibly by androgen in prostate cancer [34,37] and ovary cancer [34] at the transcriptional level. Androgen is a pro-tumorigenic factor often regarded as a target in the treatment of prostate cancer.

Interestingly, SBP1 mRNA is not detectable in androgen-insensitive prostate cancer cell lines lacking androgen receptors [37]. Of the four growth factors TGF-β, IFN-γ, TNF-α, and PDGF, only TGF-β can suppress the expression of SBP1 at both the transcriptional and translational levels while simultaneously promoting cell proliferation [24]. TGF-β acts as a tumor suppressor in early tumor stages and gradually develops into a pro-tumor factor [43]. This coincides with the gradual SBP1 loss in the later stages of cancer development. Because androgen can increase TGF-β1 expression in an androgen receptor-dependent manner [44], the effect of androgen on SBP1 may be regulated by the TGF-β pathway.

SBP1 is maximally expressed in developed normal epithelial cells. This is consistent with the fact that loss of SBP1 is accompanied with a reduction of carcinoembryonic antigen (CEA), a differentiation marker. However, it was found that the cell proliferation marker PCNA and the cell cycle inhibitor p21WAF1/cip1 (p21) were not changed accordingly [30]. The negative correlation between Ki-67 antigen and SBP1 expression is consistent with the observation that SBP1 expression is decreased in highly proliferating lung tumor [25]. A portion of tumor cells undergo the anaplastic process, which may imply that SBP1 loss endows cancer cells with strong proliferative ability by reversing cell differentiation. However, dexamethasone (an inhibitor of cell proliferation) does not increase SBP1 expression in lung cells. Dexamethasone can promote increased SBP2 (sometimes referred to as AP56) in liver [25]; however, SBP2 is found in mice but not in humans. SBP1 expression can vary in the cell lines from the same cancer tissues [42]. Two explanations for these observations are proposed here: (1) these cell lines are from different stages of cancer patients, since SBP1 is progressively reduced during cancer development [35,45]; or (2) these cell lines are from different differentiation stages, because SBP1 increases with cell differentiation [30].

SBP1 has several anticancer properties. SBP1 knockdown cells showed more mobility, proliferation, and resistance to apoptosis under oxidative stress when compared to scrambled control [29,46]. Overexpression of SBP1 sensitized tumor cells to apoptosis, oxidative stress, cellular senescence, and cisplatin [29,35,42]. The pro-tumor genes Notch1 and Cdk1 are also upregulated in SBP1 knockout mice [47]. All of the above evidence points to the critical role of SBP1 in cancer development.

20.2.4 SBP1 Loss and Cancer Prognosis

Low expression of SBP1 usually leads to poor prognosis in lung cancer [25], colon cancer [25,30,41], prostate cancer [48], breast cancer [36], malignant pleural mesothelioma [49], and liver cancer, as well as vascular invasion and recurrence [29], in spite of individual differences [34]. Low expression of SBP1 is correlated with patient age, α-fetoprotein, tumor size, tumor number, tumor encapsulation, vascular invasion, and recurrence. Most of these factors contribute to the overall survival rate, especially for the hepatocarcinoma patients beyond the Milan criteria where SBP1 works accurately as a predictor for the overall survival rate [29]. This suggests a critical role of SBP1 in determining patient survival, a topic that has been reviewed recently [13,50]. SBP1 was found to be one of those proteins overexpressed in chemosensitive ovary tumor tissues [51]. Therefore, SBP1 could be a good candidate as a target in cancer treatment.

20.2.5 SBP1 and Neuronal Diseases

SBP1 is found to be dysregulated in schizophrenic patients, in whom SBP1 expression is decreased in liver and increased in red blood cells (RBCs) [52]. The upregulation of SBP1 in peripheral blood cells (PBCs) and dorsolateral prefrontal cortex in schizophrenia at both transcriptional and translational levels is proposed to be used as a biomarker for schizophrenia [53]. The upregulation of SBP1 can be extended to individuals with episodes of psychosis and bipolar disorders, both of which suggest commonalties in the development of psychosis [54]. However, there is not a significant relationship between SBP1 expression and lifetime exposure to antipsychotic medications (fluphenazine equivalents) [54]. Another verification study showed that the upregulation of SBP1 is not significant in the PBCs but its expression level is positively correlated with age in schizophrenia patients [55]. It has been suggested that there is a possible association between genetic variation in *SELENBP1* and schizophrenia [56]. The upregulation of SBP1 is also found in patients with spontaneous equine recurrent uveitis (ERU), an incurable autoimmune disease affecting the eye [57], in which SBP1 may work as an autoantigen [58]. Limited literature makes it near impossible to extrapolate SBP1's function in these neuronal diseases, but it is an interesting topic worth further investigation.

20.2.6 SBP1 and ROS

Reactive oxygen species (ROS) can result in DNA damage and in that way induce genomic instability. There is evidence indicating a relationship between SBP1 and ROS associated with hypoxia inducing factor-1α (HIF-1α) and a selenoenzyme-glutathione peroxidase 1(GPx1). The *SELENBP1* promoter contains a hypoxia response element targeted by HIF-1α, a tumor progression factor that is both a contributor to angiogenesis as well as a tumor suppressor in some specific contexts [59]. The expression of both can be increased by hydrogen peroxide treatment, while inhibition of SBP1 can reduce the expression of HIF-1α [29]. Von Hippel-Lindau protein (pVHL)

mediates the degradation of HIF-1α in normoxia [60]. SBP1 may be involved in the HIF-1α pathway via ubiquitination/deubiquitination in a selenium-dependent manner based on the interaction with pVHL-interacting deubiquitinating enzyme 1 (VDU1) co-localizing in the perinuclear region [31]. This interaction is subjected to reducing agents like 0.1% β-mercaptoethanol possibly due to selenium dissociation. It is likely that SBP1 and HIF-1α may regulate each other in the presence of selenium. Interestingly, SBP1 is linked to another selenoenzyme type 2 iodothyronine deiodinase (D2) via VDU1/2, which catalyzes the thyroid hormone thyroxine (T4) to triiodothyronine (T3) [61]. VDU1 is also required for Slit signaling in inhibiting cell migration possibly by mediating Robo1 deubiquitination [62] (see Figure 20.3). VDU1 seems to play a pivotal role in the beneficial effect of SBP1, and is worthy of further investigation.

There is a reciprocal relationship between SBP1 and GPx1 in both prostate cells [29,33] and the prostate tissue [48]. Specifically, SBP1 can reduce GPx1 activity, while GPx1 can downregulate SBP1 at the transcriptional level. This may be due to the physical interaction between these two proteins [33]. SBP1 and GPx1 form nuclear bodies together under oxidative stress [29]. GPx1 is an important selenoprotein functioning in ROS scavenging. GPx1 is also a protein that is related with cancer risk [48,63] via its antioxidant activity in protecting cancer cells from oxidative stress, while SBP1 seems to work as a pro-oxidant instead of an antioxidant.

ROS can form a hypoxic microenvironment which will induce HIF-1α expression, while hypoxia can induce ROS itself. HIF-1α targets the promoter of SBP1 to facilitate its expression. SBP1 further decreases the antioxidant activity of GPx1 (as noted above). The final consequence is that cells become senescent with accumulated ROS. SBP1 may participate indirectly in the selenium-induced cellular senescence as an early barrier to tumorigenesis [64].

20.2.7 Other Functions of SBP1

Current research has begun to open the window needed for in-depth understanding of this mysterious protein. In addition to the above, SBP1 is involved in the aging process. Its expression was reduced in the livers of a senescence-prone inbred strain of mice (SAMP) during aging [65]. SBP1/2 increases in the kidneys of old mice [66]. SBP1/2 can also be increased by chemical pollutants [67,68], suggesting its possible roles in detoxification. The predicted transcriptional factors of SBP1 include NF-κB, Spz1, NRF-2, E74A, Snail, Androgen, and Thing1-E47 (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite/), of which only androgen has been reported to downregulate SBP1. We have attempted to include all of the above by summarizing SBP1 functions in Figure 20.3. Issues that remain to be addressed include determining the kinase that phosphorylates SBP1, and the role of selenium in the interaction between SBP1 and VDU1.

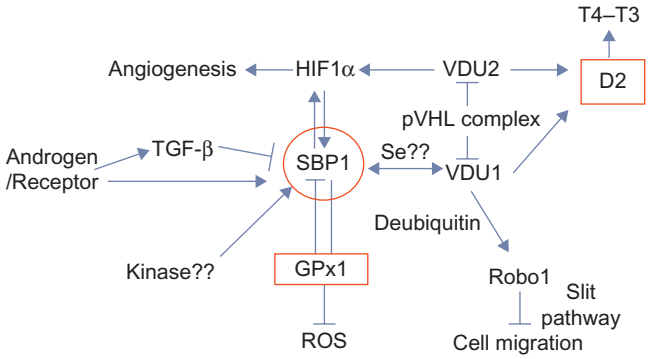

FIGURE 20.3 SBP1 pathways. The molecular pathway of SBP1 is drawn based on the literature review. The selenoproteins GPx1 and D2 are indicated with squares.

20.3 OTHER SBPS

20.3.1 AP56/SBP2

Selenium binding protein 2 (or AP56) is an acetaminophen binding protein found in mice. SBP2 may be involved in the hepatotoxicity of overdose use of acetaminophen, an analgesic that is metabolized via cytochrome P450 [69]. SBP2 can bind electrophilic chemicals, including naphthalene [70]. It should be pointed out that naphthalene can be metabolized into reactive intermediates via cytochrome P450. SBP2 is only 24 nt different from SBP1 within the 1419-nt coding region, but SBP2 mRNA is mainly found in mouse liver [27]. Interestingly, both genes are located at a close loci of mouse chromosome 3. To date, no human homolog of SBP2 has been reported. SBP2 can be decreased by peroxisome proliferators [22], typically ciprofibrate, a hypolipedemic drug that can induce hepatocellular carcinogenesis upon prolonged exposure. Based on their hepatic abundance, cellular localization, and sequence similarity, it is very likely that SBP1 and SBP2 compensate for each other functionally under some circumstances. If this assumption is true, it is no wonder that SBP1 knockout mice did not show any apparent phenotype [47]. However, SBP1 and SBP2 have distinct regulations, as evidenced by their opposite responses to aging [65]. Further research needs to be done to characterize the differences between these two "brother" proteins.

20.3.2 Liver Fatty Acid Binding Protein

In 1989, a 14-kb selenium binding protein was identified along with SBP1 in mouse liver [19]. Further

investigation confirmed that this protein is a fatty acid binding protein [20]. There are nine families of fatty acid binding proteins with tissue specificity that are involved in the regulation of fatty acid uptake and intracellular transport [71]. Mouse liver fatty acid binding protein is a cytoplasmic protein composed of 127 amino acids with different isoforms [72,73]. Its DNA clone was expressed in *E. coli* in 1991 [74]. The crystal structure and structures in the apo form and holo forms have also been determined [75,76]. Liver fatty acid binding protein metabolizes a fatty acid from a "donor" membrane into an "acceptor" membrane [77]. Besides fatty acids it can bind several other molecules, including warfarin [78], bile salts, acyl-CoA esters, bromosulfthalein, lysophospholipids, and monoglycerides [75]. The main function of liver fatty acid binding protein is to transport intracellular hydrophobic ligands throughout cellular compartments, including the peroxisomes, mitochondria, endoplasmic reticulum, and nucleus [79]. It also promotes cell division and may transport carcinogens [80]. The physiological role of this protein's selenium binding is still an enigma.

20.3.3 GAPDH

In vivo labeling with ^{75}Se was used to show that GAPDH, DPA, and deoxyribose-5-phosphate aldolase (DERA) can bind selenium in *E. coli* [21]. Both GAPDH and DPA can bind to glyceraldehyde 3-phosphate. DERA is an enzyme that catalyzes 2-deoxy-D-ribose 5-phosphate into acetaldehyde and D-glyceraldehyde 3-phosphate, a substrate of GAPDH.

GAPDH is a 37-kDa protein that catalyzes the conversion of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate as a tetramer in the sixth step of glycolysis for gluconeogenesis. GAPDH plays a critical role in providing energy for fast axonal transport [81]. Because of its stable expression, GAPDH is often used as an internal control with small within-tissue variations [82]. Interestingly, GAPDH is also a "moonlighting protein" with multiple functions probably conferred by its posttranslational modifications. It has two domains, of which one is used to bind nicotinamide adenine dinucleotide and the other to catalyze other substrates. GAPDH can assemble with other glycolytic enzymes into complexes on the erythrocyte membrane, this process being regulated by oxygenation and phosphorylation [83]. In GAPDH, 41 tyrosine phosphorylation by Src has been shown to be essential in the early secretory pathway mediated by Rab2 [84]. GAPDH is able to catalyze thiol–disulfide exchange reactions [85]. Inhibition of GAPDH can function as a switch to reroute the carbohydrate flux to the oxidative stress counteraction [86]. GAPDH is also a mediator of death via the NO/GAPDH/Siah1 pathway [87,88]. The neuroprotective agents deprenyl and TCH346 can prevent GAPDH activation in apoptosis [89]. GAPDH accumulates in the mitochondria of cells mediating the cyclosporin A-inhabitable permeability transition during apoptosis [90]. Oxidation of GAPDH has more potential to bind to nucleic acids with obscure mechanisms [91]. GAPDH is also shown to participate in the S phase-dependent histone H2B transcription [92]. The multiple functions of GAPDH continue to be "hot" research topics.

20.3.4 Mercaptopyruvate Sulfurtransferase

Mercaptopyruvate sulfurtransferase (3-MST) is an enzyme that catalyzes 3-mercaptopyruvate and cyanide into pyruvate and thiocyanate. It can also produce H_2S from 3-MP (3-mercaptopyruvate) in the presence of thioredoxin and dihydrolipoic acid [93]. Based on its sulfur delivery function, 3-MST has the ability to bind selenium [94]. 3-MST plays an important role in iron–sulfur chromophore formation in the adrenal cortex [95], the synthesis of sulfur amino acids, and the management of oxidative stress [96].

20.3.5 Rhodanese

Rhodanese can bind selenium from SeO_3^{2-} and glutathione (GSH) [97]. Rhodanese binds selenium better than GAPDH and 3-MST, both of which can more readily release selenium in the presence of a physiological dithiol [94]. Rhodanese is a mitochondrial enzyme protecting aerobic respiration from cyanide poisoning by converting it to the less toxic thiocyanate (SCN^-) [98]. A group of heterogeneous but phylogenetically related rhodanese-like proteins share a highly conserved rhodanese homology domain [99,100].

20.3.6 Hemoglobin

Hemoglobin (Hb) is an iron-containing metalloprotein in the red blood cells of all vertebrates. The main function of hemoglobin is to carry oxygen to different tissues of the body for energy metabolism, and then transport carbon dioxide back to the lungs. It can also scavenge nitric oxide [101]. Moreover, α- and β-globin have been found in mesencephalic dopaminergic neurons and glial cells, suggesting their physiological mitochondrial functions [102]. Selenotrisulfide can bind to a β subunit of hemoglobin via selenotrisulfide [103], although the physiological significance is unclear.

20.4 CONCLUSIONS

Our understanding of selenium metabolism and its molecular roles lags behind that of counterparts like iron, zinc, or copper. However, its nutritional role cannot

be neglected since selenium status is found to be associated with many disease susceptibilities, including cancer, viral infections, and cardiovascular, cerebral, and neural diseases. Selenoproteins are critical players in selenium nutrition, whereas the roles of SBPs are seldom reported. At present, no selenium transporter has been reported. For this reason, many SBPs have yet to be discovered. SBP1 has received the most attention because it has a close relationship with cancer development. To date, only GPx1 and VDU1 have been identified as interacting with SBP1 directly. Whether or not other molecules are involved needs further investigation. SBP1 is also involved in senescence, detoxification, and protein transportation. This chapter has reviewed the multiple functions of SBP1 as well as other SBPs with the hope of spurring on more scientific progress. These SBPs (like SBP1 and GAPDH) are extensively involved in several physiological pathways that apparently have little to do with selenium. They are truly moonlighting proteins in that they are taking on secondary functions in addition to binding selenium. A deeper understanding of these pathways will undoubtedly explain how they are involved in our health and disease susceptibilities.

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CHAPTER

21

Selenium and Senescence: Centering on Genome Maintenance

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21.1 INTRODUCTION

Selenium, a trace nutrient, is an element from the soil and is necessary for optimal health. The biological functions of selenium are mediated through selenoproteins and selenium metabolites. Selenium is incorporated into selenoproteins at the nutritional level. There is a total of 25 selenoproteins in humans, playing functional roles in redox regulation, thyroid hormone maturation, protein folding, and selenium storage and transportation. Physiologically, selenoproteins play critical roles in neuronal protection, immune response, viral suppression, glucose metabolism, and carcinogenesis [1–3]. After the expression of selenoprotein is saturated, excess selenium in the body can be metabolized and free radicals generated [4].

Senescence is a form of permanent cell cycle arrest induced by various cellular stimuli, including telomere attrition, DNA damage, oxidative stress, and oncogenes. Senescence limits proliferation potential, which is thought to both promote aging and suppress tumorigenesis [5]. Replicative senescence is considered to be a major contributor to normal aging as a result of telomere attrition and/or dysfunction.

21.2 SELENIUM AND SELENOPROTEINS

21.2.1 Selenium

Dietary intake of selenium at both adequate and supranutritional levels can contribute to optimal health, although caution should be taken in consideration of the benefit window of supranutritional selenium being narrow and individualized. The current recommended dietary allowance of selenium in the US is <40 and 55 μg/day for children under 13 years and above 14 years of age, respectively. The selenium requirements during pregnancy and lactation are 60 and 70 μg/day, respectively. In the US, the intake of selenium tends to fall between 50 and 200 μg/day [6]. However, extreme cases have been reported elsewhere, such as in China, where the intake ranges from 7 to 3800 μg/day, resulting in selenium deficiency or toxicity [7]. The best known selenium deficiency syndromes are Keshan disease and Kashin-Beck disease [8], the etiology of which are thought to be associated with increased virulence of Coxsackie B and fungus-produced mycotoxin, respectively. Because dietary selenium deficiency and glutathione peroxidase-1 knockout in mice similarly potentiate Coxsackie B virulence, it is thought that oxidative stress contributes to the cardiomyopathy phenotype in Keshan disease [9]. On the other hand, selenium at toxic levels can induce the formation of reactive oxygen species (ROS) and the resultant selenosis [10]. Moreover, conclusions made from the Nutritional Prevention of Cancer (NPC) clinical trial conducted in the US indicate that the daily oral administration of selenium at 200 μg (a dose three- to four-fold higher than nutritional needs) in the form of selenized yeast significantly decreases the risks of prostate, lung, and colon cancers [11]. The effect of selenium chemoprevention at supranutritional levels might result from the induction of ROS through selenium metabolism. Indeed, Wu and colleagues have shown

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that sublethal doses of selenium compounds can activate cellular senescence in a manner depending on ROS in non-cancerous but not in cancerous cells, suggesting that selenium chemoprevention may target premalignant cells through mild oxidative stress [12]. Although results from the follow-up clinical trial, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), do not support a beneficial role of selenomethione in chemoprevention, the discrepancies are largely reconciled and detailed on the National Cancer Institute's website, from which the inconsistent results are believed to be mainly attributed to the form of selenium (selenized-yeast vs selenomethionine) and the basal level of selenium in volunteers (low in NPC vs high in SELECT) [13,14]. Additional clinical studies are needed to elucidate or reconfirm a role of selenium in cancer prevention.

21.2.2 Selenoproteins

Results from the genome-wide search of selenocysteine-insertion sequence (SECIS) elements conserved in selenoproteins reveal that there is a total of 25 selenoproteins [15]. In prokaryotic and eukaryotic cells, selenium is incorporated into selenoproteins through selenocysteine, the "21st amino acid." Selenocysteine synthase replaces sulfur in Seryl-tRNA with selenium for the generation of selenocysteine-RNA [16]. Strikingly, the codon for selenocysteine, UGA, is shared with translation termination machinery. Distinguishing these two events requires the SECIS element located at the 3′-UTR of all selenoprotein mRNAs [17–19]. The SECIS element forms a stem and loop structure, which is recognized and stabilized by SECIS-binding protein 2 to position selenocysteine tRNA for decoding UGA [20]. The regulation of selenoprotein mRNA expression by selenium levels has been well studied [21–23]. Body selenium status regulates the expression of selenoproteins during translation through the post-transcriptional modification of selenocysteine-tRNA maturation and selenium availability for selenocysteine biosynthesis [18].

21.2.3 Selenoprotein Functions

Selenoproteins have numerous physiological functions in the process of redox regulation and signaling, thyroid hormone metabolism, selenocysteine synthesis, transportation and storage of selenium, and protein folding. In humans, the majority of the 25 selenoproteins exhibit antioxidative activities [19]. Interestingly, cellular localizations of redox-related selenoproteins vary, suggesting that these antioxidative selenoproteins collectively protect cells in different compartments against oxidative stress [24]. The biological and cellular functions of selenoproteins are discussed below, with a focus on those related to senescence.

21.2.3.1 The Selenium-Dependent Glutathione Peroxidase Family

The selenium-dependent glutathione peroxidases (GPxs) are critical antioxidant enzymes that use glutathione to reduce hydrogen peroxide and organic hydroperoxides [25]. It has been known since 2003 that there is a total of five selenium-containing GPxs in humans and four in mice. GPx1 was first identified to protect hemoglobin from oxidative breakdown in erythrocytes [26]. In February 1973, Rotruck and colleagues reported for the first time that GPx1 is a selenium-containing protein [27]. This seminal contribution was accompanied by a similar paper, published 3 months later, from the Floh é group [28]. GPx1 expresses ubiquitously, but is relatively abundant in kidneys, liver, and placenta. GPx2 is mainly found in the gastrointestinal tract. The extracellular GPx3 (also known as plasma GPx) is highly expressed in and secreted from epididymis and kidney. Phospholipid hydroperoxide GPx (GPx4) primarily expresses in liver and testis. GPx6 is rich in embryo and olfactory epithelium, but is not a selenoprotein in mice [15]. GPxs are localized in various cellular compartments, including GPx1 in cytoplasm and mitochondria, GPx2 in cytoplasm and possibly Golgi apparatus, GPx3 in extracellular space, and GPx4 in cytoplasm, mitochondria, and nuclei [29,30]. Little is known about the cellular localization of GPx6.

GPx1 plays a pivotal role in the mitigation of oxidative stress [31] and accounts for the majority of the peroxide-decomposing activity of selenium. GPx1 can prevent the formation of oxidative DNA damage and, in principle, suppress tumorigenesis [32–34]. Consistent with this notion, the tumor suppressor p53 is known to transactivate GPx1, resulting in declined oxidative stress [35]. Similarly, overexpression of GPx1 counteracts tumorigenesis by the induction of growth arrest and DNA damage (Gadd45) gene expression for reinforcement of cell cycle arrest [36]. GPx1 is also implicated in protection against cardiovascular disease, neurodegeneration, and autoimmune disease [2]. However, although increased oxidative stress is associated with many metabolic and age-related degenerations, GPx1 expression above the physiological level may not necessarily be beneficial. Intriguingly, GPx1 overexpression mice develop insulin resistance and glucose intolerance [37]. On the contrary, GPx1 knockout mice on a high-fat diet are more resistant than wild-type animals to development of insulin resistance and glucose intolerance [38]. These results are best explained by the notion that hydrogen peroxide is required for physiological insulin signaling [39]. GPx2 may promote early stage carcinogenesis, but later inhibits cancer cell growth, invasion, and migration [30]. Overexpression of GPx3 in mice can inhibit cancer cell growth and metastasis [34]. GPx4 overexpression in mice is known to inhibit the growth of tumor and metastasis [34].

21.2.3.2 Thioredoxin Signaling

In mammals, there are three thioredoxin reductases (TrxRs): cytosolic TrxR1 [40], mitochondrial TrxR2 [41], and testis-specific thioredoxin glutathione reductase (TrxR3) [42]. TrxRs belong to members of the pyridine nucleotide-disulfide oxidoreductase family and play critical roles in the recycling of oxidized thioredoxin. The thioredoxin system regulates the activity of NF-κB, AP-1, p53, and the glucocorticoid receptor, all of which are transcription factors that contain cysteine residue(s) in the DNA-binding domain [43]. TrxR1 is required for embryonic development. Knockout of TrxR1 in mice leads to accumulation of non-functional oxidized ribonucleotide reductase, resulting in impaired DNA synthesis [44]. Interestingly, TrxR1 is overexpressed in malignant cells [45–47]. Cells with TrxR2 mutations progress in an enhanced rate from G1 to S phases of the cell cycle, consistent with the proposed role of this selenoprotein in the regulation of cell proliferation [48]. Because the activities of TrxR are highly associated with carcinogenesis, they are promising targets for cancer therapeutics [49].

21.2.3.3 Thyroid Hormone Metabolism

Iodothyronine deiodinases (DIOs) regulate thyroid hormone maturation and maintain optimal health span. DIOs regulate the conversions between different forms of thyroid hormones: thyroxine (T4), 3,5,3′-triodothyronine (T3) and reverse triiodothyronine (rT3). DIO1 is mainly localized in liver, kidney, and thyroid. DIO2 expresses highly in brain, pituitary, thyroid, skeletal muscle, and brown adipose tissue. DIO3 is abundant in placenta and uterus during pregnancy, as well as in cerebral cortex and skin [24]. T4, the major hormone secreted from thyroid gland, requires DIO1 and DIO2 to catalyze deiodination for formation of the active T3. Levels of T3 in the serum of DIO1 or DIO2 knockout mice are normal [50], but DIO2 knockout mice retain a higher level of T4 and show impairment in hearing, thermogenesis, and neurocognition [51]. The phenotypes of DIO3 knockout mice include increased prenatal mortality, growth retardation, impaired fertility, hyperthyroidism in the prenatal period, and hypothyroidism in adulthood [51]. DIOs are also implicated in carcinogenesis. Increased expression of DIO3 correlated with the promotion of tumor proliferation [52]. While expression of DIO1 is decreased in cancers of various origins, including papillary thyroid, kidney, lung, prostate, and liver, expression of DIO2 is increased in cancers of follicular, anaplastic, and medullary thyroid, as well as astrocytes, gliocytes, and pituitary gland in the brain. DIO3 expression is increased in liver hemangioma, gliomas, gliosarcoma, glioblastoma, and pituitary tumors [53]. Because the protein expression of DIO1-3 varies in cancer cells, they are candidate biomarkers for cancer diagnosis and therapy.

21.2.3.4 Selenium Transportation and Storage

Selenoprotein P (Sepp1) plays critical roles for selenium transportation and retention in the body. There are four Sepp1 isoforms, the full-length of which contains 10 residues of selenocysteine [54,55]. Sepp1 is mainly synthesized in kidneys and secreted to extracellular fluids, including plasma [56]. Delivery of selenium to testis and brain requires Sepp1 [57,58] through the receptor-mediated uptake by apolipoprotein E receptor-2, and to kidneys via the megalin membrane protein [59,60]. Sepp1 knockout rats show an increased excretion of selenium by the urine system [61]. In addition to its well accepted role as a selenium transporter, a peroxidase activity of Sepp1 has also been reported [62].

21.2.3.5 Other Notable Selenoproteins

Proteins in the Sep15 family, including Sep15 and selenoprotein M, control the quality of protein-folding in endoplasmic reticulum (ER) [63]. Sep15 expresses highly in liver, kidney, testes, and prostate [24], whereas selenoprotein M is mainly found in brain. Sep15 cooperates with the chaperon protein UDP-glucose:glycoprotein glucosyltransferase to regulate protein-folding in ER [64]. Sep15 may also participate in disulfide bond modifications on unfolded and misfolded proteins [65]. Furthermore, a recent report implicates Sep15 in the promotion of colorectal tumorigenesis and metastasis [66].

Selenoprotein H (SelH) is considered a dual function protein involved in redox regulation and transactivation of phase II antioxidants and other stress-responsive proteins. In mice, SelH mRNA is mainly expressed in the brain, thymus, lung, testes, and uterus, as well as in certain cancer cells and during embryogenesis [67] SelH was initially identified as a thioredoxin reductase homolog with glutathione peroxidase-like activity [67]. Overexpression of human SelH in mouse HT22 neuronal cells improves cell survival after UVB irradiation through the suppression of superoxide production [68]. Moreover, SelH-overexpressed cells show elevated mitochondrial biogenesis and functions [69]. Based on protein domain search, SelH carries an AT-hook domain and thus can potentially bind DNA minor grooves for transactivation of the targeted genes [70]. By employing chromatin immunoprecipitation, Panee and colleagues have demonstrated the appearance of GFP-tagged SelH on the heat shock element (HSE) and stress response element [70]. Furthermore, metal transcription factor-1 can transactivate SelH by binding to its metal response element of the promoter [71]. Because oxidative stress can possibly be induced by heavy metals, SelH expression can in principle be upregulated by environmental toxicants. SelH is known to be localized to nucleoli under the condition of GFP-tagged SelH overexpression in NIH 3T3 cells [67], suggesting a role for SelH in rDNA metabolism.

Available lines of recent evidence suggest critical physiological roles of other less characterized selenoproteins. Selenoprotein N mutations lead to a human genetic disorder, rigid spine muscular dystrophy, potentially through the induction of protein oxidation, calcium handling abnormalities, and predisposition to oxidative stress [72,73]. Selenoprotein W is highly expressed in muscle, heart, spleen, and brain, and regulates immune responses and the thioredoxin-dependent redox pathway in humans [74]. Selenoprotein R, also known as methionine sulfoxide reductase B, is one of the enzymes in the methionine sulfoxide system and the protein expression is closely associated with body selenium status [75]. Maintenance of the functional methionine sulfoxide system prevents protein–carbonyl adducts and age-related neurodegeneration [75,76]. Future studies on mechanistic and physiological investigations of these and other newly identified selenoproteins will provide critical insight into the battle against aging and age-related chronic diseases.

21.3 SENESCENCE

Senescent cells are at a stage of permanent withdraw from the cell cycle. A complete cell division cycle includes continuous advancement in the sequence of the G0/G1, S, and G2/M phases. Single cell organisms maintain normal cell cycles in order to propagate. In multicellular organisms, proliferation helps to renew or replenish cells in organs and tissues. Senescence can be induced at least by unrepaired DNA damage, dysfunctional telomeres, acute conformational changes of chromosome, and mitogenic stimuli. Broadly speaking, there are two types of senescence: telomere-dependent replicative senescence, and stress-induced senescence.

Cellular senescence appears to be a double-edged sword regarding optimal health. Because cellular senescence completely stops proliferation, it holds promise as a strategy for tumor suppression, embryonic development, and renewal of old tissues. On the contrary, cellular senescence contributes to organismal aging by limiting proliferation of cells that need to be renewed. Hence, understanding how and when cellular senescence is regulated contributes to successful cancer and aging interventions.

21.3.1 Activation of Cellular Senescence

Mounting evidence links the DNA damage response to cellular senescence [77]. First, unrepaired DNA damage and dysfunctional telomeres can result in a persistent DNA damage response and activation of downstream mediators of cellular senescence. Second, the acute changes in chromosome structures, such as a switch from heterochromatin to euchromatin, can lead to cellular senescence through the DNA damage response to the relaxation of chromosome structure. Furthermore, mitogenic stimuli are known to promote proliferation at the expense of collapsed replication forks or misfired replications.

21.3.1.1 Dysfunctional Telomere and Persistent DNA Damage Response

Formation of endogenous DNA damage is inevitable as the errors are constantly generated during normal metabolism. By estimation, there are about 20,000 events of endogenous DNA damage per cell per day [78]. ROS, the most frequent inducers of endogenous DNA damage, are generated through the respiratory chain reaction in mitochondria and during many immune responses. Oxidative stress can induce the formation of oxidized guanine and DNA single-strand breaks (SSBs) and DNA double-strand breaks (DSBs) [79]. The so-called "end-of-replication" problem also contributes to genome instability. Replication of telomeric DNA at the very end of the lagging strand cannot be completed by DNA polymerase, resulting in telomere shortening [80]. When telomere attrition reaches a very short stage after many rounds of proliferation, the 3′ chromosomal end that was protected previously in the telomeric T-loop is exposed and triggers DNA damage response [81–83].

Acute DNA damage is usually induced by exogenous clastogens, including ionizing irradiation, UV light, mutagens, and carcinogens. UV irradiation from sunlight exposure is the most common form of clastogen and generates about 10^5 events of DNA damage per cell per day [78]. Although the majority of UV-induced DNA damage can be faithfully repaired, the same is not true for some forms of DNA damage, such as DSB and DNA crosslinks. Different from endogenous DNA damage, DNA breaks from exogenous clastogens are usually induced in a prompt manner.

The DNA damage response comprises checkpoint activation, DNA repair, senescence, and apoptosis, which collectively maintain genome stability and limit unrepaired cells from proliferation. In general, checkpoint activation allows time for DNA repair machinery to fix the lesion. If the DNA damage is too severe or the DNA repair capability is compromised, unrepaired DNA damage can induce senescence or apoptosis. Depending on the types of damage, they are addressed by different DNA repair pathways. Base excision repair corrects mutated bases generated by depurination, deamination, or oxidation through short patch or long patch sub-pathways [84]. Nucleotide excision repair fixes bulky helix distortions of DNA by global genome or transcription-coupled nucleotide excision repair [85]. Mismatch repair is a post-replication pathway that removes mis-incorporated or mutated bases and matches them with a correct one [86]. DSBs are primarily

repaired by homologous recombination (HR) and non-homologous end-joining (NHEJ). HR requires end-processing of the broken ends to generate a 3′ single-stranded tail in search of the homologous sister chromatid, resulting in error-free repair of the DSB. NHEJ is considered an error-prone repair of a DSB because it directly ligates two broken ends together that may include unrepaired DNA. Furthermore, the genome is protected by shelterin proteins that protect telomeres from being recognized as DNA breaks. Telomere elongation needs ataxia telangiectasia and Rad3 related (ATR) and ataxia telangiectasia mutated (ATM) protein kinases to activate HR for efficient telomere elongation. In the case of unprotected telomeres, these kinases can activate persistent DNA damage responses [82]. The genome stability is thus maintained by DNA repair mechanisms and by telomere stability machinery to prevent DNA damage accumulation.

Persistent DSBs and dysfunctional telomeres are the most pronounced inducers of cellular senescence [87,88]. The ATM kinase serves as the major mediator that senses and transmits the signal of DNA breaks to downstream DNA repair and checkpoint proteins. In particular, activated ATM phosphorylates p53 for proliferation arrest through the activation of p21, a gatekeeper controlling the G1–S transition. $p16^{INK4}$–retinoblastoma (Rb) is another pathway of cellular senescence by G1 arrest in the response to DNA damage [89], and is proposed as the secondary barrier to reinforce the growth arrest modulated by p53 when a telomere is dysfunctional [90].

21.3.1.2 Acute Chromosome Conformation Changes

Histone deacetylase (HDAC) inhibitors can drive acute changes in chromosome structures and activate cellular senescence. In general, histone acetyltransferase and HDAC are regulators that relax and pack the structure of chromosome, respectively [91]. HDAC inhibitors can induce the DNA damage response, heterochromatin perturbation, and p16- and/or p53-dependent senescence in human diploid fibroblasts [92]. When heterochromatin perturbation happens, DNA damage response is activated together with increased ATM phosphorylation and γH2AX formation [93]. HDAC inhibitors such as sodium butyrate and trichostatin can induce cellular senescence through induction of p16 expression and the $p16^{INK4}$–Rb pathway [92,94]. HDAC can suppress p16 transcription through its recruitment to the promoter region of p16 [95]. Although p53 is a substrate of Sirtuin 1, an HDAC [96], the role of p53 in cellular senescence induced by HDAC is not fully understood and may differ in various species and cell types [94,97].

21.3.1.3 Mitogenic Stimuli

Mitogenic stimulation is also a double-edged sword, because it can induce both cell proliferation and cellular senescence. Mitogenic stimuli are required to activate DNA replication and cell division in confluent and sparsely cultured cells, and for single cell proliferation to achieve a monolayer population. Interestingly, some mitogenic stimuli can also activate cellular senescence. For example, chronic exposure of tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, not only promotes proliferation by the activation of activator protein-1 [98] but also induces cellular senescence in endothelial progenitor cells through activation of the p38 mitogen-activated protein kinase (MAPK) pathway [99]. Furthermore, angiotensin II induces cell proliferation by induction of DNA synthesis in certain cell types, but it can also induce cellular senescence in endothelial cells through the activation of the MAPK pathway [100]. The other critical mitogenic stimulus is oncogene. When oncogenes, such as Ras, Raf, $BRAF^{E600}$, and STAT5A, are expressed in normal cells, cellular senescence can be induced [101–104]. Under the condition of constitutive activation of Ras, Raf, or $BRAFE^{600}$, the activated MAPK pathway can lead to cellular senescence through p53 and $p16^{INK4}$ accumulation. Additionally, Ras-induced senescence requires MEK, a MAPK downstream component whose activation alone can lead to cellular senescence in primary murine fibroblasts [105]. Another pathway involved in oncogene-induced cellular senescence is the DNA damage response. Oncogenic stimuli can trigger DNA hyperreplication, resulting in p53 and $p16^{INK4}$ induction, DNA damage, and checkpoint activation [104,106,107]. Thus, mitogenic stimuli at the early stage of tumorigenesis are tightly regulated by complicated signal transduction pathways for the prevention of malignant transformation through induction of cellular senescence.

21.3.2 Markers of Cellular Senescence

Senescent cells have been shown to exhibit specific phenotypes *in vivo* and *in vitro*. The phenomenon of cellular senescence was first reported by Hayflick and colleagues, who found that cultured human diploid fibroblasts divided at a declined rate after certain passages, followed by a complete stop in cell proliferation [108]. Currently, there are six major senescent phenotypes in cultured cells and mammalian tissues: expression of senescence-associated β-galactosidase (SA-β-gal), persistent DNA damage response or DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), increased $p16^{INK4}$ expression, senescence-associated heterochromatin foci (SAHF) formation, transformation into senescence associated secretary phenotype (SASP), and decreased lamin B1 expression [5].

21.3.2.1 SA-β-Gal

SA-β-gal is an abundant lysosomal enzyme with an optimal pH at 4 in young or immortal cells. However,

the activity is activated at pH 6 in senescent cells. The expression of SA-β-gal is highly correlated with cellular aging in cultured cells and in tissues from various animal species [109,110].

Persistent induction of the DNA damage response exists in senescent cells. Markers of the DNA damage response include those that appear rapidly at the proximity of the lesion, such as activation of ATM, ATR and the catalytic activity of DNA-dependent protein kinase ($DNA\text{-}PK_{cs}$), and the phosphorylation of their substrates [111]. Senescent cells continue to assemble proteins that sense, transmit, and mediate DNA damage signals at the site of unrepaired DNA damage [88,112]. In the liver of old *Terc*$^{-/-}$ mice, persistent γH2AX foci occur in shortened telomeres with senescence [113].

21.3.2.2 DNA-SCARS

DNA-SCARS is a form of permanent DNA damage associated with defects in DNA repair proteins [114]. Towards the end of the replicative lifespan of a cell, telomere attrition to a critically short level results in loss of protection by shelterin proteins and permanent activation of DNA-SCARS. Unprotected and exposed telomeres are recognized and marked by DSB repair machinery, resulting in persistent activation of the DNA damage response at chromosome ends [115]. As such, ATM constantly transduces DNA damage signals to downstream checkpoint kinases such as CHK2, which in turn activates p53 and p21 for senescence induction. Noticeably, this is distinct from another senescence pathway activated by $p16^{INK4}$–Rb [116,117]. Therefore, dysfunctional telomeres can provoke genome instability and the subsequent cellular senescence by the formation of DNA-SCARS.

21.3.2.3 Increasing $p16^{INK4}$ Expression

$p16^{INK4}$ promotes cellular senescence by the maintenance of Rb at hypophosphorylation status through the inhibition of cyclin-dependent kinases. In the human genome, $p16^{INK4}$ and $p19^{ARF}$ are encoded from the *INK4/ARF* locus on chromosome 9 by alternative splicing. These two proteins play important roles in cell cycle regulation and senescence induction. $p19^{ARF}$ mediates the p53 pathway, whereas $p16^{INK4}$ regulates the Rb pathway [118–120]. Expression of $p16^{INK4}$ results in a G1 cell cycle arrest by inhibiting Rb phosphorylation and dissociations of CDK4 or CDK6 from cyclin D and Rb [121–124]. Furthermore, Rb hypophosphorylation by $p16^{INK4}$ induction promotes cellular senescence when cells encounter persistent DNA damage or mitogenic stimuli [125,126]. Because the turnover of $p16^{INK4}$ mRNA is decreased in senescent than in proliferating cells, $p16^{INK4}$ can also serve as an indicator of cellular senescence [127–131].

21.3.2.4 SAHF

Senescence-associated heterochromatin foci (SAHF) are structurally different from regular heterochromatin, and can be induced in senescent cells. SAHF were first observed in senescent human diploid fibroblasts [132]. Like other characteristics of heterochromatin, SAHF formation is accompanied by the expression of histone H3 methylation on lysine 9 and heterochromatin protein 1 (HP1) on the pericentric regions of chromosomes. However, SAHF distinctly accumulate phosphorylated HP1γ and high-mobility group A (HMGA) proteins in a manner requiring HIRA, ASF1, and macroH2A proteins [133,134]. Another distinctive feature of SAHF is the loss of histone H1, which can be explained by the observation that HMGA protein is a stronger competitor than histone H1 in binding linker DNA in senescent cells [135]. SAHF can also be formed by the induction of mitogenic stimuli. This chromatin conformation change needs the $p16^{INK4}$–Rb pathway activation to inhibit the transactivation function of E2F. The binding of Rb to some of the E2F responsive genes is critical for heterochromatin formation and gene silencing [132]. Additionally, inhibition of the Wnt signaling pathway leads to cellular senescence and SAHF formation in a manner independent of p53 or Rb [136]. It is thus believed that chromosome reorganization and the formation of SAHF are guided by pathways involved in cellular senescence but not proliferation.

The formation of SAHF depends on types of cells and stimuli. MRC-5 and BJ human normal fibroblasts and primary keratinocytes form SAHF upon oncogenic stimulation. However, only MRC-5 cells can form SAHF after etoposide, doxorubicin, and hydroxyurea exposure, bacterial intoxication, and telomere attrition [137]. These findings raise important questions on the mechanistic basis of SAHF formation and how to appropriately consider SAHF as a senescence marker. Although SAHF do not universally appear, they are useful to distinguish senescent from proliferative or quiescent cells [132].

21.3.2.5 Lamin B1 Underexpression

Lamins are nuclear structural proteins that provide critical barriers against genome instability in the nuclear envelope through DNA repair, DNA replication, transcription control, and chromatin organization [138–142]. The nuclear envelope is composed of an outer membrane, an inner membrane, nuclear pore complexes, and the lamina. The A- and B-type lamins collectively comprise lamina. In mammals, A-type lamins, lamin A and C, are encoded by the *LMNA* gene, whereas lamin B1 and lamin B2 are transcribed from the *LMNB1* and *LMNB2* genes, respectively. A-type lamins are mainly expressed in fully differentiated cells, while B-type lamins are abundant during cell development [143–145]. Interestingly, mutations in the *LMNA* gene lead to several human

diseases, including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, atypical Werner syndrome, and Hutchinson-Gilford progeria. However, human genetic diseases are more likely to be developed by mutations in A-type than B-type lamins because of the prominent role of lamin A in the maintenance of nuclear structural stability. Nonetheless, lamin B1 level does not decrease in stress-induced senescence in cancer cells.

Lamin B1 is a newly identified marker of cellular senescence. Although lamin B1 is not as ubiquitous as lamin B2 in human tissues, its expression is highly associated with cell proliferation. Although lamin B1 is expressed in proliferative, quiescent, and senescent cells [146], the level of lamin B1 is decreased when p53 or Rb is activated and the turnover of lamin B1 protein and mRNA is enhanced in senescent cells [147].

21.3.3 Transforming into SASP

The senescence associated secretary phenotype (SASP) controls the senescence microenvironment through non-autonomous regulation. SASP was first described in oncogene-expressing cells displaying a persistent DNA damage response and increased secretion of a list of chemokines and cytokines [148]. Knockdown of chemokine receptor CXCR2 alleviates replicative and oncogene-induced senescence and decreases the DNA damage response, suggesting that chemokines are secreted in senescent cells to reinforce permanent cell cycle arrest in the surrounding non-senescent cells [148,149]. Cells with SASP secrete soluble factors that include interleukins, chemokines, growth factors/regulators, inflammatory factors, proteases, receptors, ligands, PGE2, nitric oxide, and ROS. Furthermore, SASP is thought to counteract tumorigenesis through modulation of the innate immune response for tumor clearance and senescence induction in the nearby precancerous cells [150–152].

Although senescence induction requires the activation of p16–Rb and/or p53–p21 pathways for cell cycle arrest, the activation of SASP is not p16-dependent or simply an inevitable consequence of p16-induced senescence [153]. In contrast, activation of p53 inhibits SASP and promotes autonomous regulation of the cells [154]. Such knowledge is helpful for the extrapolation of cellular senescence to organismal aging. While p53 signaling subsides with age, its inhibition of the SASP is alleviated such that the inflammatory signaling is activated. As such, SASP-mediated non-autonomous regulation dominates over the p16- or p53-dependent autonomous pathway. This supports the microenvironment theory of SASP through cell non-autonomous regulation [155].

The p38–NF-κB pathway also plays a pivotal role in SASP. The p38 MAPK is a stress-induced kinase greatly activated upon induction of senescence. Differently from the DNA damage response, which may not always activate SASP, p38 induction is sufficient to turn SASP on [156]. The p38–MAPK pathway activation transmits signals to NF-κB, serving as an effective transactivator for the expression of multiple secretary factors. Interestingly, senescence induction by the oncogenic Ras can be reversed by knocking down NF-κB [155,157,158]. Thus, inflammatory factors from a senescent cell can stimulate senescence in non-senescence cells through circulation.

21.3.4 Diverse Dealings of Cellular Senescence

Studies have shown that cellular senescence plays critical roles in embryonic development and patterning, tissue regeneration, and chemoprevention [159–162]. Conversely, cellular senescence arrests damaged cells from proliferation, eventually removing them from the organs or tissues through macrophage-mediated clearance or apoptosis to facilitate replenishing new cells from stem cells [163,164]. Interestingly, the removal of senescent cells by inducible apoptosis can protect tissues and organs against age-associated disorders in older mice [165]. Similarly, cellular senescence can be activated by trace elements such as selenium. Wu and colleagues have shown that selenium compounds at doses less than LD_{50} can induce senescence by the activation of DNA damage responses in non-cancerous but not cancerous cells, suggesting that tumorigenesis can be stifled by selenium-induced activation of early tumorigenesis barriers [12,166].

In contrast, SASP can promote both aging and carcinogenesis. Non-autonomous regulation by SASP may accelerate cellular senescence in surrounding normal cells toward tissue or organismal aging and the proliferation of precancerous cells through the secretary cytokines and chemokines. With such changes in secretory profile, premalignant cells are prone to transform into malignant cells. In lamin A-defective mice, SASP induces NF-κB dependent systematic inflammation in association with ATM activation [167]. Thus, cellular senescence in a broader perspective can prevent carcinogenesis and tissue regeneration, yet promote aging and tumorigenesis, depending on the timing and location of senescence activation.

Is cellular senescence beneficial or detrimental to multicellular organisms? Proteins in the senescence-associated responses are critical in determining how the cell senesces. For example, the ATM–p53 pathway activation counteracts tumorigenesis by the induction of cellular senescence [161]. Although ATM and p53 play central roles in the activation of senescence response to damaged cells, the detailed molecular mechanism through which they modulate senescence is largely unknown. Understanding how ATM and p53 function in the interphase between aging and longevity, or between carcinogenesis and cancer suppression, will advance future biomedical research, in an attempt to redefine or update the remedy of cancer and aging interventions [162].

21.4 ATM ACTIVATION IN DNA DAMAGE RESPONSE

ATM plays a central role in transducing DNA damage signals to downstream effectors for checkpoint activation, DNA repair, and programmed cell death. The most important DSB sensor is believed to be poly(ADP-ribose) polymerase 1, which recruits Mre11-Rad50-NBS1 (MRN) protein complex to the damaged site [78,168]. In turn, ATM is recruited by MRN and subsequently phosphorylates NBS1 and other proteins for S-phase checkpoint activation [169–171]. ATM pathway activation is initiated by ATM autophosphorylation at Ser-1981 in response to DSB or oxidative stress [172,173]. Another two kinases with similar DSB sensing and signaling functions are DNA-PK_{cs} and ATR. Together with the Ku70/Ku80 sensor proteins, DNA-PK_{cs} initiates the NHEJ pathway of DSB repair [174]. For ATR, the DNA damage sensor is ATRIP [175]. ATM can functionally interact with DNA-PK_{cs} and ATR. ATM and DNA-PK_{cs} collaborate in response to DSB [176–178]. Moreover, the MRN complex cooperates with ATM to resect a DNA end for the generation of a 3′-single strand tail, which recruits replication protein A to set the stage for ATR-dependent HR repair. Similarly, if ATR-mediated HR is incomplete, ATM can be involved in the DNA damage response. Furthermore, p53 phosphorylation by ATM results in increased p53 stability and induction of cellular senescence or apoptosis [179,180]. Because ATM can potentially phosphorylate hundreds of substrates [181], this kinase plays critical upstream roles in the control of various types of cellular stress.

Activated ATM functionally regulates p53 by multiple post-translational modification events. Because p53 is constitutively associated with murine double minute 2 (MDM2) and delivered to cytosol for proteolytic degradation, its half-life is short (30 minute) in proliferating cells [182,183]. However, activated ATM phosphorylates MDM2 on Ser-395, resulting in a dissociation of p53 from MDM2 and the subsequent increase in p53 steady-state level. p53 is further stabilized by CHK2 phosphorylation on Ser-20, the residue for MDM2 binding [184,185], resulting in increased steric hindrance of the MDM2–p53 interaction. Furthermore, activated ATM can phosphorylate p53 on Ser-15 for increased p53 stability [180,186,187]. The direct or indirect modifications by ATM determine the transactivation and other activities or stability of p53, promoting cellular senescence and apoptosis. The various functions of p53 are discussed below.

21.5 ROLES OF P53 IN DNA DAMAGE AND SENESCENCE RESPONSES

Although p53 is generally considered to be a tumor suppressor, this protein is usually mutated during tumorigenesis and was originally recognized as a tumor cell-specific antigen or an oncogene in both humans and mice [188,189]. Most human cancers harbor p53 mutations, more than 80% of which are missense mutations [190]. Thus, p53 mutations are validated biomarkers for cancer diagnosis [191]. In particular, in some cases of breast cancer, at least three mutation sites can be found in the p53 gene [191,192]. In fact, p53 contributes to the transformation-activating feature of the oncogenic *Ras* or *Myc* [193], yet overexpression of wild-type p53 compromises this event of tumorigenesis [193,194]. Expression of the carboxyl-terminal p53 in mice reduces the incidence of tumor [195]. Furthermore, overexpression of p53 renders the "super p53" mice increased resistance to carcinogens but aged normally [196].

p53 transactivates or represses its target genes through transactivation, DNA binding, tetramerization, and nuclear localization domains of p53. Activated p53 forms a tetramer and serves as a transactivator, targeting the p53 response element that is composed of two 10-nucleotide decamers with a 0–13 nucleotide spacer in between. Although functional p53 response elements usually contain only less than three nucleotides at the spacer, sometimes a non-canonical element exists in the active p53 response element [197]. The N-terminal p53 contains the transactivation and DNA-binding domains for the transcriptional regulation of genes involved in redox regulation, metabolism regulation, DNA repair, autophagy, cell cycle arrest, cellular senescence, and apoptosis. A list of known p53 transactivation targets is shown in Table 21.1 [35,198–272]. However, in other cases, p53 functions differently and suppresses the transcription of downstream genes [234,246]. For example, when cells with UV-induced p53–p21 activation, p53 can bind to the promoter region of the p202 gene, which encodes an interferon-inducible negative regulator, and leads to gene repression [246].

Furthermore, p53 can serve as an effector protein in the response to DNA damage to activate or sustain cellular senescence. Activated p53 transactivates p21 expression that inhibits CDK2-cyclin E, resulting in G1/S arrest [272]. Additionally, p53 can possibly promote G2/M arrest through the 14–3–3 complex [241]. Because the activation of cell cycle arrest is a prerequisite for cellular senescence, p53 is a critical regulator of cellular senescence that receives and transmits signals of DNA damage response.

21.6 PATHWAY CROSSTALK IN SENESCENCE

Evidence ranging from cultured cells to mammals suggests that cellular senescence can be induced by various forms of nuclear stress, including DNA damage and

TABLE 21.1 p53-Regulated Genes in Anti-stress, Metabolism, DNA Repair, Cell Cycle, and Apoptosis

Target	Activation/Inhibition	Species	Reference
Anti-stress			
FLJ11259/DRAM	Activation	Human	[198]
Glutathione S-transferase P1 (GSTP1)	Activation	Human	[199]
Interferon regulatory factor-2-binding protein-2 (IRF2BP2)	Activation	Human	[200]
Serine/threonine kinases 17A	Activation	Human	[201]
Glutathione peroxidase 1	Activation	Human	[35]
Metabolism			
Tissue inhibitor of metalloproteinase-3 (TIMP3)	Activation	Mouse	[202]
Brain-specific angiogenesis inhibitor 1	Activation	Human	[203]
Smooth muscle alpha-actin	Activation	Human	[204]
Human metalloproteinase-1	Inhibition	Human	[205]
Thiamine transporter 1	Activation	Mouse	[206]
Staf50 (TRIM22)	Activation	Human	[207]
Podocalyxin	Inhibition	Human	[208]
Fatty acid synthase	Activation	Worm to human	[209]
Vitamin D receptor	Activation	Human	[210]
Polycystic Kidney Disease-1 Gene	Inhibition	Human	[211]
Notch1	Activation	Human	[212]
Brain-expressed RING finger protein	Activation	Human	[213]
SPATA 18	Activation	Human	[214]
Parkin	Activation	Human or mouse	[215]
Thrombospondin-1	Inhibition	Human	[216]
TIGAR	Activation	Human	[217]
DNA damage repair			
GADD45	Activation	Human	[218]
Phosphotyrosyl phosphatase activator	Activation	Human	[219]
Ribonucleotide reductase (p53R2)	Activation	Human	[220]
DinB	Activation	Mouse	[221]
DNA polymerase eta (PolH)	Activation	Human	[222]
Pierce 1	Activation	Mouse	[223]
Damage-specific DNA binding protein 2	Activation	Human	[224]
Fanconi anemia, complementation group C	Activation	Human	[225]
PMS2	Activation	Human	[226]
MLH1	Activation	Human	[226]
Proliferating cell nuclear antigen	Activation	Human	[227]
Xeroderma pigmentosum, complementation group C	Activation	Human	[228]
Cell cycle			
Cyclin G	Activation	Rat	[229]
p22/PRG1	Activation	Rat	[230]
PTGF-β	Activation	Human	[231]

(*Continued*)

TABLE 21.1 (Continued)

Target	Activation/Inhibition	Species	Reference
DDA3	Activation	Mouse	[232]
Wig-1	Activation	Human	[233]
Protein regulator of cytokinesis	Inhibition	Human	[234]
SMART1	Activation	Human	[235]
DEC1	Activation	Human	[236]
Prl-3 (phosphatase of regenerating liver-3)	Activation	Human	[237]
DUSP11 (dual specificity phosphatase 11)	Activation	Human	[238]
Necdin	Activation	Mouse	[239]
Murine double minute 2	Activation	Mouse	[240]
14-3-3 protein	Activation	Mouse	[241]
B99	Activation	Mouse	[242]
Insulin growth factor-binding protein 3	Activation	Human	[243]
GPI-anchored molecule-like protein	Activation	Human	[244]
Apoptosis			
MCG10	Activation	Human	[245]
P202 (Interferon-inducible phosphoprotein)	Inhibition	Human	[246]
mRTVP-1	Activation	Mouse	[247]
Snk/Plk2	Activation	Mouse	[248]
CD200	Activation	Mouse	[249]
SIVA	Activation	Mouse	[250]
Decoy receptor 2	Activation	Human	[251]
Epithelial cell kinase	Activation	Human	[252]
TIS11D	Activation	Human	[253]
RhoE (Inhibit ROCK I)	Activation	Human	[254]
APLP1	Activation	Human	[255]
Beta 1,4 GalTII	Activation	Human	[256]
Apoptosis-enhancing nuclease (AEN)	Activation	Human	[257]
TNFSF10 (TRAIL)	Activation	Human	[258]
S100 calcium-binding protein A9 (S100A9)	Activation	Human	[259]
AlphaB-crystallin	Activation	Human	[260]
PUMA	Inhibition	Human	[261]
Foxo3	Activation	Mouse	[262]
Bax	Activation	Human	[263]
Fas (APO-1/CD95)	Activation	Human or mouse	[264]
KILLER/DR5	Activation	Human	[265]
PAG608	Activation	Mouse	[266]
p53-regulated apoptosis-inducing protein 1 (p53AIP1)	Activation	Human	[267]
p53-dependent damage-inducible nuclear protein (p53DINP)	Activation	Human	[268]
Apoptosis protease-activating factor 1	Activation	Human	[269]
PERP	Activation	Mouse	[270]
PIDD	Activation	Human	[271]

chromatin alterations [273–275]. Interactions between p53 and other pathways represent novel autonomous or non-autonomous regulation in senescent cells. As described above, "super p53" mice with additional copies or constitutive expression of p53 are resistant to tumorigenesis [195,196]. As aging progresses, the DNA damage response and dysfunctional telomeres can activate p53, senescing the cells in a premalignant stage. However, in some cases p53/p21 and p16 are thought to initiate and maintain cellular senescence, respectively [89,90]. Crosstalk between p53 and Rb pathways may need the intermediate p16 protein, which regulates p21 accumulation by transcriptional or post-translational regulation to reinforce cellular senescence [276,277]. Interestingly, in immortalized cells, overexpression of exogenous p21 promotes demethylation at the promoter of p16 gene. Thus, the expression of p16 is restored for induction of growth arrest or cellular senescence [278]. The p16–Rb pathway is not a redundant pathway to activate cellular senescence for tumor suppression; rather, it is important and meant to secure the cooperation with p53 and p21. If the regulation of cellular senescence is properly controlled by these effector proteins, the individual may live longer with a decreased incidence of cancer.

Furthermore, not all pathways of senescence are associated with the DNA damage response. For example, the senescence-associated lamin B decline is independent of the p38MAPK–NF-κB pathway, ROS, or DNA damage response. Rather, p53 and p16 pathways are activated. On the contrary, cellular senescence induced by chromatin perturbation is p53- and p16-independent. Also, there may be cell type-specific senescence phenotypes in the response to various stimuli [137,147,153].

21.7 SELENIUM AND CELLULAR SENESCENCE

Bruce Ames and Joyce McCann estimated that at least 11 selenoproteins in mammals are linked to senescence, aging, and age-related disorders [279]. In contrast, excessive amounts of selenium may activate cellular senescence and prevent carcinogenesis [12]. Few studies have directly investigated the link between selenium and senescence, but evidence suggests that both selenoproteins and selenium metabolites are involved. Cellular and body selenium status alter the redox status and the expression of selenoproteins. The majority of selenoproteins contribute directly or indirectly to lowering oxidative stress, potentially safeguarding the cells and the body from stress-induced senescence. As such, selenium deprivation in principle can promote senescence as a result of selenoprotein deficiency and increased oxidative stress. Consistent with this notion, fibroblasts derived from GPx1 knockout mice show senescence-like phenotypes with reduced proliferative capacity [280]. Since severe DNA damage is a major contributor of senescence, deficiency in antioxidant selenoproteins may sensitize cells to oxidative stress. LNCaP prostate cancer cells with GPx1 knockdown show increased sensitivity to UV-induced DNA damage, suggesting a protective role of GPx1 against genome instability [281]. Moreover, a study based on a population in central Europe demonstrates that levels of selenium in plasma are inversely associated with the extent of DNA damage [282]. Another epidemiological study conducted in New Zealand, where the body selenium levels of the residents are among the lowest in the world, shows a positive correlation between low plasma selenium and DNA damage accumulation, and suggests that residents may be prone to developing age-related disorders [283].

Induction of DNA damage and senescence responses are key events at the precancerous stage for halting cell proliferation. The observations that selenium compounds can activate cellular senescence in non-cancerous cells and that GPX1-deficient cells show senescence-like phenotypes indicate a possibility of chemoprevention by supranutritional selenium [12,280,284]. According to the conclusion made from the NPC clinical trial, selenium supplementation reduces the incidence of carcinoma development in several organs [11]. ROS induction by supranutritional selenium is likely to activate a mild DNA damage response. With a persistently activated DNA damage response, cell proliferation can be halted permanently in the form of cellular senescence through p53 and ATM [166]. In human diploid fibroblasts, selenium compounds at doses lower than that which will kill 50% of the population can induce oxidative stress and the DNA damage response for senescence induction. On the contrary, selenium treatment does not induce a senescence response in human prostate and colorectal cancer cells [12,285]. Thus, supranutritional selenium may play a role in the suppression of proliferation in premalignant cells to ensure chemoprevention [12]. Altogether, understanding the mechanism by which selenoproteins and selenium metabolites regulate senescence processes is necessary to rationalize selenium intervention for protection against senescence-associated chronic disorders, and will advance the prime goal in achieving optimal health.

21.8 FUTURE PERSPECTIVES

Personalized nutrition should be taken into consideration regarding selenium needs. The recommendation of dietary selenium consumption needs to be adjusted based on body selenium status and the form of dietary selenium. Although the NPC and SELECT trials provided inconsistent results on selenium being

a chemoprevention agent, the causes of such confusing data have been addressed to a large extent. The discrepancy may be attributed to different basal levels of body selenium in participants, and the form of selenium [14,286]. If basal body selenium status prior to entering the trials is high, individuals do not benefit from selenium supplementation; however, in the population with lower body selenium status, selenium supplementation indeed confers cancer chemoprevention. Additionally, the forms of selenium supplementation are different in the two clinical trials. Different selenium compounds have their preferred targets of metabolism. Supported by results from animal studies, it is clear that selenomethione is not an ideal selenium speciation for chemoprevention [287,288]. Moreover, the risk of diabetes after selenomethionine supplementation was found to be increased in those who have a high basal level of body selenium. Thus, only those with suboptimal body selenium status benefit from supplementation of certain forms of selenium. The beneficial effect occurs only within a narrow range surrounding the level of nutritional adequacy. However, the best form of selenium for chemoprevention in humans is not known.

The stage of the life cycle also plays a critical role in selenium supplementation. In human normal aging, defects accumulated in the body gradually contribute to symptoms of aging and age-related disorders. An optimal body selenium status in principle mitigates disadvantages but maximizes advantages towards healthy aging. It is possible that, early in life, supranutritional selenium is beneficial to secure the full expression of selenoproteins for maintenance of physiological functions and mild oxidative stress for cancer prevention by activation of senescence in premalignant cells. At the mid-life stage, the need for selenium in the body may be decreased and intake should be curtailed to an adequate level. Since genome instability can be induced by ROS and accumulates with age, oxidative stress generated from excess selenium may exacerbate the aging process. On the other hand, previous studies have shown that increased selenium consumption or overexpression of specific selenoproteins such as GPx1 and selenoprotein P may promote diabetes-like symptoms [37,289]. As such, lowering dietary selenium intake to an adequate level may maintain functional glucose homeostasis and thus healthy aging. Later in life, dietary selenium deficiency may be beneficial. This is supported by our unpublished findings in that selenium deficiency activates anticarcinogenesis miRNAs and that selenoprotein H deficiency promotes early onset of cellular senescence, a tumorigenesis barrier. Altogether, it is equally important to adjust the recommendation of dietary selenium consumption according to the stage of life cycle, body selenium status, and selenium speciation for an optimized health span and lifespan.

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CHAPTER

22

Nutritional Strategies Against Sarcopenia of Aging: Current Evidence and Future Directions

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22.1 INTRODUCTION

The age-related loss of muscle mass and function (sarcopenia) is one of the most dramatic changes that accompany aging, due to its association with a number of adverse health outcomes (e.g., frailty, falls, disability, institutionalization, and mortality) [1]. The complex and incompletely understood pathogenesis of sarcopenia explains the current lack of effective pharmacological remedies. In this scenario, an adequate protein and energy intake together with physical exercise (resistance, aerobic, or their combination) is presently indicated as the most effective strategy to manage sarcopenia [2].

After a concise overview of common age-associated changes in nutrition, this chapter summarizes the current nutritional recommendations to protect against sarcopenia, with a particular emphasis on those released by the Society for Sarcopenia, Cachexia, and Wasting Disease [3]. The following sections introduce novel nutritional candidates and dietary strategies that may be harnessed to preserve muscle mass and function into old age. The last part discusses possible threats resulting from the inappropriate prescription of nutritional supplements commonly considered as remedies against sarcopenia, and the prospect of tailoring dietary recommendations through the implementation of multimodal approaches.

22.2 AGE-RELATED CHANGES IN EATING HABITS

Food intake declines gradually throughout adulthood. Several factors, including masticatory disability and some medications, contribute to reducing food consumption in later life [4]. Furthermore, advancing age in itself is associated with a physiologic reduction in appetite ("anorexia of aging") that can eventually evolve into pathologic anorexia and malnutrition [5]. In addition, older people often develop a predilection for energy-dilute foods, such as grains, vegetables, and fruits, in place of energy-dense sweets and protein-rich nutrients [5]. As a consequence of these factors, the prevalence of malnutrition ranges from 5% to 20% in community-dwelling older adults, and exceeds 60% in the institutionalized elderly [6].

22.3 CURRENT NUTRITIONAL RECOMMENDATIONS AGAINST SARCOPENIA

22.3.1 Proteins, Amino Acids, and Derivatives

The balance between protein synthesis and breakdown is critical to muscle tissue homeostasis [7]. Indeed, the

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ingestion of an adequate amount of dietary protein is necessary to maintain muscle functionality in advanced age [8]. Older persons are at high risk for insufficient protein intake, with approximately 40% of men and women older than 50 years consuming less than the recommended dietary allowance (0.8 g/kg per day) [9]. Older people may actually need to ingest a higher quantity of protein than that recommended for younger subjects in order to overcome age-related changes in protein metabolism, such as high splanchnic extraction and declining anabolic responses to dietary proteins [3].

The European Union Geriatric Medicine Society (EUGMS), in cooperation with other scientific organizations, recently appointed an international study group to review dietary protein needs in the elderly (PROT-AGE Study Group) [8]. The group recommended an average daily intake of at least 1.0–1.2 g/kg [7]. A higher protein intake (1.2–1.5 g/kg per day) is advised for those practicing physical exercise or suffering from acute or chronic diseases, unless presenting with severe kidney failure [8].

Two recent complementary clinical trials reported the effects of protein supplementation (alone or in combination with resistance training) on physical function and body composition in community-dwelling frail elderly [10,11]. Overall, results showed that protein supplementation alone may have a positive effect on physical function, and that a higher protein intake may significantly modify body composition when associated with resistance training.

As for the feeding pattern, the available evidence does not allow provision of definite recommendations [12]. Pulse-feeding (i.e., a main high-protein meal, usually at midday) has been reported to be effective at improving whole-body protein anabolism [13,14]. However, a spread-feeding pattern (i.e., ingestion of 25–30 g of high-quality protein at each meal) may be easier to maintain over the long term, while ensuring a greater 24-hour anabolic response [15].

The amino acid composition of dietary proteins has a great impact on muscle protein metabolism. The essential amino acid (EAA) leucine is recognized as the master dietary regulator of muscle protein turnover, due to its ability to activate the mammalian target of rapamycin (mTOR) pathway and inhibit the proteasome [16]. It is therefore recommended that older people consume protein sources containing a relatively high proportion of leucine and other EAAs (i.e., high-quality proteins) [3].

β-Hydroxy β-methylbutyrate (HMB) is a metabolite of leucine that is receiving increasing attention as a potential nutritional aid against sarcopenia [17]. In skeletal muscle, HMB inhibits protein breakdown, stimulates protein synthesis through the activation of the mTOR pathway, and increases fatty acid oxidative capacity [16]. Studies have shown HMB supplementation is able to reverse deficits in net anabolism and improve muscle strength in sarcopenic older adults, especially when combined with resistance exercise [18–20]. In addition, HMB supplementation may be considered to attenuate acute muscle atrophy in bedridden elderly patients [21].

22.3.2 Vitamin D

Serum levels of vitamin D decline steadily with aging, due to inadequate dietary intake, reduced sunshine exposure, impaired capacity of the skin to synthesize vitamin D under the influence of UV light, and diminished renal conversion of 25-hydroxy vitamin D to its active form [22]. Strikingly, 40–100% of US and European community-dwelling older adults show vitamin D deficiency, and the situation is even worse in the institutionalized elderly, as a consequence of poorer dietary intake, decreased sunshine exposure, and higher multimorbidity rates [23].

Low serum concentrations of vitamin D and high serum levels of parathyroid hormone are associated with reduced muscle mass and strength in older adults [24]. Furthermore, serum levels of vitamin D were found to be strong independent predictors of changes in muscle mass and strength in older community-dwellers over 2.6 years of follow-up [25].

The mechanisms whereby vitamin D influences muscle metabolism and trophism are still under debate. Actions of vitamin D are performed through binding to specific receptors (vitamin D receptors, VDRs) located on the cell surface and in the nucleus. The activation of VDRs modulates calcium uptake, phosphate transport across the cell membrane, phospholipid metabolism, production of inflammatory cytokines, satellite cell proliferation, and protein synthesis [26]. Interestingly, similar to steroid and thyroid hormones, the constitutive presence of VDRs within mitochondria has been detected in several cell types [27]. However, the function of mitochondrial VDR and its actual existence in skeletal myocytes have not yet been clearly established.

Regardless of the precise mechanism(s) of action, vitamin D supplementation in old age has shown to improve muscle function, reduce the incidence of falls, and ameliorate muscle fiber composition and morphology [28]. Based on these findings, it is currently recommended to measure serum levels of 25-hydroxy vitamin D in all sarcopenic patients and to prescribe vitamin D supplements (800 IU (20 μg)/day) to those with values lower than 100 nmol/l (40 ng/ml) [3].

22.3.3 Creatine Monohydrate

Creatine monohydrate (Cr) is often recommended as an ergogenic aid due to its function as a temporal and spatial energy buffer [29]. Studies have also found that Cr administration stimulates muscle anabolism by

acting on protein kinetics, satellite cell function and content, and anabolic hormone secretion [30,31].

Cr supplementation to older adults engaged in strength training programs has shown to improve muscle mass and strength, with possible long-term residual beneficial effects after the end of the intervention [32–35]. Interestingly, the combination of Cr and whey protein appears to potentiate the muscle anabolic effects of the two nutrients during resistance exercise [32], suggesting a synergistic effect on anabolic pathways. Other studies, however, did not find additional benefits of Cr supplementation in terms of muscle mass and strength buildup in older persons engaged in resistance training over exercise alone [36,37].

Given these uncertainties, further investigations are required to establish the optimal dosing and timing of Cr supplementation in the sarcopenic elderly. As of now, Cr administration may be considered in older people who are Cr-deficient or at risk of Cr deficiency [3,8].

22.4 NEW DIETARY CANDIDATES FOR THE MANAGEMENT OF SARCOPENIA

22.4.1 Omega-3 Fatty Acids

In recent years, supplementation with omega-3 fatty acids (ω-3 FAs) has been proposed as a possible aid against sarcopenia [38]. The consumption of fatty fish (the richest dietary source of ω-3 FAs) is positively associated with grip strength in older persons [39]. In addition, supplementation with ω-3 FAs could enhance the anabolic effects of protein/amino acid supplementation and exercise training [40–43]. The daily administration of a formulation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 8 weeks increased the rate of muscle protein synthesis in healthy older persons [40]. Moreover, 12-week supplementation with α-linolenic acid (α-LNA) combined with resistance training reduced circulating levels of interleukin-6 (a cytokine involved in muscle wasting), although the effects on muscle mass and strength were marginal [41].

Although there is no established dietary reference intake (DRI) for ω-3 FAs, the adequate intake for EPA is set at 1.6 and 1.1 g/day for men and women over 50 years of age, respectively [44]. The DRI for α-LNA in the same age group is established at 11 g/day for men and 14 g/day for women. Such an intake is ensured by the consumption of at least two servings of fish a week. Fatty fish, such as salmon, mackerel, herring, lake trout, sardines, and albacore tuna, and their oils are the best food sources of ω-3 FAs (and, interestingly, of vitamin D).

The risk for adverse events associated with long-term ω-3 FA supplementation (e.g., prolongation of bleeding time, atrial fibrillation and flutter, and liver toxicity) in older people needs to be clearly established. Finally, a warning has recently been raised regarding the existence of an association between high circulating levels of ω-3 FAs and increased risk of prostate cancer in elderly men [45]. These observations impose a special caution in the prescription of ω-3 FAs as a remedy against sarcopenia.

22.4.2 Ursolic Acid

Ursolic acid is a lipophilic pentacyclic triterpenoid, especially abundant in apple peel, with anti-inflammatory and antihyperlipidemic properties [46]. Recently, ursolic acid has been identified as a possible remedy for muscle atrophy in a screen for small-molecule inhibitors of skeletal muscle wasting in mice [47]. Interestingly, supplementation with ursolic acid to mice on a high-fat diet increased muscle mass and strength, improved glucose tolerance, promoted brown adipose tissue deposition, and decreased white adipose tissue mass and the severity of hepatic steatosis [48].

These findings suggest that ursolic acid might represent an effective nutritional aid to counteract age-related changes in body composition and metabolic derangements. However, the effect of supplementation with ursolic acid in older adults with sarcopenia has yet to be investigated.

22.4.3 Nitrates and Nitrate-Rich Foods

Recent evidence indicates that nitrate (NO_3^-) from both endogenous and dietary sources is metabolized to bioactive nitric oxide (NO) [49]. The latter, in turn, could promote muscle trophism by modulating several processes involved in the pathogenesis of sarcopenia. For example, dietary NO_3^- can increase gastric NO levels, which could relieve the early satiety typical of old age, therefore eliminating one of the components of anorexia of aging. In addition, the increase in circulating NO levels following NO_3^- ingestion could improve endothelial function and muscle perfusion. In a recent study, 7-day supplementation with nitrate-rich concentrated beetroot juice ameliorated excitation–contraction coupling at low frequencies of stimulation and enhanced evoked explosive force production in untrained young adults [50]. Supplementation with dietary inorganic NO_3^- has also shown to improve muscular mitochondrial bioenergetics in young, healthy subjects [51]. This effect may have potential implications for the management of sarcopenia, given the central role postulated for mitochondrial dysfunction in the pathogenesis of age-related muscle degeneration [52]. However, these results have not been confirmed in older adults supplemented with NO_3^--rich concentrated beetroot juice for 3 days [53].

Given these contrasting findings, further research is necessary to establish whether NO_3^- supplementation can offer a therapeutic advantage against sarcopenia.

22.5 NUTRITIONAL STRATEGIES TO COUNTERACT MUSCLE AGING

22.5.1 Calorie Restriction Mimetics, Exercise Mimetics, and Gymnomimetics

Exposure to moderate physiological stressors such as calorie restriction (CR) without malnutrition and regular physical exercise exerts synergistic beneficial effects on muscle health [54]. However, the implementation of such behavioral interventions in older persons is hampered by difficulties in maintaining substantial food restrictions for the long term, and the frequent lack of motivation in engaging in exercise programs [55]. More importantly, weight loss may be harmful in non-obese older persons as it can accelerate muscle loss and increase the risk of disability and mortality [56].

A possible strategy to overcome some of these issues could be based on supplementation with dietary-derived CR mimetics (CRMs) and exercise mimetics (EMs). CRMs and EMs are phytochemicals with antioxidant properties that have been shown to partially mimic the signaling events that mediate some of the beneficial effects of CR and physical exercise [57]. Examples of mimetic compounds include resveratrol (found in grapes and red wine), quercetin (found in apples, onions, and berries), epigallocatechin-3-gallate (found in green tea), and nootkatone (found in grapefruit). Studies *in vitro* and in animal models have shown that these bioactive substances exert their beneficial actions through several pathways, including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), sirtuin 1, and AMP-activated protein kinase (AMPK) [57,58]. In addition, specific CRMs or EMs are able to promote skeletal muscle anabolism, attenuate muscle wasting, and improve muscle recovery both in aged rodents and in cellular models of muscle atrophy [59–62]. However, the effects of these mimetic agents on human muscle aging have not yet been investigated. Unexpectedly, a recent report has cast doubts on the actual efficacy of resveratrol supplementation in humans [63]. Indeed, resveratrol ingestion reduced the positive effect of exercise training on blood pressure, blood cholesterol, and maximal oxygen uptake, and did not affect the retardation of atherosclerosis in elderly men [63].

Interestingly, the exposure of cultured myotubes to a combination of metabolites, the levels of which were increased in the circulation in response to exercise (glycerol, niacinamide, glucose-6-phosphate, pantothenate, and succinate), resulted in upregulation of nur77 [64]. The latter is induced in skeletal muscle after exercise, and functions as a transcriptional regulator of genes involved in glucose and lipid metabolism. Notably, the "gymnomimetic" effect was not obtained when the metabolites were used separately.

Despite these intriguing findings, more research is needed to fully characterize the bioactive properties of CRMs, EMs, and gymnomimetics, and clearly identify the cellular pathways mediating their effects. Studies are also necessary to establish whether these compounds can be harnessed as a safe and effective therapeutic approach against sarcopenia.

22.5.2 Manipulation of the Gut Microbiota

The human body may be envisaged as a living ecosystem characterized by complex host–bacterial interactions. Symbiotic bacterial inhabitants are found in several discrete host niches, such as the skin, the oral cavity, and the respiratory, gastrointestinal (GI), and urogenital tracts. The GI tract in particular houses over 1000 distinct bacterial species, for a total of about 10^{14} microorganisms, collectively known as the gut microbiota. In recent years, it has become increasingly clear that host–microbe interactions influence several aspects of human physiology, including nutrient bioavailability, glucose and lipid metabolism, immune system conditioning and response, drug metabolism and toxicity, and the development and maturation of the central nervous system [65,66].

Recently, the existence of a gut–muscle axis has been postulated which could be influenced by the gut microbiota [67]. Studies in rodents have shown that the gut microbiota can modulate the activity of master regulators of muscle energy metabolism, such as AMPK and PGC-1α [68] In addition, the gut microbiota influences the bioavailability of amino acids by regulating their synthesis and catabolism, and the activation of muscle-wasting pathways and intramuscular fat deposition [69,70]. The gut microbiota may also influence (and be influenced by) the bioavailability and bioactivity of most nutritional factors proposed as remedies against sarcopenia. For instance, the colonic microbiota could modulate the metabolic fate of candidate CRMs and EMs (e.g., resveratrol, quercetin, epigallocatechin-3-gallate) by converting these compounds into bioactive substances [71]. In turn, these phytochemicals could modify the gut microbial composition and/or activity [71].

In a mouse model of cancer cachexia, compositional alterations of the gut microbiota have been associated with muscle atrophy induced by overactivation of the ubiquitin–proteasome system (UPS) and autophagy [72]. Oral supplementation with specific *Lactobacillus* species reduced systemic levels of inflammatory cytokines and suppressed the expression of muscle-atrophying systems [72]. It is worth noting that chronic inflammation and the activation of autophagy and UPS are implicated in the development of sarcopenia [73]. Their modulation through the manipulation of gut microbiota (e.g., administration of prebiotics, probiotics, and synbiotics, and

fecal transplant) might therefore represent a new venue for the management of age-associated muscle wasting.

22.6 ARE NUTRITIONAL SUPPLEMENTS ALWAYS BENEFICIAL FOR MUSCLE? THE CASE OF ANTIOXIDANTS

Accumulation of oxidative damage to macromolecules over the life course is considered to be one of the central mechanisms driving the aging process [74]. The popularity of this theory has offered the rationale for the widespread prescription of antioxidants as an anti-aging remedy [75]. Sarcopenia is no exception.

Although studies in animal models seem to support the use of antioxidants as a countermeasure to age-related muscle loss [76], no clinical trials have yet confirmed these findings in humans. The available evidence is indeed based on preclinical investigations and cross-sectional observations [77–79]. When prescribing antioxidants, one should consider that redox physiology is not as straightforward as it appears. While excessive reactive oxygen species (ROS) generation in skeletal myocytes likely contributes to the development of sarcopenia, low oxidant levels are essential for optimal cell signaling and stress adaptation [80]. Notably, during moderate-intensity exercise, ROS stimulate force generation [81]. This hormetic response is maintained into old age [82]. On the other hand, supplementation with vitamins C and E prevented the muscular metabolic adaptations elicited by physical exercise in young adults [83]. More important, meta-analyses and systematic reviews have shown an increased mortality risk associated with antioxidant supplementation in both healthy individuals and patients with various diseases [84–86].

The incomplete knowledge of human redox physiology and the possible harms resulting from uncontrolled antioxidant supplementation warn against the implementation of this intervention to prevent or treat sarcopenia. Indeed, a recent statement from the Society on Sarcopenia, Cachexia and Wasting Disease does not even mention antioxidants as possible nutritional agents to manage sarcopenia in older persons [3]. Hence, unless a deficiency is ascertained, antioxidant supplements are not to be prescribed to sarcopenic subjects, especially if practicing physical exercise.

22.7 A NEW WAY TO LOOK AT THE NUTRITIONAL REGULATION OF MUSCLE PHYSIOLOGY: THE "PACHINKO MODEL"

Taken together, results from studies assessing the influence of nutrition on muscle physiology depict an extraordinarily complex scenario. The plethora of factors and mechanisms involved in the nutritional regulation of muscle trophism could be envisaged as a Pachinko machine, after the world-famous Japanese pinball device (Figure 22.1). The "Pachinko model," proposed for the first time by Nicholson and Wilson in 2003 to describe the complexity of xenobiotic metabolism [87], depicts nutrition as a flow of balls, each corresponding to single dietary constituents, through the "human system." The metabolic fate of nutrients and their effects on muscle physiology are not preordained but probabilistic and influenced by several factors, pictured in the model as the pins of the machine. Two types of pins may be distinguished. The first corresponds to non-modifiable factors, such as age, sex, and genetic background. The other set includes modifiable factors, such as epigenetic and/or transcriptional regulation of genes, posttranslational modifications of enzymes, physicochemical conditions, hemodynamics, etc., which could be tuned through hypothetical "control knobs." These represent external factors (e.g., meal timing, type and amount of physical activity, and gut microbiota composition and function) that can modulate the effects of nutrients on muscle. This dynamic model epitomizes the complexity that needs to be deciphered to develop a personalized multimodal intervention against sarcopenia. In this scenario, the implementation of integrated "multiomic" approaches

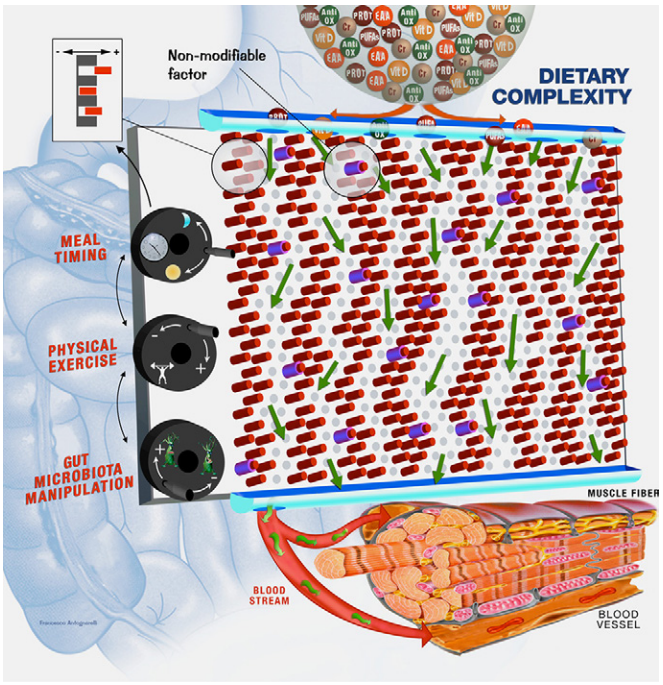

FIGURE 22.1 Dynamic "Pachinko model" of nutritional regulation of muscle physiology. Anti-Ox, antioxidants; Cr, creatine; EAA, essential amino acids; Prot, proteins; PUFAs, polyunsaturated fatty acids; Vit D, vitamin D. Reproduced from Calvani *et al*. [2], *J Frailty Aging* 2013;2:38–53.

may enable identification of the outcomes (exit holes) of the "metabolic pinball." This information, combined with functional and imaging assessments, could allow development of a full and personalized characterization of the sarcopenic patient, identifying the system(s) compromised and designing targeted treatments.

22.8 CONCLUSION

Specific nutritional interventions, especially when combined with physical exercise, produce significant improvements in muscle mass and function in older adults. However, the clinical application of research findings requires overcoming several issues. First, no unique operational definition of sarcopenia exists, and functional endpoints have not been established on which to base the interpretation of study results [88]. One important caveat of current nutritional recommendations for sarcopenia is that no evidence exists about the duration of the intervention that maximizes the benefits and reduces the risk for adverse effects. Likewise, the optimal dosage of most dietary agents has yet to be established. Finally, most studies have explored the efficacy of single nutritional components, but no information is currently available on the effects of comprehensive dietary interventions.

As discussed previously, significant advances in the field could be promoted by the implementation of multidimensional approaches combining multiomic profiling with functional and imaging assessments. The information obtained may be used to design dietary interventions that match the actual requirements of the sarcopenic subject, and to follow the nutritional and functional trajectories over time.

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CHAPTER

23
Minerals and Older Adults

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23.1 INTRODUCTION

Adequate intake of minerals plays an important role in the prevention and treatment of many illnesses. Many chronic diseases commonly seen in older adults are directly affected by mineral intakes, including calcium and osteoporosis, iron and anemia, and magnesium and diabetes.

A diet low in calories and lacking sufficient nutrient-dense foods puts many older adults at particular risk for mineral deficiencies. Inadequate intake can be precipitated by a number of factors, including physical and financial restrictions that limit the ability to purchase and prepare foods, resulting in overreliance on easy to prepare and convenience items and a decrease in the intake of fresh, nutrient-dense foods. Physical limitations may include impaired eyesight, and neurological or motor disorders resulting in limited mobility, weakness, tremors, or parasthesias.

These physical limitations, as well as chewing and swallowing difficulties and impaired taste and smell sensation, may also reduce the ability to consume adequate nutrition orally. Polypharmacy, more common in aging adults, can result in early satiety, dry mouth, and gastrointestinal symptoms, which also inhibit consumption of adequate nutrients. Further, many older adults have one or more chronic medical conditions that may alter the digestion, absorption, and utilization of minerals.

23.2 CALCIUM

Calcium is the most abundant mineral in the human body, largely found in bones and stored as hydroxyapatite, which accounts for 99% of total body calcium; the rest is located in the serum and soft tissues. There are three forms of serum calcium: ionized; in complex with other non-protein anions such as phosphate; or protein-bound, primarily with albumin. The ionized form of calcium is the most physiologically active, and is thus the most accurate measurement of serum calcium abnormalities. In addition to providing structure for bones and teeth, calcium is also necessary for endocrine, neurological, muscular, and cardiovascular functions [1].

Calcium is absorbed in the small intestine; absorption is reduced in the presence of low vitamin D and estrogen levels, and hypochlorhydria (increased gastric pH caused by reduced production of gastric acid) [2]. Vitamin D deficiency in recent years has been recognized as a widespread problem, and seniors are at particular risk, especially those that are institutionalized or who otherwise have limited exposure to sunlight [3,4]. Hypochlorhydria is also a common occurrence in the aging population [5]. Age is an important factor in calcium absorption; in young children absorption rates can be as high as 60%, but decline to 15–20% in adulthood and continue to drop with increasing age [6].

Calcium is excreted in the urine; excretion may be accelerated by increases in caffeine, sodium, and protein intake. In a study of over 3000 subjects, Taylor *et al*. [7] found that caffeine intake was positively associated with urinary calcium excretion. Similarly, high sodium intakes have been linked with increased urinary calcium excretion [8], estimated by Zarkadas *et al*. [9] at 40 mg of calcium lost for every 2.3 g of sodium consumed. While protein intake has been widely reported to increase calcium excretion, evidence suggests that it also enhances calcium absorption. Hunt and colleagues, in a study on postmenopausal women, reported that the increase in calcium absorption nearly compensated for the increase in calcium excretion when protein intake was increased from 10% to 20% of total calories [10].

23.2.1 Osteoporosis

Osteoporosis is the most common bone disease in the US, affecting over 10 million Americans; an additional 34 million people have reduced bone mass, putting them

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at significant risk for the disease. Inadequate calcium intake, most commonly seen in individuals with lactose intolerance or milk protein allergy, and in vegans, is a primary risk factor for the development of osteomalacia and osteoporosis. Other risk factors include female gender, older age, family history, smaller body frame, low levels of estrogen in women and testosterone in men, inactivity, smoking, alcohol abuse, and use of corticosteroid medications [6].

Inadequate calcium intake, osteomalacia, and osteoporosis cannot be diagnosed by measurement of serum calcium, as these levels are maintained by hormones that regulate the amount of calcium deposited or withdrawn from bone. Thus, osteoporosis is diagnosed via measurement of bone mineral density (BMD) or via the presence of a fragility fracture. The primary treatment goal is the prevention of fractures, which includes slowing bone loss or increasing BMD; calcium supplementation is a necessary component of treatment in those who cannot maintain adequate intake of calcium via diet alone [11].

In a meta-analysis comparing post-menopausal women receiving calcium supplementation with those consuming a placebo, Shea and colleagues demonstrated a small but significant improvement in reduction of bone loss in those subjects consuming the supplement [12]. Calcium supplements included calcium carbonate, citrate, gluconate, citrate malate, and lactate, and doses were at least 400 mg/d. Increases in BMD have also been demonstrated in non-osteoporotic older men supplemented with 1200 mg/d of calcium for 2 years; the authors found no such improvements in BMD with calcium supplementation of 600 mg/d [13].

Fracture risk and calcium supplementation has also been studied. In a trial of over 36,000 women from the Women's Health Initiative (WHI), researchers studied supplementation of 1000 mg/d of calcium and 400 IU/d of vitamin D and found an insignificant risk reduction for hip fracture and a small but significant increase in BMD. The authors postulated that the dose of vitamin D was insufficient to effect a significant reduction in fractures [14].

Supporting this conclusion is a meta-analysis conducted by Tang *et al*. [15]. Pooled results of 17 studies investigating calcium or calcium and vitamin D supplementation and fracture risk revealed an overall risk reduction of 12–13% for all fracture sites. More significantly, in studies reporting the highest compliance rate with supplementation guidelines (>80%), the risk reduction for all fracture sites was 24%. Risk reduction was also greater in those studies in which calcium supplementation was ≥1200 mg, and vitamin D supplementation was ≥800 IU daily.

Similarly, in a review of nine studies Parikh *et al*. [16] found a significant reduction in fracture risk and an increase in BMD in those nursing home residents who were supplemented daily with 1200 mg calcium and 800 IU of vitamin D.

Although definitive conclusions are difficult to draw because of differences in study design, including the population studied, supplementation given, and outcomes measured, it does appear that increased calcium intake is correlated with reduced incidence of osteoporosis, higher BMD, and lower risk of bone fractures. It has been suggested that dietary calcium intake has a stronger effect on BMD than calcium supplementation, as absorption is improved when calcium is consumed as part of a meal, and because dietary calcium is generally consumed in smaller, more frequent doses [17].

23.2.2 Cardiovascular Health

It has been suggested that increased calcium intake may reduce blood pressure, although research results are mixed. In a meta-analysis, Allender *et al*. [18] concluded that higher calcium intake was correlated with lower blood pressure, but that the effect was too small to support the recommendation of calcium supplementation in treating hypertension. In another meta-analysis, investigators concluded that increased calcium intake resulted in a small decrease in systolic but not diastolic blood pressure [19]. Similarly, authors of a more recent systematic review concluded that the relationship between calcium and blood pressure was weak at best, and that better quality studies of longer duration were needed to fully elucidate the effect of calcium on blood pressure [20]. Daily supplementation with 1000 mg of calcium and 400 IU of vitamin D in the WHI study also did not reduce blood pressure or the risk of developing hypertension [21].

Researchers have also examined the relationship between calcium intake and stroke. The WHI study showed no effect on coronary or cerebrovascular risk with calcium and vitamin D supplementation over a 7-year period [22]. Limitations of the study include the participants' adherence to the supplement regimen, a potentially inadequate dose of vitamin D, and the fact that the trial was designed to examine the effect of calcium supplementation on risk of bone fracture. In a review of observational, experimental, and clinical studies, researchers determined that the data were not sufficient to make recommendations supporting the use of calcium supplements for the prevention of stroke [23].

In more recent years it has been suggested that calcium supplementation actually increases the risk of both myocardial infarction (MI) and stroke. In a meta-analysis of 13 studies with over 30,000 participants, the authors concluded that calcium supplementation increased the risk of MI by 25% and stroke by 15–20%, and suggested that calcium supplementation targeted to specific populations should not be endorsed [24]. However, other

analyses have not reached this conclusion, citing study shortcomings, including small sample size, scarcity of data on calcium supplementation in men, and the fact that the relationship between calcium supplementation and cardiovascular event risk was not the intended endpoint in most of the studies [25]. Clearly, further research is needed to elucidate the role calcium supplementation plays in cardiovascular disease and events.

23.2.3 Cancer

Several observational studies have reported an inverse relationship between calcium intake and incidence of colorectal cancer [26–28]. In an analysis of cohort studies, Cho *et al*. [29] concluded that an increase in calcium intake via supplementation reduced colon cancer risk by 10–15%. In a large study of nearly 88,000 women and over 47,000 men, higher daily calcium intake (>1200 mg vs <500 mg) was found to be significantly associated with a lower incidence of proximal colon cancer [30]. In a more recent European study, researchers followed over 477,000 men and women and found a correlation between higher dietary calcium intake from dairy products and reduced rates of colorectal cancer [31].

Conversely, in the WHI study no effect on the incidence of colorectal cancer was demonstrated; however, the authors speculated that the lack of positive results may have been due to the potentially inadequate dose of vitamin D and the relatively short study duration of 7 years [32]. Although not all results are consistent most research appears indicate that increased dietary calcium intake does reduce colorectal cancer risk, but further research is needed to clarify the relationship.

Calcium intake has been implicated as a risk factor for the development of prostate cancer, although research results are mixed. In one prospective study of over 29,000 men between the ages of 55 and 74, researchers discovered a modest association between calcium intake and low-fat dairy product intake and non-aggressive prostate cancer [33]. In other study of over 47,000 men, daily calcium intake exceeding 1500 mg was associated with an increased risk of advanced or fatal prostate cancer [34]. Other studies have found similar results [35–37]. However, in a large meta-analysis examining the results from 45 observational studies, Huncharek *et al*. [38] concluded that there was no association between intake of dairy products and prostate cancer risk.

Although results are not definitive, many studies do suggest higher intakes of calcium may be a risk factor for prostate cancer. Although there are no recommendations at this time for men to limit calcium intake or dairy products, these data, the potential benefits for bone health, and the individual's risk for osteoporosis and prostate cancer should be weighed before a decision is made to initiate calcium supplementation.

23.2.4 Weight Management

Recent studies have suggested that increased calcium intake, from dietary sources or supplementation, aids in weight control. In a retrospective study, Gonzalez *et al*. [39] examined over 10,000 men and women between the ages of 53 and 57, and used linear regression to assess calcium's effect on weight changes, taking into account energy intake and physical activity. Researchers found an inverse relationship between calcium supplementation and weight gain over 10 years, but in women only [39].

However, in a randomized controlled trial, Yanovski *et al*. [40] found no benefits in supplementing overweight and obese adults with 1500 mg of calcium for 2 years [40]. In another study, overweight and obese premenopausal women followed a calorie restricted diet and physical activity regimen for 12 weeks, then were randomized to receive 800 mg of calcium phosphate, or 800 mg of calcium lactate, or 1% milk, or a placebo. The researchers concluded that there were no statistically significant differences in weight loss among any of the groups [41]. Although some study results show benefits, data are inconsistent and further research is needed before specific recommendations can be made with regard to calcium intake and weight control.

23.2.5 Supplementation

The Recommended Dietary Allowance (RDA) for calcium is 1000 mg for adult men and women, and 1200 mg for men over the age of 70 and women over the age of 50 [42] (see Table 23.1 for the RDAs for select minerals for adults over 50 years of age). Calcium intake generally declines with age; in Americans over the age of 60, calcium intake is reported to be only 80% and 55% of estimated needs for men and women, respectively [43]. The best dietary sources of calcium are dairy products and sardines, but lesser amounts can be found in dark leafy greens and broccoli; foods commonly fortified with calcium include soy milk, tofu, orange juice, and cereals.

Indications for calcium supplementation include suboptimal calcium intake with the presence of osteoporosis

TABLE 23.1 RDA for Select Minerals for Individuals Over the Age of 50 [42]

Mineral	RDA for men	RDA for women
Calcium	1000 mg/d (51–70 years) 1200 mg/d (>70 years)	1200 mg/d
Iron	8 mg/d	8 mg/d
Magnesium	420 mg/d	320 mg/d
Zinc	11 mg/d	8 mg/d
Selenium	55 μg/d	55 μg/d

or osteopenia, chronic corticosteroid therapy, or menopause [2]. Vegans and those with lactose intolerance are most likely to have diets deficient in calcium [44]. It should be noted that concurrent vitamin D supplementation is also necessary in individuals with a vitamin D deficiency, as calcium absorption will be impaired.

Calcium supplements come in tablets, chewables, powders, capsules, and liquids, and the most common forms are calcium carbonate and calcium citrate [2]. Both are readily absorbed, although calcium carbonate requires an acidic environment and thus is better taken with food [45]. Medications reducing stomach acidity, such as proton pump inhibitors, may also reduce calcium absorption, although evidence is mixed [46]. As hypochlorhydria is common in aging adults, calcium citrate may be the preferred form of calcium supplementation for these populations.

TABLE 23.2 Dietary Sources of Selected Minerals [6,47]

Mineral	Dietary sources
Calcium	Milk, cheese, yogurt, calcium fortified orange juice, sardines with bones, pudding, fortified cereals, instant breakfast drink, spinach, kale, turnip greens
Iron	Liver, meat, fish, oysters, clams, poultry, fortified cereals, spinach, beans, molasses, tofu
Magnesium	Nuts, seeds, beans, peas, unrefined grains, fortified cereals, spinach, potato with skin, yogurt
Zinc	Oysters, meat, poultry, lobster, crab, fortified cereals, beans, nuts, unrefined grains
Selenium	Fish, shellfish, red meat, chicken, eggs, milk, fortified cereals

23.3 IRON

Iron is an important component of many proteins and enzymes and thus is required for numerous metabolic reactions, including cell growth and differentiation. Iron is also a vital component of hemoglobin, which is responsible for oxygen transport; insufficient iron stores result in reduced tissue oxygenation, causing fatigue and decreased immune function. The total amount of iron in the body varies from about 2 to 4 grams, with differences dependent on age, body size, nutritional status, and gender; women generally have less iron than men. Iron is stored bound to transferrin in the blood, as ferritin in intestinal cells, and as myoglobin in muscle cells [47].

Iron is found in its heme form in meat, poultry, and fish, and in its non-heme form primarily in plant foods (see Table 23.2 for good food sources of select minerals). Heme iron is more readily absorbed in the gastrointestinal (GI) tract because it does not require conversion to its ferrous form prior to absorption as does non-heme iron. Absorption of heme iron is about 40%, while non-heme iron absorption can vary from 10% to 50%. Absorption is dependent on iron status; iron deficiency anemia (IDA) results in an increase in enterocyte transferrin receptors, thus enhancing iron absorption. Acidity aids in maintaining iron in its ferrous form; ingestion of foods containing compounds such as vitamin C, and aspartic and glutamic acids, increases acidity and thus aids non-heme absorption [47].

23.3.1 Iron-Deficiency Anemia

The World Health Organization considers IDA the most prevalent nutritional deficiency, affecting as much as 30% of the world's population [48]. It has been estimated that IDA accounts for about 16% of all cases of anemia in the US [49]. Symptoms include tachycardia, fatigue and pallor, reduced mental performance, reduced resistance to infection, and impaired thermoregulation. The etiology of IDA is often multifactorial, and includes insufficient intake, impaired absorption, increased needs, or chronic blood loss [47,50].

Insufficient iron intake is most commonly seen in vegetarians and vegans, as the most easily absorbed food sources include liver, oysters, red meat, and dark poultry meat. Further, absorption of non-heme sources such as fortified cereals, beans, lentils, tofu, spinach, raisins, and molasses can be reduced in the presence of phytates, tannins, calcium, and polyphenols. Presence of hypochlorhydria, use of proton pump inhibitors, and the absence of gastric acid secretion as seen with gastrectomy and bariatric surgery patients may also limit iron absorption [5,51].

Increased iron losses most commonly occur in menstruating women; in the aging population, iron loss may be seen in those with chronic slow blood loss, as from GI bleeding. Individuals with chronic kidney disease undergoing hemodialysis are also at increased risk for IDA, as they are unable to produce sufficient amounts of erythropoietin, necessary for the formation of red blood cells, and because of blood lost during the hemodialysis process, which can be as high as 3 g of iron per year [52].

In a meta-analysis of 20 studies, Qu *et al*. [53] concluded that those with *H. pylori* infection were 2.2-fold more likely to develop IDA [53]. In the five randomly controlled trials assessed, eradication of *H. pylori* improved hemoglobin and serum ferritin levels; however, the results seen were not significant. Further research on IDA and *H. pylori* is needed before specific screening or treatment recommendations can be made.

IDA is a microcytic hypochromic anemia; diagnosis can be challenging, especially in the presence of inflammation, which may result in altered levels of storage and transport proteins (see Table 23.3). Diagnosis may also

TABLE 23.3 Diagnosis of IDA [6,47]

Test	Normal range*	Comments
Serum iron	50–170 μg/l (males) 28–160 μg/l (females)	Low with Fe deficiency Low during inflammation, during and 1–2 weeks after injury Day-to-day and diurnal variations – highest mid-morning, lowest mid-afternoon
Transferrin (transport protein)	170–340 mg/dl	High with Fe deficiency Low during inflammation
Ferritin (storage protein)	11–307 ng/ml	Low with Fe deficiency High during inflammation
Transferrin saturation	20–50%	Low with Fe deficiency
Total iron binding capacity	261–478 μg/dl	High with Fe deficiency High with pregnancy and hepatitis Low with malignancy and hemolytic anemia

**Normal range values will vary by laboratory.*

be problematic in the elderly population, as serum ferritin has been shown to increase with age; Rimon *et al*. [54] demonstrated that serum iron, ferritin, and transferrin saturation were poorly sensitive to capturing iron deficiency in patients over the age of 80 [54]. The transferrin receptor–ferritin index or ratio has been shown to be a more sensitive test in diagnosing iron deficiency, and should be considered when screening elderly patients [54]. An increase in the red cell distribution width has also been proposed as a sensitive indicator of iron deficiency anemia [55]. Regardless, inflammatory status should be considered when interpreting laboratory values, and the individual's medical and nutritional history should also be considered in the diagnostic process.

23.3.2 Supplementation

The RDA for iron is 8 mg for adult males and females over the age of 50 years [42]. Recommended doses for supplementation are 150–200 mg of elemental iron per day. Supplementation is necessary with symptomatic IDA, and should be considered if dietary intake is insufficient to meet estimated needs. Supplementation should continue in those who are unable to meet needs orally, or in those with chronic malabsorption. Supplements are typically available in ferrous sulfate, gluconate, or fumarate forms, although fumarate is most easily absorbed. Product manufacturers are required to list the elemental dose on the label, or the amount that is expected to be absorbed, so that consumers can more easily determine how much they need to take [47].

Iron supplements should be taken in divided doses, as absorption decreases as the amount ingested increases. Absorption is also inhibited in foods containing phytates, such as whole grains; oxalic acid, which is found in spinach, tea, and chocolate; and polyphenols, which are found in coffee and tea [47,55]. Absorption is also reduced in the presence of other minerals, so iron should be taken separately from calcium and multivitamin supplements. Common side effects include nausea and constipation [6].

Intramuscular and intravenous forms can also be given in the acute care setting, or in the event that deficiency does not improve with oral supplementation; the available forms are iron dextran, sodium ferric gluconate, iron sucrose, and ferumoxytol, with the latter two being associated with fewer side effects [47]. Iron should not be supplemented in individuals with hemochromatosis, a genetic condition in which iron is very efficiently absorbed, resulting in deposition of excess iron in tissues and organs.

Iron should be supplemented cautiously in critically ill patients, as it can contribute to microbial growth and increased oxidative reactions [47]. However, in a recent randomized double-blind trial, Pierraci *et al*. [56] supplemented anemic, critically ill patients with 325 mg of ferrous sulfate three times daily and found no difference between the iron-supplemented group and the placebo group in antibiotic days, rate of infection, length of stay, and mortality rate [56].

23.4 MAGNESIUM

Total body magnesium is approximately 25 grams, 50–60% of which is found in the bone; the rest is primarily in muscles and intracellular fluid, with trace amounts in other fluids and soft tissues. Serum magnesium is protein bound or in an ionized form, and small amounts are complexed with phosphate, citrate, and other compounds. Magnesium is necessary for over 300 biochemical reactions, including those associated with protein, glucose, and DNA metabolism, as well as neuromuscular transmissions, muscle contraction, and cardiovascular excitability. Magnesium is also critical in the production of parathyroid hormone, and plays an important role in calcium homeostasis and vitamin D production [1].

Magnesium absorption occurs in the jejunum and ileum, and about 30–40% of dietary magnesium is absorbed. Absorption may be impeded in diets high in fiber, oxalate, phytate, and phosphate, as magnesium may become bound to these substances. Magnesium is excreted in the kidneys, which are the primary organs that maintain normal serum magnesium levels [1].

23.4.1 Cardiovascular Health

Research has suggested that diets rich in magnesium may reduce the incidence of hypertension; many of these studies investigated efficacy of the DASH (Dietary Approaches to Stop Hypertension) diet. While high in magnesium, this diet is also high in potassium, calcium, fiber, and low in sodium, and thus it is difficult to determine to what extent magnesium independently played a role in the successful reduction of blood pressure in these trials [6]. However, Song *et al*. [57], in a prospective study of over 28,000 women over the age of 45, did find that dietary magnesium intake was inversely associated with the risk of developing hypertension [57].

In a meta-analysis of 12 studies of 545 hypertensive subjects receiving magnesium supplements, Dickinson *et al*. [58] found a significant reduction in diastolic blood pressure but not systolic blood pressure. The authors concluded that the association between magnesium and blood pressure was weak, and likely due to bias because of the poor quality and heterogeneity of the studies [58].

Another meta-analysis of 44 studies concluded that magnesium supplementation of 10 mmol (243 mg) daily may significantly reduce blood pressure in those patients with stage 1 hypertension already receiving pharmaceutical treatment for at least 6 months. In untreated patients, results suggested that magnesium supplementation of 20 mmol (486 mg) daily was necessary to effect a statistically significant decrease in blood pressure. The authors concluded, however, that because much of the current research has been conducted on normotensive subjects, and many studies have small sample sizes, these results are not conclusive and further research is needed [59].

The relationship between serum magnesium levels and dietary magnesium intake and cardiovascular disease (CVD) has also been studied. In a recent meta-analysis, researchers noted that individuals with the highest serum magnesium levels had a 20% lower incidence in CVD events than those with the lowest serum magnesium levels. Dietary magnesium intake was also inversely associated with CVD; intake nearest 400 mg/d was associated with the lowest incidence of CVD, although no further benefit was seen for intakes above that level [60].

23.4.2 Osteoporosis

It has been suggested that magnesium has a multifactorial effect on bone health. It is necessary for osteoblast function, thus depletion is associated with decreased bone mass and altered hydroxyapatite crystal structure, which reduces bone strength. Further, magnesium deficiency has also been correlated with reduced levels of parathyroid hormone and vitamin D; in osteoporotic women, magnesium supplementation has successfully corrected these abnormalities. However, elevated serum magnesium levels, as commonly seen in patients with chronic kidney disease, have also been correlated with bone mineralization defects, so serum magnesium should be monitored and corrected in high-risk patients [61,62].

Dietary magnesium supplementation has been shown to suppress bone turnover in both postmenopausal women and young men [63,64]. Higher intakes of magnesium have also been associated with increased bone mineral density (BMD) in the elderly [65]. In a recent study, Genuis *et al*. [66] supplemented 77 patients with osteoporosis or osteopenia with a combination of micronutrients for 1 year; the supplement included 25 mg of elemental magnesium, as well as docosahexanoic acid, vitamin D3, vitamin K, and strontium citrate. Overall, BMD significantly increased; however, it is difficult to determine what role magnesium played in these results. Further, the study had a small sample size, and there was no placebo-controlled group [66].

Sufficient studies examining the affect of magnesium supplementation on BMD are lacking. While it is clear that adequate amounts of magnesium are important for bone health, further research is needed to determine the effect of magnesium supplementation in the prevention and treatment of osteoporosis.

23.4.3 Diabetes

Magnesium plays an important role in blood glucose control, as deficiency can decrease insulin sensitivity in peripheral tissues and insulin production in pancreatic beta cells. In turn, hyperinsulinemia may lead to or worsen magnesium deficiency by increasing renal magnesium excretion [67].

Magnesium intake is inversely associated with incidence of diabetes. In a large study of over 85,000 women aged 30–55 and over 42,000 men aged 40–75, subjects' diets were analyzed and followed for 18 and 12 years for the women's and men's groups, respectively. Researchers discovered an inverse relationship between magnesium intake and risk of diabetes [68].

Similar results were seen in a smaller study which measured metabolic impairments, defined as impaired fasting glucose, impaired glucose tolerance, or insulin resistance, at baseline and after 7 years. For subjects with no impairments at baseline, those with the highest intake of magnesium had a 37% lower incidence of metabolic impairments; for subjects with metabolic impairments at baseline, those with the highest magnesium intake had a 32% lower incidence of diabetes [67].

The effect of magnesium supplementation on blood glucose control in subjects with diabetes has also been studied. In a meta-analysis of nine randomized controlled

trials involving a total of 370 subjects with diabetes, results showed that a median supplement dose of 360 mg of magnesium resulted in significantly improved fasting blood glucose levels, but not A1C. This may, however, have been due to the shorter duration of some of the studies; treatment duration was 4–16 weeks. It is difficult to draw definitive conclusions on these data, given the small sample size and heterogeneity of subjects and treatment. Further, magnesium status at baseline was not described; it is possible that positive effects were seen because of magnesium deficiency at baseline [69].

While diabetes and magnesium research is mixed, it does appear that correction of magnesium deficiency will help control blood glucose levels in people with diabetes. In a position statement from the American Diabetes Association the authors do not advocate for routine magnesium supplementation, although they do acknowledge micronutrient deficiencies are common with poorly controlled diabetes, and these deficiencies should be treated with a healthful diet and supplementation if necessary [70].

23.4.4 Supplementation

The RDA for magnesium for adults 31 years and older is 420 mg/d and 320 mg/d for men and women, respectively [6]. Good food sources of magnesium include green vegetables, unrefined grains, legumes, nuts, and seeds. Magnesium intake in the US has been shown to be inadequate, especially in the elderly, African Americans, and Hispanics [71].

Hypomagnesemia can be caused by inadequate intake or absorption, increased renal excretion, or redistribution from extracellular to intracellular fluid. Symptoms include loss of appetite, nausea, vomiting, and weakness; as deficiency progresses, numbness and tingling, muscle cramps, cardiac arrhythmias, and seizures can occur. Because only a small amount of magnesium is found in the extracellular fluid, serum magnesium does not necessarily correlate with total body magnesium stores. Insufficient stores of magnesium may adversely affect bone health, but may not result in any acute symptoms of deficiency [47].

Magnesium deficiency may be caused by medications, including certain diuretic, antineoplastic, and antibiotic medications, which either increase excretion or decrease absorption of magnesium. Medical conditions in which deficiency is common include GI malabsorptive disorders such as short bowel syndrome, inflammatory bowel disease, and celiac disease; alcoholism; and poorly controlled diabetes [6]. Individuals with these conditions and the elderly are at particular risk for magnesium deficiency.

Magnesium can be supplemented parenterally or orally (see Table 23.4 for supplementation recommendations for select minerals). For hypomagnesemia in the acute care setting, parenteral supplementation of magnesium sulfate, in doses of 1–3 g, is the preferred method of replacement, because of poor GI tolerance and the slow onset of action seen with administration of oral doses. Oral forms of magnesium supplementation include magnesium oxide, carbonate, hydroxide, citrate, lactate, chloride, and sulfate. The amount of elemental magnesium as well as the bioavailability should be considered when choosing a magnesium supplement. Magnesium oxide has the highest amount of elemental magnesium, at 60%, while magnesium sulfate has the lowest, at 10% [6].

Oral dosage of magnesium supplements may vary based on the cause and severity of the deficiency, the presence of comorbid conditions, and the chosen form of supplement. Caution should be used in those with chronic kidney disease, as magnesium is primarily excreted by the kidneys. For the treatment of hypomagnesemia in patients with diabetes, studied doses vary widely, and the dose required for repletion of stores will depend on the individual's magnesium intake from food, as well as kidney function. Ideal magnesium dosage for the treatment or prevention of hypertension is also unclear. In most studies, doses ranged from approximately 240 to 970 mg/d for 8 to 26 weeks [57–59].

23.5 ZINC

Zinc is a trace mineral with total body stores of only 2–3 grams; as most zinc is widely distributed in all cells of the body there is no storage system for the mineral, and thus it can more easily become depleted than some other minerals. Zinc plays a role in immune function, wound healing, protein and DNA synthesis, growth, and the metabolism of over 200 enzymes [6,47].

Approximately 20–40% of dietary zinc is absorbed. Absorption occurs in the small intestine and zinc is then carried to the liver bound primarily to albumin, thus hypoalbuminemia may impair hepatic release of zinc. Serum zinc levels may also decrease in acute illness and infection as zinc is redistributed from the blood to the cells, and absorption decreases [47].

23.5.1 Immunity

Aging negatively impacts the immune system, most notably T cells, whose function and production are impaired, largely due to shrinkage of the thymus gland typically seen with aging. Humoral immune response is also affected, characterized by decreased production of effective and specific antibodies, resulting in increased susceptibility to bacterial and viral infections. Zinc deficiency also causes immune impairment via reductions in thymus gland size, lymphocyte proliferation,

TABLE 23.4 Supplementation Recommendations for Select Minerals [42,47]

Mineral	Supplement	Dose	Duration	Comments
Calcium	Calcium citrate Calcium carbonate (gluconate, lactate, phosphate also available)	Dependent on dietary intake and type of calcium supplement; goal is 1200 mg/d total Most common forms are tablets, chewables, capsules	Indefinitely; until needs can be met with dietary intake alone	Citrate preferred for adults with hypochlorhydria Carbonate contains more elemental calcium (40% vs 21%)
Iron	Ferrous gluconate Ferrous sulfate	325 mg tablets three times daily, or dosed to provide 150–200 mg/d elemental iron Ferrous gluconate contains 60 mg elemental iron, sulfate 36 mg	3 months for repletion; until needs can be met with dietary intake alone; longer in the presence of continued iron losses	Do not take with other mineral supplements or high phytic acid foods; 1 h before and 2 h after meals preferred Take in divided doses
Magnesium	Magnesium oxide, carbonate, hydroxide, citrate, lactate, chloride, or sulfate	Dose dependent on degree of deficiency, current intake from dietary sources, and form of supplement IV: 1–3 g/d magnesium sulfate for treatment of hypomagnesemia	Daily until serum levels are corrected; until needs can be met with dietary intake alone	Magnesium also used as an antacid, laxative, and a treatment for migraines Use cautiously in renal dysfunction Magnesium oxide, carbonate, and hydroxide have the highest % of elemental magnesium
Zinc	Zinc sulfate Zinc gluconate	Orally: 200–220 mg/d IV: 6–10 mg/d Add 12.2 mg/l of small bowel fluid lost, 17.1 mg/kg of stool or ileostomy output	No established parameters; in the acute care setting, 10–14 days is common Indefinite supplementation for those with chronic losses	Large doses may induce copper and iron deficiency Zinc gluconate may make some antibiotics less effective Zinc sulfate contains more elemental zinc than gluconate (23% vs 14%)
Selenium	Selenious acid	Orally: 50–200 μg/d; available in tablets or capsules IV: 20–40 μg/d; up to 100 μg/day for deficiency	24–31 days	Oral doses should be taken with food

interleukin-2 production, antibody response, and natural killer cell activity [72]. Decreased zinc stores have been frequently documented in the elderly population; zinc absorption decreases with age and oral intake is often reduced as well [43].

The effect of zinc supplementation on immune function has been investigated. Boukaiba supplemented elderly institutionalized subjects with 20 mg of zinc gluconate for 8 weeks, resulting in an increase in thymulin levels (a measure of T cell function) [73]. In a later study, supplementation with 45 mg elemental zinc daily for 12 months resulted in a decreased incidence of infection in elderly subjects, as well as improvements in parameters of cell-mediated immunity and oxidative stress markers, compared to a control group of younger subjects [74].

While a number of other studies have shown similar results, it is still unclear whether these effects can be sustained in the long term, and to what degree zinc deficiency affected the results. In some studies zinc status was not measured at baseline, so improvements seen may be a result of correction of deficiency, leaving unanswered the question of zinc supplementation effectiveness in the presence of adequate zinc stores. The studied doses and duration of supplementation vary widely, which also makes interpretation of results problematic.

23.5.2 Wound Healing

Zinc is necessary for the synthesis of granulation tissue and re-epithelialization, and thus is vital for the wound healing process. Zinc deficiency delays wound healing, and can also result in decreased appetite and altered taste and smell perception; the resultant reduction in oral nutrition intake then exacerbates the deficiency [47].

The effect of zinc supplementation on healing has been studied in various types of wounds, including pressure ulcers, burns, and chronic lower extremity stasis ulcers. It is difficult to draw definitive conclusions because of the heterogeneity of subjects, including age, disease state, type of wound, and zinc status, as well as study size and the differences in study design, such as the type and amount of zinc being investigated.

Many studies do not investigate the effects of zinc alone; zinc is often given with a host of other nutrients,

including energy, protein, arginine, glutamine, vitamins, and other minerals. Although some studies have demonstrated positive results with patients supplemented with arginine, vitamin C, and zinc, it is impossible to determine to what extent zinc had a role in study outcomes [75–77]. As yet, there is no evidence that routine zinc supplementation facilitates wound healing, except in those with zinc deficiency.

23.5.3 The Common Cold

Zinc has been shown to inhibit replication of the rhinovirus, and is thus commonly found in many over-the-counter cold remedies, including throat lozenges and nasal sprays. In a recent Cochrane review of 18 placebo-controlled trials with a total of over 1700 otherwise healthy subjects, the authors concluded that zinc lozenges and syrup, if taken within 24 hours of onset of symptoms, were effective in reducing the duration of colds. The study doses varied; however, amounts greater than 75 mg/d are recommended for greatest effect. Although some studies have shown positive results, at this time there is insufficient evidence to recommend zinc supplementation for prevention of the common cold [78].

23.5.4 Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness and visual impairment in the US in individuals 65 years and older. There is currently no cure, and treatment does not reverse damage already present. Because zinc is a primary component of many antioxidant enzymes, and because it is found in high concentrations in the part of the retina that is affected by AMD, it has been postulated that zinc intake may play a role in both development of and treatment for the disease [79].

In perhaps the largest and most well-known study, the Age Related Eye Disease Study (AREDS), researchers supplemented 3640 subjects, aged 55–80 years, with the antioxidants vitamins C and E, beta carotene, 80 mg of zinc oxide, or antioxidants with zinc, daily. Supplementation of both zinc and antioxidants with zinc resulted in a significantly reduced risk of developing advanced AMD in high-risk groups. A reduction in the rate of development of moderate visual acuity loss was also seen in the antioxidants with the zinc group [80].

A recent meta-analysis examining 11 studies on zinc and AMD concluded that while there was insufficient evidence to suggest that zinc intake was correlated with the development of AMD, supplementation does appear to help prevent progression to advanced AMD in those that already have the disease. Citing data primarily from the AREDS study, the authors also concluded that it appears that zinc with added antioxidants was effective in improving visual acuity in AMD patients. Because the form and dose of zinc, as well as the duration of supplementation, varied from study to study, no specific recommendations can be made regarding the ideal zinc treatment for AMD [79].

23.5.5 Supplementation

The RDA for zinc is 11 mg/d and 8 mg/d for adult men and women, respectively [42]. Zinc is found in a wide variety of foods, including meat, poultry, nuts, seeds, beans, whole grains, and fortified cereals; oysters in particular are extremely high in zinc. It should be noted that phytates found in plant foods, such as whole grains, cereals, and legumes, inhibit the absorption of zinc, thus animal sources are more readily absorbed and vegetarians and vegans are at higher risk for deficiency. Other minerals may also significantly decrease zinc absorption, so zinc should not be taken with calcium or other vitamin and mineral supplements [6,47].

Groups at risk for zinc deficiency include those with malabsorptive diseases such as celiac disease, inflammatory bowel disease, and short bowel syndrome. Bariatric surgery, chronic kidney disease, excessive GI fluid losses such as high-output GI fistulas and diarrhea, excessive alcohol intake, sickle cell disease, and burns are also risk factors for deficiency [6,47]. The elderly have been shown to have suboptimal intakes of zinc [43].

Zinc deficiency may cause diarrhea, fatigue, skin lesions, alopecia, alterations in immune function, taste abnormalities, and impaired wound healing. Zinc status is often difficult to assess, in part because of the changes in zinc metabolism during the acute phase response, and the unreliability of serum zinc as a marker of zinc status [81]. Risk factors, physical signs and symptoms, and nutrition intake should also be assessed when screening for deficiency.

Zinc supplements, including zinc sulfate and gluconate, come in a variety of forms such as tablets, lozenges, and intravenous doses. Supplemental doses to treat deficiency are usually 220 mg twice daily, as enteral zinc is not 100% bioavailable; parenteral zinc, on the other hand, should be limited to 40 mg/d or less (see Table 23.5 for the Tolerable Upper Levels for select minerals). Excessive zinc administration may cause nausea and diarrhea, interfere with copper and iron absorption, and impair wound healing [47,82].

There are no specific recommendations regarding how long zinc supplements should be taken, although common practice in the acute care setting is to supplement for 10–14 days and then reassess zinc status. Because of its effects on copper and iron absorption, zinc supplementation should not be continued long term; however, it may be necessary for those who have

TABLE 23.5 Tolerable Upper Levels (TUL) of Select Minerals for Adults Over the Age of 50 [6]

Mineral	TUL
Calcium	2000 mg/d
Iron	45 mg/d
Magnesium	350 mg/d
Zinc	40 mg/d
Selenium	400 μg/d

a chronic insufficient dietary intake of zinc, excessive GI fluid losses, or impaired absorption. In these cases, copper and iron status should be monitored closely.

23.6 SELENIUM

Selenium is a trace element that is complexed with proteins, called selenoproteins, which play a role in immune function and thyroid function, and act as antioxidants [6]. Dietary selenium is primarily in the selenomethionine form, and 90% is absorbed in the duodenum and proximal jejunum. Inorganic forms of selenium are not as readily absorbed. Selenium absorption does not appear to be linked to selenium status; rather, excretion is the method by which selenium status is maintained. Selenium is excreted in both urine and feces [83].

23.6.1 Immunity

Selenoproteins are necessary for the activation, proliferation, and differentiation of many immune cells, and thus play a role in the initiation and enhancement of immunity, as well as immunoregulation. Because of these functions, selenium has been investigated as a treatment for immune conditions such as viral infections, including human immunodeficiency virus (HIV) infection, and systemic inflammatory response syndrome (SIRS) [84].

Serum selenium levels have been shown to decrease significantly in HIV-infected individuals, and are associated with increased mortality. Research has suggested that selenium supplementation in HIV may result in fewer hospitalizations, suppressed viral load, increased CD4 T cell counts, and lower risk of diarrhea; however, some studies have also shown increases in viral shedding with selenium supplementation. Further research is needed before recommendations can be made for zinc supplementation in HIV [85].

Several studies have demonstrated a correlation between selenium status and clinical outcomes in patients with SIRS; serum levels decrease with SIRS, and it has been postulated that these levels may serve as a prognostic indicator for the syndrome. Several studies examining the effect of selenium supplementation in SIRS have shown positive effects, including reduced mortality, rate of infection, and severity of disease [86–89]. These data are promising, but results were inconsistent and the timing and dosage of selenium varied, thus further research in this area is needed.

The effect of selenium supplementation on general immunity has also been studied. Broome *et al*. [90] supplemented individuals with marginally low serum selenium levels with 50 or 100 μg/d of sodium selenite for 14 weeks. Subjects demonstrated improved selenium status and an increase in cellular immune response to an administered vaccine. Researchers concluded that a dose of 100 μg of sodium selenite was beneficial in optimizing immune function in individuals with suboptimal selenium status [90]. Improvements in humoral immunity in response to a vaccine were demonstrated by Girodon *et al*. [91] when elderly subjects were supplemented with 20 mg of zinc sulfide and 100 μg of selenium sulfide daily [91]. However, it should be noted that most of the subjects had both selenium and zinc deficiency at baseline; at present, there is no evidence that supplementation in the absence of deficiency results in immunologic benefits.

23.6.2 Cancer

It has been noted that people living in areas with low soil selenium levels have a higher incidence of certain kinds of cancer, including skin cancer [92]. Lower serum selenium levels are also associated with higher risk of colorectal, lung, and prostate cancers. It has been hypothesized that selenium may reduce the risk of cancer via its antioxidant protection against free radicals, and it also has been shown to slow tumor growth [6].

In the Nutrition Prevention of Cancer Trial (NPC), researchers investigated the effects of 200 μg/d of selenium supplementation on skin cancer incidence. While there were no apparent effects on rates of skin cancer, selenium supplementation was positively correlated with lower mortality rates and lower incidence of prostate cancer. Of note, subjects with the lowest serum selenium levels at baseline had a lower risk of cancer while those that had the highest serum selenium levels at baseline had a higher risk of cancer, suggesting that supplementation may only be beneficial in those with deficiency [93].

In a later study, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), researchers supplemented healthy men with 200 μg/d of selenium, 400 IU/d of vitamin E, or both. Results indicated that vitamin E supplementation increased risk of prostate cancer, while selenium supplementation had no effect [94]. It has been postulated that the conflicting results from the

NPC and SELECT trials may be a result of differences in the subjects' serum selenium levels at baseline (114 μg/l vs 136 μg/l). Authors of a recent review suggested that serum selenium status was more important than selenium intake in decreasing cancer risk, recommending a serum concentration of 120–160 μg /l, which was felt to be generally achievable via consumption of a healthful diet without selenium supplementation [95].

In a meta-analysis of studies examining nutritional supplementation and prostate cancer risk, Jiang *et al*. [96] concluded that supplementation of selenium did not result in a lower incidence or mortality from prostate cancer. In another study, intake of dietary selenium was not associated with prostate cancer risk [97]. However, in work by Penney *et al*. [98] poor selenium intake was associated with a higher risk of poor-prognosis prostate cancer, but only in men with specific genes that influence the requirement for selenium. Further research is needed to fully understand the role of selenium in prostate cancer risk. Selenium supplementation has also not been shown to reduce the risk of colon cancer [99].

23.6.3 Diabetes

Several epidemiological studies have suggested a positive correlation between higher serum selenium levels and incidence of type 2 diabetes. In the NPC trial, investigators found as a secondary measure that those subjects with the highest serum selenium levels had a significantly higher risk for development of type 2 diabetes than those with lower levels; however, this correlation was not seen in the SELECT trial. It should be noted that the diagnosis of diabetes was not based upon serum biomarkers, but rather self reported or based on the use of diabetes medication. Further, these studies were on men only, and serum selenium levels at baseline were significantly different in the two trials. It has been suggested that the relationship between type 2 diabetes and selenium is U-shaped, with an increased risk of diabetes at both low and high levels of serum selenium, but further research is needed to examine this relationship [100].

23.6.4 Supplementation

The RDA for selenium is 55 μg/d for adult men and women [42]. Selenium is found in a variety of foods, including both plant and animal sources, such as fish, shellfish, organ meats, red meat, chicken, eggs, and milk. The amount of selenium from plant sources depends on the amount of selenium in the soil in which the plant grew; concentrations in animal products also vary depending on the amount of selenium in the animal feed [42,47]. Although evidence indicates that selenium intake is marginal in some areas of the world, including parts of China, Northern Europe, New Zealand, and Russia, research suggests that most Americans obtain adequate amounts of selenium via their usual diets [101,102].

Individuals at risk for selenium deficiency include those with suboptimal dietary selenium intake, generally as a result of living in an area of the world with low soil selenium levels, and severe malabsorptive GI diseases [6]. Selenium deficiency has also been reported in individuals receiving long-term parenteral nutrition without selenium, and in patients with high-output chylous fistula losses [47,103].

Selenium deficiency may lead to Keshan's disease, a form of cardiomyopathy, as well as oxidative injury and altered thyroid metabolism. Serum selenium is an indicator of short-term selenium status; depressed levels have been observed in the acute phase response, so this should be taken into consideration when assessing selenium status. Erythrocyte selenium levels may also be measured to assess long-term selenium status [47].

The ideal dosage for selenium supplementation is unknown, and likely depends on both the degree of deficiency and comorbidities. In studies on patients with SIRS, selenium was given as a continuous infusion at doses ranging from 500 to 1600 μg/d for 9 to 14 days [86–89].

The Tolerable Upper Limit for selenium is 400 μg/d, and frequently studied doses range from 100 to 200 μg/d. While no adverse affects were noted in most intervention studies, further research is needed to more accurately determine timing, dosage, and duration for selenium supplementation. Because results of some trials suggest supplementation of selenium may increase the risk of type 2 diabetes and cancer, it is advised that only selenium-deficient individuals receive supplementation.

23.7 CONCLUSION

A healthful diet high in a variety of nutrient-dense foods is necessary for older adults to maintain an adequate intake of minerals. However, physical limitations, financial difficulties, polypharmacy, chronic diseases, and acute illness often result in suboptimal intakes and altered absorption, excretion, and metabolism of these micronutrients may occur. A thorough nutritional assessment, including diet history and physical exam, should help identify these issues, and proper nutrition treatment, via alterations in food intake or the addition of supplements, can improve mineral levels and thus help treat and prevent many chronic diseases.

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CHAPTER

24

Vitamin D and Immunity

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24.1 INTRODUCTION

Vitamin D is a fat-soluble vitamin that in recent years has been gaining increased attention amongst nutrition experts, the media, and, consequently, the general public. It is currently one of the most widely researched vitamins, and this can be attributed to its proposed wide range of beneficial effects on the human body.

There are only a few naturally occurring food sources of vitamin D, including, for example, fatty fish, fish liver oil, and egg yolk. However, it is the only vitamin that, depending on the availability of ultraviolet B radiation (sunlight), can be synthesized by the human body. Due to the scarce natural sources of vitamin D, in some countries certain foods are fortified with vitamin D, with food fortification starting as early as 1920 when vitamin D was recognized to be important for the prevention of rickets. Examples of foods fortified with vitamin D include milk, margarine, breakfast cereals, yogurt, cheeses, juices, and spreads [1].

Despite the well-established role of vitamin D in calcium and phosphorus homeostasis and its resulting importance in bone health, the actions of vitamin D have more recently been linked to a number of other health aspects, including its role in the immune system. Vitamin D's role in immunity was first established based on the results of several observational studies linking lower levels of vitamin D to an increased risk of respiratory infections in infants, children, and adults. In this context, large population-based studies in the United States and Great Britain have also shown a robust dose–response relationship between lower levels of vitamin D and an increased risk of upper respiratory infections. Additionally, the severity of acute lower respiratory infections in children and mortality from pneumonia in adults has been linked to vitamin D deficiency [2].

The goal of this chapter is to review the immunomodulatory effects of vitamin D, thereby briefly providing an overview of the immune system and vitamin D in general, followed by a summary of the available evidence concerning the effects of vitamin D supplementation on immunity.

24.2 OVERVIEW OF THE IMMUNE SYSTEM

The immune system has evolved to protect the host from pathogenic microbes that are themselves constantly evolving. It also helps the host eliminate toxic or allergenic substances that enter through mucosal surfaces. While eliminating pathologic microbes and toxic or allergenic proteins, the immune system must avoid responses that produce excessive damage of self-tissues or that might eliminate beneficial commensal microbes. The host uses both innate and adaptive mechanisms to detect and eliminate pathogenic microbes, and both of these mechanisms include self–non-self discrimination.

Immune cells, like other cell types, need to be fed. They require an adequate supply of energy, nutrients, and micronutrients serving as co-factors in the development, maintenance, and expression of the immune response. There are two types of immune responses that help protect the body from invading pathogens: innate (or natural, non-specific) and adaptive (or acquired, specific).

Innate immunity is present from birth and provides the first barrier against invaders. Its role is to prevent the entry of infectious agents into the body. The skin, respiratory tract, gastrointestinal system, mucus secretions, and acidity of the stomach are examples of innate

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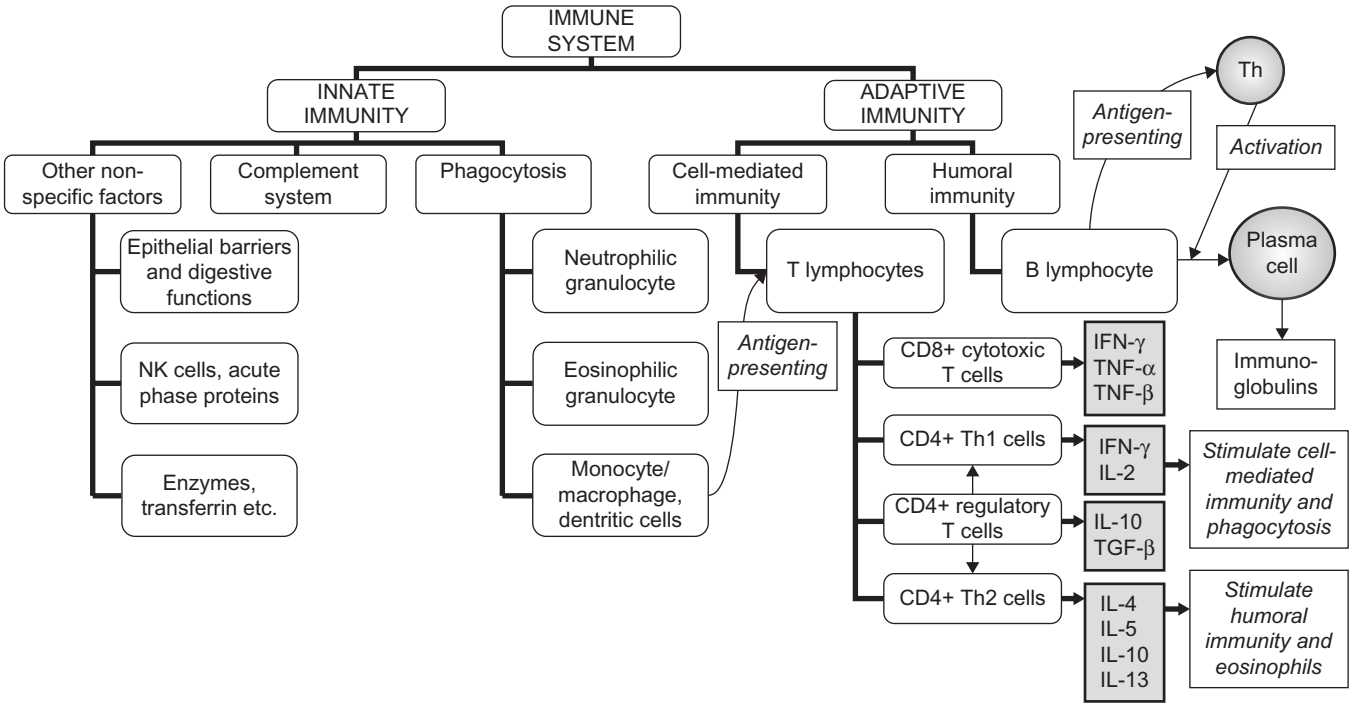

FIGURE 24.1 Schematic representation of the human immune system. CD, cluster of differentiation; DC, dendritic cell; IFN-γ, interferon γ; IL, interleukin; NK cell, natural killer cell; Th, T helper lymphocyte; TGF, transforming growth factor; TNF, tumor necrosis factor [4].

immunity. Should these defenses fail, resulting in pathogens being able to enter the body, innate immune system cells (e.g., phagocytic cells such as monocytes, macrophages, neutrophils, and dendritic cells) come into play and rapidly eliminate invading microbes, thereby keeping the infection at bay. In addition, natural killer (NK) cells are implicated in the control of autoimmunity and resistance to tumors [3].

The secreted mucus layer that overlays the epithelium in the respiratory, gastrointestinal, and genitourinary tracts, and the epithelial cilia that sweep away this mucus layer, enable these regions to be constantly refreshed after they have been contaminated with inhaled or ingested particles. The innate response also includes soluble proteins and bioactive small molecules that are either constitutively present in biological fluids (e.g., complement proteins) or released from cells as they are activated (including cytokines that regulate the function of other cells, chemokines that attract inflammatory leukocytes, lipid mediators of inflammation, reactive free radical species, and bioactive amines and enzymes that also contribute to tissue inflammation). Lastly, the innate immune system includes membrane-bound receptors and cytoplasmic proteins that bind molecular patterns expressed on the surfaces of invading microbes. Some aspects of the innate host defenses are constitutively active (e.g., the mucociliary blanket overlying many epithelia) while others are activated after interactions of host cells or host proteins with chemical structures that are characteristic of invading microbes but are absent from host cells.

Adaptive immunity is the second barrier to infection taking over if the innate immune response cannot clear the infection in a short time. This more sophisticated immunity involves the specific recognition of antigens on an invading pathogen, which characterize it as being foreign. Adaptive immunity is acquired later in life, such as after an immunization or successfully fighting off an infection. It retains a memory of all the invaders it has previously faced, ensuring a faster and stronger response in case of a second encounter with the same antigen. The majority of the cells of adaptive immunity are T lymphocytes and B lymphocytes. These cells carry receptors on their surfaces that distinguish "self" from "non-self". Each T and B cell recognizes antigens specific to particular infectious agents. Following maturation, these cells enter the pool of naïve cells in the peripheral lymph nodes ready to respond to foreign pathogens. An overview of the main components of the immune system is given in Figure 24.1 [4].

24.2.1 Aging of the Immune System

According to WHO, the proportion of the world's population aged over 60 years will have doubled from about 11% to 22% between 2000 and 2050 [5]. The

absolute number of people aged 60 years and over is expected to increase from 605 million to 2 billion over the same period. Only in the late phase of life does the progressive decline of immune function create vulnerability, with a resultant increase in morbidity and mortality due to infection in the elderly. In light of this, it is becoming critical to improve our understanding of immunological aging, or "immunosenescence."

Age-related changes to the functioning of the cells of the innate immune system dramatically affect the ability to combat bacterial and viral infections in old age. In aged subjects, a breakdown in the integrity of innate physical barriers such as the skin, gastrointestinal tract, and lung occurs, resulting in increased susceptibility to invasion by pathogens and an increased burden on the cells of the innate immune system.

Moreover, cells of the innate immune system, notably neutrophils, monocytes/macrophages, and dendritic cells, undergo changes that lead to an overall compromised functioning of the immune system with age. Additionally, monocytes have been shown to increase in numbers with increased age [6]. Defective toll-like receptor (TLR, a receptor that activates immune response) function has also been studied in monocytes, where the production of cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have been shown to be reduced when induced by TLR1/2 [7]. Age also has a general suppressive effect on dendritic cell function. With aging, neutrophils display reduced functions – for example, slowed response to chemotaxis, phagocytosis, generation of superoxide, alterations in signal transduction, and membrane lipid rafts [8]. One study by Lord *et al.* [9] demonstrated that the age-related decline in neutrophil functions is in part explained by the reduced Fc-γ receptor (a receptor that binds to antibodies) expression [9]. The response to CD16 triggering, as well as to granulocyte macrophage colony-stimulating factor and N-formyl-methionine–leucine–phenylalanine stimulation, is reduced, suggesting that even when receptor expression is maintained, as it is for granulocyte macrophage colony-stimulating factor receptor, the signaling cascade leading to activation is impaired. Animal studies also confirmed the hypothesis that proximal events of the receptor may explain the reduced activation level of the cells following stimulation [10]. Some events associated with signaling and activation may also interfere with neutrophil functions. For instance, as dehydroepiandrosterone sulfate levels are highly reduced with aging (adrenopause), this could greatly impact neutrophil activation. Lord and colleagues have shown that dehydroepiandrosterone sulfate increases superoxide generation of neutrophils via a PKC-β/p47(phox) pathway [11]. This suggests that antibactericidal activity may be altered due to extrinsic reasons, and that modulation of neutrophil activity is possible.

Although the past decade has seen a lot of research in the field of immunosenescence, the effect of aging on innate immune responses is still not fully understood. Moreover, our understanding of how the changes in the immune system with age are influenced by micronutrients is an area that is still in its relative infancy.

The ability of the adaptive immune system to cope with age-associated changes is limited. The continuous decrease in diversity of the antigen repertoire and the accumulation of functionally impaired memory lymphocytes with age can lead to gaps in the body's defenses, and pathogens can exploit these gaps.

At the cellular level, the most prominent features of immunosenescence include a substantial decrease in the number of naïve lymphocytes, as a result of a reduction in thymic output of T cells [12], as well as fewer bone marrow early progenitor B cells [13] and an accumulation of oligoclonally expanded and functionally incompetent memory lymphocytes. The causes of age-associated decline in the generation of naïve cells are likely to be multifactorial and to involve changes in growth factors and/or hormones, hematopoietic progenitor cells, and their surrounding microenvironment. The accumulation of memory cells with age may reflect an adaptive response to the decline of production of naïve lymphocytes through homeostatic expansion, as well as the cumulative effect of past and persistent viral infections [14].

Functionally, the decline of adaptive immunity with age can be attributed to impairment at the systemic and microenvironmental levels in lymphoid and non-lymphoid organs involving multiple types of cells other than lymphocytes (extrinsic defects), and to the specific impairment in the function of lymphocytes (intrinsic defects). The age-associated extrinsic changes often affect multiple systems, such as the neuroendocrine system [15] and even the innate immune system [16], which can impact the functioning of the adaptive immune function; therefore, cause and effect are not always clear cut.

Our knowledge of aging of the adaptive immune system and the role of micronutrients therein is so far not fully understood.

24.3 VITAMIN D – OVERVIEW OF ITS BIOLOGICAL FUNCTIONS

Vitamin D, which is also known as calciferol, describes a group of fat-soluble seco-sterols of which the major ones are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamins D2 and D3 differ only in their side-chain structure, as shown in Figure 24.2. These minor differences do not affect their metabolism, and the two forms have been reported to have similar

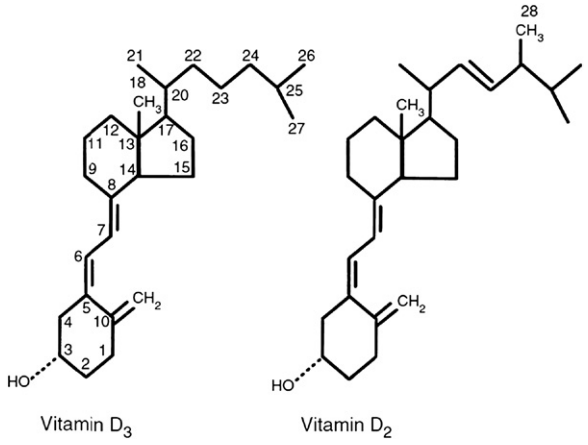

FIGURE 24.2 Molecular structures of vitamins D2 and D3.

potency in the body. Vitamin D3 can be synthesized in the skin of humans from 7-dehydrocholesterol and is also present in animal-based foods; therefore nowadays it is the preferred source used in supplements. Vitamin D2 is largely synthesized commercially [1,17].

Vitamin D is considered biologically inactive until it undergoes two enzymatic hydroxylation reactions at carbon 25 and carbon 1 (see Figure 24.2 for numbering). The first reaction takes place in the liver where vitamin D is converted to 25-hydroxyvitamin D (25-OHD), which is the major circulating form of vitamin D and is therefore also regarded as the most reliable marker of vitamin D status. The second hydroxylation reaction takes place in the kidney, which converts 25-OHD to the biologically active hormone 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), also known as calcitriol [1,17]. This is the active metabolite responsible for the proposed widespread functions of vitamin D. One of its most established and well-known functions is its role in the regulation of serum calcium and phosphorus homeostasis with, more specifically, its regulation of intestinal calcium transport and mineralization of bone therefore being essential for maintaining bone health [1,18]. Numerous studies and reviews have described the role and benefits of vitamin D in bone health, as well as in fall and fracture prevention [19].

More recently, due to the presence of vitamin D receptors throughout the body, vitamin D has been associated with a wide array of other functions, such as the regulation of cell proliferation, cell differentiation, and apoptosis. Therefore the actions of vitamin D may be quite diverse, and it has been suggested that vitamin D may have potential preventive or therapeutic roles in cancer and chronic conditions such as autoimmune conditions (e.g., type 1 diabetes), cardiovascular disease, and infections [1,20]. The next section of this chapter will focus on vitamin D's role in immunity.

24.4 VITAMIN D AND ITS IMPORTANCE IN IMMUNITY

Over the past decade the perspective on how vitamin D influences human health has changed dramatically, based on identification of the vitamin D receptor (VDR) in peripheral blood mononuclear cells. This, in combination with epidemiological evidence linking vitamin D status to respiratory infections, sparked the early interest in vitamin D as an immune system regulator [21–23]. The discovery of significant quantities of vitamin D receptors in monocytes, macrophages, and thymus tissue suggests a specific role of vitamin D and its metabolites in the immune system. Most cells of the immune system express vitamin D receptors [24].

24.4.1 Vitamin D in Innate Immunity

In the innate immune system, vitamin D has a role as a mediator of human host defense mechanisms against microbial disease. First insights into vitamin D-induced antimicrobial activity by human monocytes and macrophages against *M. tuberculosis* were gained in experiments by Rook *et al.* [25] in 1986 and by Crowle *et al.* [26] in 1987. These experiments were performed adding 1,25-$(OH)_2$D to the extracellular medium of *M. tuberculosis*-infected human monocytes and macrophages *in vitro* with a resultant reduction of the intracellular proliferation of the bacteria. This effect was enhanced when interferon-γ was present. Interferon-γ is known to stimulate the vitamin D-activating enzyme CYP27B1 that is expressed in monocytes/macrophages and dendritic cells.

More recent insights into the underlying mechanisms suggest that activation of toll-like receptors (TLR, 2/1) results in the induction of key genes in the vitamin D pathway, including VDR and CYP27B1. Under conditions where the extracellular concentration of 25-OHD is present at sufficient levels, TLR2/1 activation of monocytes results in a CYP27B1- and VDR-dependent expression of the antimicrobial peptide cathelicidin, and direct microbicidal activity against intracellular *M. tuberculosis*. The induction of CYP27B1 and VDR in monocytes was subsequently demonstrated to be mediated through the actions of TLR2/1-induced IL-15 expression [27]. Inhibition of the VDR resulted in ablation of the TLR2/1-induced antimicrobial activity, implicating that VDR activation is a critical step in the innate immune response against *M. tuberculosis* and potentially explaining the association of 25-OHD serum levels with susceptibility to tuberculosis – for example, where low 25-OHD levels in the circulation cannot provide sufficient substrate 25-OHD for CYP27B1-mediated production of 1,25-$(OH)_2$D to activate the VDR-dependent antimicrobial response. Through rheostatic regulation of CYP27B1

activity and conversion of substrate 25-OHD to product 1,25-$(OH)_2$D, the macrophage directly controls its intracellular level of 1,25-$(OH)_2$D [28]. It is also now recognized that TLR-induced antimicrobial activity can be inhibited by blocking CYP27B1 activity [29]. These data suggest that it is serum 25-OHD and not the 1,25-$(OH)_2$D concentration that controls the intracellular 1,25-$(OH)_2$D level and is essential for the TLR-induced antimicrobial activity. This explains why in previous experiments *in vitro*, a supraphysiologic concentration of 1,25-$(OH)_2$D in the conditioning extracellular media was required to generate sufficient intracellular levels of the metabolite to affect the VDR and to achieve an antimicrobial effect in human macrophages.

24.4.2 Vitamin D in Adaptive Immunity

Early studies of vitamin D and the immune system demonstrated VDR expression in both T and B cells [30]. Notably, VDR expression by these cells was only immunologically functional in active, proliferating cells, suggesting an antiproliferative role for 1,25-$(OH)_2$D on these cells [31]. T helper (Th) cells appear to be the principal target of 1,25-$(OH)_2$D, which can suppress Th cell proliferation as well as modulate cytokine production by these cells [32]. Activation of naïve Th cells by antigens in turn leads to the generation of Th subgroups with distinct cytokine profiles: Th1 (IL-2, IFN-γ, TNF-α) and Th2 (IL-3, IL-4, IL-5, IL-10) that, respectively, support cell-mediated and humoral immunity [33,34]. *In vitro* 1,25-$(OH)_2$D inhibits Th1 cytokines [32] whilst promoting Th2 cytokines [35]. A third group of Th cells known to be influenced by vitamin D are interleukin-17 (IL-17)-secreting T cells (Th17 cells). Indeed, recent studies have shown that 1,25-$(OH)_2$D suppresses IL-17 production via direct transcriptional suppression of IL-17 gene expression [36]. Another group of T cells known to be potently induced by 1,25-$(OH)_2$D is regulatory T cells (Tregs) [37]. Although part of the Th cell family, Tregs act to suppress immune responses by other T cells as part of the machinery to prevent over-exuberant or autoimmune responses [38]. Recent studies have underlined the importance of Tregs in mediating the immunoregulatory actions of vitamin D. Administration of 1,25-$(OH)_2$D systemically to patients with renal disease has been shown to expand circulating Treg populations [39].

Despite the fact that expression of VDR by B cells has been recognized for many years [30], the ability of 1,25-$(OH)_2$D to suppress B cell proliferation and immunoglobulin (Ig) production was initially considered to be an indirect effect mediated via Th cells [31]. However, more recent studies have confirmed direct effects of 1,25-$(OH)_2$D on B cell homoeostasis [40], with notable effects including inhibition of plasma cell and class-switched memory cell differentiation. These effects lend further support for vitamin D's proposed role in B cell-related autoimmune disorders such as systemic lupus erythematosus (SLE). Other B cell targets known to be modulated by 1,25-$(OH)_2$D include IL-10 [41] and chemokine receptor type 10 (CCR10) [42], suggesting that the repertoire of B cell responses to vitamin D extends beyond its effects on B cell proliferation and Ig synthesis.

As shown by a number of studies, vitamin D in its active form 1,25-$(OH)_2$D is a potent immune system modulator. The VDR is expressed by most cells of the immune system, and some cells of the immune system are also capable of producing the enzyme responsible for the conversion of 25-OHD to 1,25-$(OH)_2$D. The evidence described above demonstrates that 1,25-$(OH)_2$D has a variety of effects on immune system function, which may enhance innate and adaptive immunity as well as inhibit the development of autoimmunity. The effects of 1,25-$(OH)_2$D on various different immune cells are summarized in Table 24.1 [43,44].

TABLE 24.1 Effects of Vitamin D on Immune Cells [43,44]

Immune cell	Effects of 1,25-$(OH)_2$D on various immune cells
Dendritic cell	• Suppresses antigen presentation to T cells • Regulates negatively DCs' differentiation, maturation, and immunostimulatory capacity • Decreases MHC class II, CD40, CD80, CD86 expression • Decreases CD1a, CD83 maturation • Decreases T cell stimulation • Decreases IL-6, IL-12, and IL-23 synthesis • Effect on IL-10 production (controversial data) • Enhances level of FOXP3 expression • Displays antigen unspecific suppressor activity. • Inhibits NF-κB family transcription factors' activation and expression • 1,25-$(OH)_2$D + LPS generates tolerogenic DCs • Induces VDR, enhancing DCs' tolerogenicity and decreasing IL-12p70 production • Upregulates CD152 • Indirect Th1 response inhibition • Impairs IFN-γ production

(*Continued*)

TABLE 24.1 (Continued)

Immune cell	Effects of 1,25-$(OH)_2$D on various immune cells
Macrophage	• Autocrine, intracrine, and paracrine effects • Stimulates the differentiation of monocytic precursors in mature cells • Downregulates GM-CSF expression • Stimulates PGE_2 production • Increases peripheral macrophage-specific surface antigens and acid phosphatase expression • Stimulates "oxidative burst" • Enhances chemotaxis and phagocytosis • Regulates the expression of CD14 (co-receptor of TLR4) • Inhibits TLR2, TLR4, and TLR9 expression • Induces hyporesponsiveness to pathogen-associated molecular patterns • Alters the TLR9-dependent production of IL-6 • Induces the expression of a splice variant form CYP24-SV • Decreases TNF-α, IL-1, IL-6, and IL-23 production • Modulates expression of MMP-7, MMP-9, and MMP-10 in M. tb-infected PBMCs • Induces IL-10 and PGE2 secretion from M. tb-infected PBMCs • Induces defensins and cathelicidin • Regulates iNOS expression (contradictory data) • TLR2 or TLR4 enhances vitamin D signaling by upregulating VDR and CYP27B1 expression
T lymphocyte	• Increases VDR levels • Regulates T cell development and migratory function • Regulates TCR signaling • Activates PLC-γ 1 • Alters cytokine secretion patterns • Suppresses effector T cell activation • Induces regulatory T cells • Regulates T cell trafficking and homing • Induces CCR10 expression • Decreases Th1 cells' proliferation • Promotes epidermal T cell homing • Inhibits chemokines and chemokine receptors • Promotes T cell shift from Th1 to Th2 in CD41 cells • Inhibits production of IL-2, IFN-γ, TNF-α, and IL-5 • Enhances TGF-β1 and IL-4 transcripts • Increases Th2 cells' function • Inhibits T cell surface expression of CLA • Inhibits TGF-β mediated Foxp3 on CD4+ T cells • Negatively regulates CCR6 expression on Th17 cells • Decreases levels of IL-2 in CD4+ T cells • Regulates Th17 cells and decreases IL-17 expression
Treg lymphocyte	• Promotes Treg cells' development • Influences Treg cells' differentiation and functions • Increases the suppressive activity and expansion of antigen-specific Treg cells • Negatively regulates the expression of CCR6 on the Th17 cells • Inhibits the lineage commitment of Th17 cells • Cooperates with IL-6 or TGF-β to mediate IL-10 production • Increases IL-27-mediated IL-10 producing CD4+ T cells • Induces Foxp3+ Treg cells' expansion • Decreases IL-2 levels • Vitamin D3 + glucocorticoids stimulate the generation of IL-10-producing CD41/CD251 and TLR9 expression • Actively regulates proliferation and cytokine production of CD81 cells
B lymphocyte	• Regulates VDR expression • Upregulates CYP24A1 • Inhibits B cell proliferation by upregulating p27 • Decreases CDK4, CDK6, and cyclin D expression • Mediates death of proliferating B cells • Inhibits plasma cells' differentiation and reduces Ig-secreting cells and Ig production • Inhibits NF-κB • Inhibits XBP1 and ERN1

CCR6, CCR10, chemokine receptors; CLA, conjugated linoleic acid; CDK-4, -6, cyclin-dependent kinases; ERN1, endoplasmic reticulum to nucleus signaling-1; FOXP3, forkhead box P-3; GM-CSF, granulocyte–macrophage colony-stimulating factor; DCs, dendritic cells; CYP24-SV, 24-hydroxylase splice variant form; CYP27B1, 25-hydroxyvitamin D-1 α-hydroxylase; Ig, immunoglobulin; iNOS, inducible nitric oxide synthases; IFN-c, interferon-c; IL-1, interleukin-1; LPS, lipopolysaccaride; MHC-2, major histocompatibility complex-2; MMP-7, matrix metalloproteinase-7; M. tb, *Mycobacterium tuberculosis*; NF-κB, nuclear factor-κB; Tregs, regulatory T cells; PBMCs, peripheral blood mononuclear cells; PLC-c1, phospholipase C-c1; PGE2, prostaglandin E2; TCR, T cell receptor; TLR-2, toll-like receptor-2; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; VDR, 1,25(OH)2D3 receptor; Th-1, T helper type 1; XBP1, X-box binding protein-1.

24.5 VITAMIN D DEFICIENCY: A GLOBAL PROBLEM

Vitamin D deficiency is a global concern, even in very sunny climates, and is a major public health problem [45]. The blood level of 25-OHD is the best method to determine vitamin D status and reveal whether somebody is getting enough vitamin D from endogenous synthesis and/or dietary sources. The 25-OHD levels recommended as "cut-offs" to define vitamin D deficiency, insufficiency, and sufficiency differ between various expert groups such as the US Institute of Medicine (IOM) and the US Endocrine Society. One reason for these discrepancies is that there is still a degree of uncertainty in this area due to continuously emerging research, which makes it difficult to define an "optimal" or "sufficient" level. Aspects to be considered when setting these levels include the amount of sunlight various population groups require to achieve a given level of vitamin D, the outcome measure to be used to define an optimum level (e.g., bone health, or chronic diseases such as cancer, heart disease, and diabetes), and the benefits and risks of widespread supplementation. There is consensus that 25-OHD blood serum levels below 25 nmol/l (10 ng/ml) qualify as "deficient" [46], but beyond this there is currently no standard definition of "optimal" 25-OHD levels [47,48]. It is quite likely that an optimal vitamin D level may vary considerably depending on the specific biological and clinical functions, which makes the task of setting specific cut-off values more challenging [49]. In general, "desirable" levels of vitamin D are classified as blood concentrations of above 75 nmol/l (30 ng/ml); "inadequate" levels are classified between 50 and 75 nmol/l (20–30 ng/ml); "insufficient" between 25 and 5 nmol/l (10–20 ng/ml); and "deficiency" below 25 nmol/l (10 ng/ml). There is also lack of standardization of methods used to measure 25-OHD status, with different tests producing very different results [50]. Recently, the IOM has reassessed dietary reference intakes for vitamin D in order to help achieve these desired levels. For adults the recommended dietary allowance (RDA) was set to 600 IU/day, with the amount being increased to 800 IU/day for the elderly, highlighting the increased requirements for this population group [1].

There are a number of factors that place individuals at risk for vitamin D deficiency, among them geographic location [51], skin tone [52,53], sunscreen use [54], age [55], body weight [56], and lifestyle [57,58]. Vitamin D deficiency is very common among patients with malabsorption syndromes, including inflammatory bowel disease and prior gastric bypass [59,60]. A recent study [61] has demonstrated that patients with Crohn's disease, despite quiescent disease status, had on average a 30% decrease in their ability to absorb oral vitamin D. Several reviews have found high prevalence of vitamin D deficiency worldwide [62–64] and in all age groups – even in industrialized countries, where vitamin D fortification has been implemented now for years. However, prevalence of vitamin D deficiency worldwide is still uncertain, as there remain many data gaps for many geographies. About 1 billion people have low vitamin D levels, found in all ethnicities and age groups [65]. The elderly populations of Europe, the USA, and Australia, however, present special problems. With increasing age, solar exposure is usually limited because of changes in lifestyle factors such as clothing and outdoor activity. Diet may also become less varied, with a lower natural vitamin D content. Most importantly, however, the dermal production of vitamin D following a standard exposure to UVB light decreases with age because of atrophic skin changes with a reduced amount of its precursor. Finally, the renal production of 1,25-$(OH)_2$D decreases because of diminishing renal function with age. These changes in vitamin D metabolism render the aging population in general at risk of vitamin D deficiency, especially in winter seasons and when living indoors and at higher latitudes. This deficiency may lead to severe consequences that negatively impact an individual's lifespan and health span [66].

24.6 HUMAN STUDIES INVESTIGATING THE EFFECTS OF VITAMIN D ON IMMUNITY

24.6.1 Autoimmune Diseases

Autoimmune diseases include diseases such as insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), and rheumatoid arthritis (RA). They are described as diseases in which the body mounts an immune response against its own tissue, rather than a foreign pathogen. As described in previous paragraphs, autoimmune responses are mediated by T cells, and 1,25-$(OH)_2$D has been shown to diminish autoimmune responses by modulating T cell responses. Epidemiological studies have revealed that the prevalence of autoimmune diseases increases with increasing latitude, indicating that serum vitamin D levels, which generally decrease with increasing latitude, may be a factor in the pathology of these diseases. Prospective studies appear to back up this association such that, for example, a case–control study in US military personnel, including 257 cases of diagnosed MS, found that white subjects in the highest quintile of serum 25-OHD had a 62% lower risk of developing MS [67]. Moreover, another study showed that, in two large cohorts of US women followed for at least 10 years, vitamin D supplement use was associated with a significant reduction

in the risk of developing MS [68]. With regard to RA, one study showed that postmenopausal women with the highest total vitamin D intakes were at significantly lower risk of developing RA after 11 years of follow-up than those with the lowest intakes [69]. On the topic of IDDM, a large cohort study in the UK has shown that 81.6% and 96.1% of IDDM patients in the UK are vitamin D deficient in the summer and winter, respectively [70]. Also, a large cohort study from the US military showed a 3.5-fold higher risk of developing IDDM in soldiers if 25-OHD levels were below 17 ng/ml prior to diagnosis compared to those with a level of above 28 ng/ml [71]. These studies, along with experimental research findings previously described, point towards a potential important role of vitamin D in autoimmune diseases.

24.6.2 Influenza and Respiratory Tract Infections

Many observational studies have shown low serum 25-OHD levels to be associated with an increased risk of respiratory infections in adults, as well as at-risk groups such as the elderly, children, and infants [2]. For instance, in one prospective cohort study, serial monthly concentrations of 25-hydroxyvitamin D were measured over the fall and winter 2009–2010 in 198 healthy adults, and participants were evaluated for the development of any acute respiratory tract infections. The incidence of infection in participants with different concentrations of vitamin D was determined, and revealed that concentrations of 38 ng/ml or more were associated with a significant ($P < 0.0001$) two-fold reduction in the risk of developing acute respiratory tract infections, and with a marked reduction in the percentages of days ill. The authors concluded that maintenance of a 25-OHD serum concentration of 38 ng/ml or higher should significantly reduce the incidence of acute viral respiratory tract infections and the burden of illness caused thereby [72]. Also, large population-based studies in the US have revealed an increased risk of upper respiratory infections with low levels of 25-OHD [73]. For instance, a secondary analysis of the Third National Health and Nutrition Examination Survey, a probability survey of the US population conducted between 1988 and 1994, examined the association between 25-OHD levels and recent upper respiratory tract infections (URTIs) in 18,883 people. Results showed that recent URTI was reported by 24% of participants with 25-OHD levels less than 10 ng/ml, by 20% with levels between 10 and 30 ng/ml, and by 17% with levels above 30 ng/ml ($P < 0.001$). Vitamin D deficiency has been linked not only with increased incidence but also with increased severity of acute lower respiratory infections and increased mortality from pneumonia, as shown by studies carried out in children and adults [74–76].

So far, randomized controlled trials have not unequivocally been able to show benefits of vitamin D supplementation, although several trials do point towards vitamin D playing a role in reducing the risk of respiratory infections. For instance, a randomized controlled trial showed that supplementation with vitamin D (1200 IU/day for 4 months) was associated with a reduced risk of seasonal influenza ($P = 0.04$) [77]. An additional study found a lower rate of upper respiratory infection and influenza in African American subjects taking a vitamin D supplement (2000 IU/day) for 3 years [78]. In 2007, as an adjunct to the RECORD trial (a blinded, randomized, placebo-controlled trial assessing vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people), Avenell *et al.* [79] examined whether vitamin D was associated with a reduction in self-reported infections. The study included 3444 elderly participants who received either 800 IU (20 μg) daily vitamin D3, 1000 mg calcium (calcium carbonate), both, or placebo, and were followed up for 24–62 months. Of the respondents randomized to vitamin D3, 17.2% (300/1740) reported an infection, compared with 18.8% (321/1704) on placebo, which yields an adjusted odds ratio of 0.90. Despite not being statistically significant, the observed differences were consistent with previous observations that vitamin D may reduce the risk of infection. In 2009, Yamshchikov *et al.* [80] conducted a systematic review on vitamin D and its use for treatment and prevention of infectious diseases. According to this review, the strongest evidence for the effectiveness of vitamin D in prevention or treatment of infectious diseases is for the reduction of risk of acute respiratory illness and influenza.

The combined evidence of epidemiological, observational, and randomized controlled studies clearly points towards an immunity-promoting role of vitamin D in humans. Research to date has shown that levels of vitamin D can affect not only the incidence but also the severity of, for example, respiratory tract infections. Vitamin D levels have also been linked to the occurrence of autoimmune diseases, and evidence is mounting regarding a beneficial role of this vitamin in regulating autoimmune responses.

24.7 CONCLUSION

Vitamin D is currently one of the most widely researched vitamins, and this can be attributed to its proposed wide range of beneficial effects on the human body. There is a large body of research demonstrating the beneficial roles of vitamin D in various aspects of the immune system, including both innate and adaptive immunity. The VDR is expressed by most cells of the immune system, and some cells of the immune system

are also capable of producing the enzyme responsible for the conversion of 25-OHD to 1,25-$(OH)_2$D, further pointing towards the essential role of vitamin D in preparing an immune response. Aging has pronounced effects on the immune system such that age-related changes to the functioning of the cells dramatically affect the ability to combat bacterial and viral infections. With increasing age, cells of the innate system, notably neutrophils, monocytes/macrophages, and dendritic cells, undergo changes that lead to compromised functioning of the immune system. Moreover, the adaptive immune system deteriorates with age such that its ability to cope with age-associated changes is limited. The continuous decrease in diversity of the antigen repertoire and the accumulation of functionally impaired memory lymphocytes with age can lead to gaps in the body's defenses, and pathogens can exploit these gaps. Overall, immunosenescence causes increased susceptibility to infections and autoimmune diseases, ultimately leading to increased morbidity and mortality.

Unfortunately, in part because of the scarcity of vitamin D content in foods and the limited sun exposure in many regions of the world, vitamin D deficiency is a major public health concern that cannot be ignored, especially in the elderly. Due to its wide variety of functions in the body vitamin D deficiency can have many adverse health implications, many of which are currently being intensely researched around the globe. Research in the area of immunity points towards several benefits of achieving and maintaining optimal vitamin D levels, although the optimal level of vitamin D is yet to be precisely determined. Studies have shown vitamin D levels above certain thresholds (such as >30 ng/ml) to be beneficial in the reduction of the incidence and severity of, for example, respiratory tract infections, and the occurrence of autoimmune diseases is also likely to be diminished with adequate vitamin D status. However, despite the already large body of research demonstrating the importance of vitamin D for immunity, more rigorous research studies with large populations and outcome measures, including 25-OHD serum status post-supplementation, are needed to further elucidate the relationships between vitamin D and the immune response. While more research is indeed needed, the current level of evidence strongly suggests that vitamin D supplementation can serve to prevent vitamin D deficiencies and in turn positively affect immune health, especially in the elderly.

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CHAPTER

25

Micronutrients and Ginseng for Immune Support in Older Adults

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25.1 INTRODUCTION

Aging is a complex process affecting a wide variety of physiological functions, including the development and maintenance of the immune system. Aging of the immune system, or immunosenescence, is multifaceted and impairs both innate and adaptive responses. It is characterized by changes in T cell subsets, cellular and molecular level alterations, and thymic atrophy. Overall, immunosenescence attenuates the host's ability to mount a robust or effective immune response [1–4]. As a consequence resistance to pathogens is impaired and the elderly are 2–10 times more likely to die of infection than younger adults [2,5]. Immunosenescence and concurrent health problems involving the lungs and heart increase the risk of complications and death from viral infections in the elderly [2]. Influenza is the fifth leading cause of death among people aged 50 years and older, and this group is a major target of vaccination campaigns [2]. However, as a result of immunosenescence, when vaccinated, seniors experience a lower antibody response than that observed in young healthy cohorts [6,7]. Experts estimate that the detrimental effects of aging on response to vaccination will increase in importance as a public health concern in the 21st century [8].

Finally, immunosenescence also results in a loss of the ability of recognition of "self" and "foreign" antigens, and is accompanied by increased autoantibody production and autoimmune disorders [1]. Interestingly, autoimmune disorders show signs of premature aging of the immune system, and chronic inflammatory states often seen in aging also help to explain characteristic alterations in patients with autoimmune diseases [1].

Current research demonstrates that nutrition plays a key role in health maintenance in the general population but particularly in older adults, and that many elderly are at increased risk of inadequate nutrient intakes [5,9]. Ensuring adequate intake of vitamins and minerals is one of the most efficient preventive measures (together with physical activity) against health deterioration due to aging. This prevention should start early in life, continue in the older population, and be sustained throughout aging [10]. Already mild micronutrient deficiencies can result in lack of well-being and general fatigue, negatively affect mental processes (e.g., memory, concentration, attention, and mood) resulting in a reduced mental and physical vitality, and, finally, negatively impact immune function and resistance to infections [5,10–12].

Immune defenses are constantly active and consist of a complex network of coordinated biological responses. The general nutritional status of an individual modulates immune functions, and immunocompetence is regarded as a measure of adequate nutrition [13]. Micronutrient deficiency impairs immunity by affecting innate, T cell-mediated and adaptive antibody response, leading to a deregulation of the balanced host response. This increases susceptibility to infections, with increased morbidity and mortality overall showing a similar picture to the one observed in immunosenescence [13]. In turn, infections aggravate micronutrient deficiencies by reducing nutrient intake, increasing losses, and interfering with their utilization by altering metabolic pathways. Insufficient intake of micronutrients commonly occurs in people with eating disorders and/or unhealthy eating habits, in smokers (active and passive), in individuals

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with chronic alcohol abuse, in certain diseases, during pregnancy and lactation, and especially in the elderly [5,13–15].

In addition to essential micronutrients, a number of herbal extracts or nutraceuticals such as *Panax ginseng*, Echinacea, propolis, or probiotics are commonly used to boost immune function. For more than 2000 years *Panax ginseng* has been used in traditional Chinese medicine for different purposes: as a general tonic and prophylactic agent to build resistance, reduce susceptibility to illness, and promote health, vitality and longevity, or as an aid during convalescence. A range of *in vitro* animal and human data show that *Panax ginseng* supports the immune system [16,17].

This chapter reviews the roles of selected micronutrients and *Panax ginseng* in relation to immune function support, particularly in the context of aging.

25.2 AGING AND IMMUNE FUNCTION

Since the beginning of recorded human history, young children have outnumbered older people. However, for the first time in history, people aged 65 years and older are projected to outnumber children under the age of 5 before 2020 [18]. This trend is emerging globally: in 2007 almost 500 million people were aged 65 years and older, accounting for 8% of the world's population. By 2030 the world is likely to have 1 billion older people, accounting for 13% of the total population. While today's proportions of older people typically are highest in more developed countries, the most rapid increases in older populations are occurring in the less developed world. Between 2006 and 2030, the number of older people in less developed countries is projected to increase by 140%, as compared to an increase of 51% in more developed countries [18].

Aging is accompanied by a variety of physiological, psychological, economic, and social changes [18]. The physiological changes in particular affect the need for several micronutrients [19]. The elderly are widely considered as a target group exposed to a higher risk for micronutrient deficiencies as age-related requirements of micronutrients generally encounter mostly decreased intakes due to lower caloric requirements [20,21], alterations in taste, smell, and salivary function [20], ever less effective vitamin and mineral absorption, and increasingly frequent digestive tract disorders [22,23]. At the same time, micronutrient requirements are unchanged or even increased (for example, for vitamins B6 and B12) [24]. The situation is further exacerbated due to frequent use of medicines with many different interactions with vitamin and mineral absorption [25–28], a reduced ability to retain vitamins, and increased levels of loss through excretion.

The age-related impairment of immune function, or immunosenescence, causes increased susceptibility to infections, cancer, and autoimmune diseases overall, leading to increased morbidity and mortality in older individuals [3,4,29,30]. The causes of immunosenescence have also been investigated [3,4]. Since the oxidative theory of aging is the most widely accepted one, several parameters of oxidative stress and antioxidant defenses have been studied in relation to immunosenescence in animals and humans alike. Indeed, with aging immune cells revealed an increase in oxidant and inflammatory compounds and a decrease in antioxidant defenses, which was more evident in phagocytic cells [31–33]. In a murine model of premature aging, immune cells showed marked oxidative stress whereas leukocytes from very old animals or healthy centenarians showed levels of both oxidative and antioxidant compounds and defenses similar to those found in the cells of younger adult mice or younger humans [31,34,35]. This chronic oxidative stress, which has among its intracellular mechanisms the activation of NF-κB in the leukocytes, affects all cells and especially those of regulatory systems such as the nervous, endocrine, and immune systems. Accordingly, the administration of antioxidants such as vitamins C and E, zinc, selenium, beta-carotene, polyphenols, and soy isoflavones in animals and in humans was found to improve both the nervous and immune functions, decreasing their oxidative stress and consequently leading to a significant increase in longevity [31–33,36–41].

In conclusion, undernutrition in general is a detrimental factor in immune responses [42,43], and mature adults are particularly at risk for micronutrient deficiencies [19,20]. Many decreased immune responses previously attributed to the aging process are actually linked to other factors, such as poor nutritional status or ongoing disease which is not clinically apparent [44–46]. The immune system theory of aging states that the rate of aging is largely controlled by the immune system. Some researchers propose the interesting concept of the immune system as a marker of general health and longevity [5,44–46].

25.3 MICRONUTRIENTS AND IMMUNE FUNCTION

It is well established that the general nutritional status of individuals modulates their immune function. Both overnutrition that results in obesity and undernutrition affect functions of innate and acquired immunity detrimentally. Furthermore, obesity (BMI $>30\,kg/m^2$) can be associated with chronic inflammation. Particular aspects of the habitual diet, including fat and protein intakes, multivitamin and mineral supplements, and alcohol consumption, as well as age, exert a significant

influence on immune function [13,15]. Overall, inadequate intake and status of vitamins and minerals may lead to suppressed immunity [44–46], which predisposes to infections and aggravates malnutrition. Micronutrient supplementation can aid the body's natural defense system on three levels by supporting physical barriers (skin/mucosa), cellular immunity, and antibody production [5,13–15]. Several reviews have recently evaluated the role of selected vitamins and minerals in immune function [5,13–15,47–50], and these are summarized in Tables 25.1 and 25.2 [5,13–15,51]. In the following, we will focus on the three key immune supporting micronutrients: vitamins C, B6, and zinc.

Vitamin C is highly concentrated in leukocytes, and is used rapidly during infection. In fact, it has been defined as a stimulant of leukocyte functions, especially of neutrophil and monocyte movement [52–54]. Vitamin C supplements have been shown to enhance neutrophil chemotaxis in healthy adults (1–3 g/day) and children (20 mg/kg per day) [55]. In addition, supplementation with vitamin C has been demonstrated to stimulate the immune system by enhancing T lymphocyte proliferation in response to infection, and increasing cytokine production and synthesis of immunoglobulins [56]. Vitamin C may also play a significant role in regulation of the inflammatory response [57].

Administration of vitamin C results in improvement of several components of the human immune response, such as antimicrobicidal and natural killer (NK) cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity (DTH) response [58–61]. Based on its immune-stimulating properties [55], vitamin C was postulated to be effective in ameliorating symptoms of upper respiratory tract infections, especially the common cold. Further, since plasma and leukocyte vitamin C concentrations fall rapidly with the onset of the infection and return to normal with the amelioration of the symptoms, it was suggested that vitamin C could be beneficial for the recovery process [62].

Hemilä [63] reviewed the literature to determine whether vitamin C given regularly at doses ≥1 g/day had any effects on the course of the common cold. Regular vitamin C use consistently and significantly reduced the duration of episodes and the severity of symptoms of the common cold by 23%, and further reduced common cold incidence by 9% [63]. A further analysis was carried out to identify factors contributing to the variation of benefits observed in the above trials [64]. It appeared that the higher the dose per kg body weight, the greater the benefit. It was found that, on average, vitamin C produced greater benefit in terms of cold duration for children than adults, and that the magnitude of the benefit of vitamin C with doses of ≥2 g/day was greater than that of 1 g/day. The latest Cochrane Review on vitamin C and the common cold published in 2013 evaluated whether oral doses of ≥200 mg/day of vitamin C reduced the incidence, duration, or severity of the common cold when used either as continuous prophylaxis or after the onset of symptoms [65]. The review confirmed the benefit of high doses of vitamin C taken regularly in reducing the duration of cold symptoms. In adults, the duration of colds was reduced by 8% (range 3–12%). The severity of colds was also reduced by regular vitamin C administration. The reduction of cold duration in adults (8%) is lower than the 23% reported previously by Hemilä [63]; however, the Hemilä 1994 review included only studies using ≥1 g vitamin C; hence the higher vitamin C dose may explain the different findings. The Cochrane Review also confirmed that there appears to be no preventive effect regarding common cold incidence in the general population. However, a subgroup of six trials involving a total of 642 marathon runners, skiers, and soldiers on sub-arctic exercises showed a pooled relative risk of 0.50 (95% CI 0.38–0.66) whilst taking prophylactic vitamin C [65].

Recently, the issue of the optimal vitamin C intake has been debated in the literature [66–68]. Experts, including Balz Frei and co-workers from the renowned Linus Pauling Institute, concluded that, based on the combined evidence from human metabolic, pharmacokinetic, and observational studies and randomized trials, 200 mg per day is the optimum dietary intake of vitamin C for the majority of the adult population to maximize the vitamin's potential health benefits with the least risk of inadequacy or adverse health effects [66]. This is about twice the current recommendations, and considering that elderly have and impaired intestinal vitamin C absorption [65] and lowered vitamin C status they have even higher intake requirements to maintain health.

Overall, data presented here indicate that vitamin C supplementation plays a role in the respiratory defense mechanisms. It appears that the elderly, who have been shown to be at risk for vitamin C deficiency and may therefore be more prone to infections, may benefit from a moderate continuous vitamin C intake in relation to respiratory infections such as the common cold [53,54,66,68].

Vitamin B6 is essential for nucleic acid and protein biosynthesis, hence an effect on immune function is logical, since antibodies and cytokines build up from amino acids and require vitamin B6 as coenzyme in their metabolism [69,70]. Aside from protein-calorie malnutrition, vitamin B6 deficiency is perhaps the most extensively investigated nutrient deficiency in immunology. Both cell-mediated and humoral immune responses are impaired in vitamin B6 deficiency. Of the lymphoid tissues studied, those most severely affected by lack of vitamin B6 are the thymus, followed by the spleen and lymph nodes. Thymic hormone activity is depressed in vitamin B6 deficiency [13,71,72].

TABLE 25.1 Water-Soluble Vitamins: Main Roles in the Immune System and Consequences of Deficiency [5,13–15,51]

Vitamin	Main roles in the immune system	Consequences of deficiency
Vitamin C	• Supports integrity of epithelial barrier by promoting collagen synthesis • Maintains redox integrity of cells and protects against reactive oxygen species generated during respiratory burst and inflammatory response • Stimulates leukocyte functions (neutrophil, monocyte movement) • Regulates immune response via its antiviral and antioxidant properties • Decreases duration/severity of common cold • Reduces incidence of common cold and pneumonia in subjects engaged in strenuous exercise or who live in crowded situations	• Decreased interferon, T lymphocyte activity, collagen production • Decreased resistance to disease • High supplemental intakes stimulate phagocytic and T-lymphocytic activity
Vitamin B6	• Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B12 and folate • Adequate intake is required for maintaining a Th1 immune response	• Decreased lymphocyte proliferation, suppression of Th1 and promotion of Th2 cytokine-mediated activity, decreased IL-2, DTH response and antibody production • Overall, suppressed immune response and atrophy of lymphoid organs
Vitamin B12	• Interferes with immune function through involvement in nucleic acid and protein biosynthesis in concert with vitamin B6 and folate • Acts as immunomodulatory for cellular immunity, especially with effects on cytotoxic cells (NK, CD8 T lymphocytes)	• Suppression of NK cell activity, decreased number of lymphocytes and CD8 cells and proportion of CD4 cells leading to an abnormally high CD4/CD8 ratio • Decreased neutrophil phagocytosis • Leads to megaloblastic anemia (characterized by large and immature red blood cells) and defective DNA synthesis in cells
Folate/ folic acid	• Interferes with immune function through involvement in nucleic acid and protein biosynthesis in concert with vitamins B6 and B12 • Maintains innate immunity (NK cell activity)	• Cell-mediated immunity especially affected: decreased circulating lymphocytes, lymphocyte proliferation and cytotoxic T lymphocyte activity • Overall impaired resistance to infections • Atrophy of lymphoid organs
Vitamin B1	• Co-factor in a number of reactions contributing to energy-yielding metabolism	• Reduced production of antibodies and decreased number of lymphocytes
Vitamin B2	• Co-factor in a number of reactions contributing to energy-yielding metabolism • Contributes to the maintenance of normal skin and mucous membranes • Contributes to the protection of cell constituents from oxidative damage	• Impaired antibody responses • Decrease in thymic weight
Biotin	• Co-factor in a number of reactions contributing to energy-yielding metabolism • Contributes to the maintenance of normal skin and mucous membranes	• Functional biotin deficiency results in impaired immune system function and increased susceptibility to bacterial and fungal infections • Impaired antibody responses • Impaired cell-mediated responses • Thymic atrophy
Pantothenic acid	• Co-factor in a number of reactions contributing to energy-yielding metabolism	• Impaired antibody production
Nicotinamide	• Contributes to the maintenance of normal skin and mucous membranes • Co-factor in a number of reactions contributing to energy-yielding metabolism	• Reduces the immunosuppressive effects of sunlight

TABLE 25.2 Selected Minerals: Main Roles in the Immune System and Consequences of Deficiency [5,13–15]

Mineral	Main roles in the immune system	Consequences of deficiency
Calcium	• Contributes to normal energy metabolism	• Calcium signaling is required to enhance T cell proliferation and the stability of the immunological synapses
Magnesium	• Is important in development, distribution, and function of immune cells and soluble factors which are critical for humoral and cell-mediated immunity • Participates in immune responses in numerous ways: as a co-factor for immunoglobulin synthesis, other enzymes, macrophage response, immune cell adherence, antibody-dependent cytolysis, IgM lymphocyte binding, T helper–B cell adherence, etc. • Plays a protective role in acute allergic reactions • Magnesium supplements have been recommended to ameliorate hypersensitivity and to prevent infection	• Magnesium deficiency impairs IgG synthesis and cell-mediated immunity; complications include thymus atrophy and elevated IgE
Zinc	• Acts on cellular and humoral immunity, helps to maintain skin and mucous membrane integrity • Cell protection from damaging effects of reactive oxygen radicals and reactive nitrogen species produced during immune activation	• Deficiency impairs phagocytosis of macrophages and neutrophils, NK cell activity, oxidative burst generation, complement activity • Thymus involution, depressed lymphocyte proliferation, Th1 cytokine production, DTH skin responses, and antibody response • Increased susceptibility to infections, especially for children and the elderly • Atrophy of lymphoid organs • Adverse effects on bone marrow

Human studies demonstrate that vitamin B6 deficiency impairs lymphocyte maturation and growth, antibody production, and T cell activity. Lymphocyte mitogenic response is impaired by dietary vitamin B6 depletion in elderly subjects and restored by administration of vitamin B6. Deficiency leads to a decrease in antibody DTH response; IL-1β, IL-2, IL-2 receptors; NK cell activity; and lymphocyte proliferation [72–74]. Marginal vitamin B6 deficiency altered the percentage of T helper (Th) cells and slightly decreased serum immunoglobulin D [75]. Marginal vitamin B6 deficiency in the elderly is associated with decreased numbers and function of circulating T lymphocytes, which can be corrected by short-term (6 weeks) supplementation with 50 mg of vitamin B6 per day [14]. Decreased IL-2 production, T lymphocyte numbers, and T lymphocyte proliferation are observed in subjects undergoing vitamin B6 depletion, indicating that vitamin B6 deficiency suppresses Th1 and promotes Th2 cytokine mediated activity, whereas repletion reverses this suppression of activity [76]. Finally, supplementary vitamin B6 improved immunocompetence in healthy elderly persons and in a number of clinical conditions in which immune suppression could be related to vitamin B6 deficiency [71,72,77].

The immune system is strongly influenced by the availability of zinc, because it is one of the most highly proliferative organs and zinc is a co-factor of >300 enzymes involved in a variety of general cellular functions, including signal transduction, transcription, and replication. Furthermore, zinc specifically interacts with components of the immune system. Zinc is considered key for optimal functioning of both innate and adaptive immunity, and impaired immune function due to inadequate zinc status may be the most common cause of secondary immunodeficiency in humans [15,53,78–81]. Zinc homeostasis influences the development and function of immune cells, activity of stress-related and antioxidant proteins, and helps to maintain genomic integrity and stability. Zinc is involved in the cytosolic defense against oxidative stress (superoxide dismutase activity) and is an essential co-factor for thymulin, which modulates cytokine release and induces proliferation. Adequate zinc intake supports a Th1 response and helps to maintain skin and mucosal membrane integrity, and unbound zinc ions exert a direct antiviral effect on rhinovirus replication. Zinc supplementation increases cellular components of innate immunity (e.g., phagocytosis by macrophages and neutrophils, NK cell activity, generation of oxidative burst, DTH activity), antibody responses, and the numbers of cytotoxic CD8+ T cells (Th1 response) [52,53,82,83].

Zinc steady-state status is regulated not only by uptake but also by fluctuations in zinc excretion associated with a number of diseases and inflammation. Healthy elderly subjects have decreased serum zinc levels, which may be due to decreased resorption or increased excretion.

Acute infections lead to a redistribution of zinc to the liver, decreasing the immunologically important serum pool. All these factors may result in an immune deficiency without specific immunological defects [84]. Low zinc ion bioavailability and impaired cell-mediated immunity are common in aging and may be restored by physiological supplementation with zinc for 1–2 months, impacting upon morbidity and survival [85]. Zinc status is of major importance for the maintenance of an effective immune response, particularly a T cell-mediated response. Subnormal plasma zinc levels (below 17 μg/dl) in men and women volunteers produced a decrease in lymphocyte counts, impaired leukocyte chemotaxis, and clinical signs indicative of decreased resistance to infection (sore throat, aphthous stomatitis, seborrheic dermatitis, acne flare-up, stye, and furunculosis) [86]. Recognition is growing that zinc deficiency increases susceptibility to a number of bacterial, viral, and parasitic challenges [52,53,78,87]. Normal serum zinc concentrations in nursing home elderly are associated with a decreased incidence and duration of pneumonia, a decreased number of new antibiotic prescriptions, and a decrease in the days of antibiotic use. Zinc supplementation to maintain normal serum zinc concentrations in the elderly may therefore help reduce the incidence of pneumonia and associated morbidity [88,89].

In addition to literature supporting individual micronutrients, Lesourd and co-workers have reviewed the effects of multiple micronutrients interventions to improve immune function in the elderly [44–46]. Their work confirms that immune responses may be enhanced in elderly individuals by the use of micronutrient supplements. This outcome is associated with correction of micronutrient deficits in most cases, but has also been observed, in a few studies, in individuals with "normal" micronutrient levels. The effect has also been found to be associated with clinical efficacy (i.e., a reduction in infection rate or length) in some studies and with reduction in the consequences of free radicals in other studies, indicating that part of the effect is associated with the antioxidant action of the micronutrients used [44–46].

In conclusion, immune defense consists of a highly complex biological response that involves cellular proliferation, enhanced protein synthesis and production of inflammatory mediators, cell-to-cell interactions, production of reactive oxygen species (ROS), and modulation of gene expression. Therefore, the immune system needs to be appropriately fed with energy sources (macronutrients) and essential micronutrients serving as co-factors in the development, maintenance, and expression of the immune response [5,13]. There is an essential role for micronutrients such as B vitamins, vitamin C, calcium, magnesium, and zinc for an adequate immune response (Tables 25.1 and 25.2). Micronutrients are required for proper functioning of the natural defenses of the body and inadequate intake has been shown to lead to suppressed immunity, predisposing the organism to increased susceptibility towards otherwise rather harmless pathogens and thus infections and malnutrition. The elderly represent a group at high risk for micronutrient deficiencies. They further experience changes in a number of immune responses, which can be described as a progressive occurrence of immune dysregulation [44,45,90]. As a consequence, aged individuals are more sensitive to infections than younger adults and hence infection is a common problem among the elderly. Therefore micronutrient supplementation in elderly subjects is recommended to restore their micronutrient levels, to re-establish their immune function, and to improve resistance to infections.

25.4 GINSENG AND IMMUNE FUNCTION

Herbal remedies known as "ginseng" are based on the roots of several distinct species of plants, mainly Korean or Asian ginseng (*Panax ginseng*), Siberian ginseng (*Eleutherococcus senticosus*), and American ginseng (*Panax quinquefolius*). These species have their own specific effects on the body. *Panax ginseng* C.A. Meyer is the most famous of all ginsengs, and of all Asian medicinal plants in general. Used in China for more than 2000 years, numerous scientific studies over the past 40 years have concentrated on the chemistry, pharmacology, and clinical aspects of ginseng use. The main active agents in *Panax ginseng* are ginsenosides, which are triterpene saponins. The majority of published research on the medicinal activity of *Panax ginseng* has focused on ginsenosides, and these are the compounds to which some ginseng products are now standardized [16,17,91,92]. Ginsenosides have been shown to interact with numerous membrane proteins such as ion channels, transporters and receptors, resulting in a broad range of physiological activities [93].

Panax ginseng is used primarily to improve psychological function, physical vitality, and activity [17]. It is further known to support immune function though its uses as an aid during convalescence and as a prophylactic to build resistance and reduce susceptibility to illness [16,17,92]. Overall, *Panax ginseng* is described in various pharmacopoeias (for example, in Germany, France, Austria, Switzerland, etc.) and considered particularly beneficial to promote health and longevity [16,17,92]. Ginseng has been described as an "adaptogen," a substance that is innocuous, does not impair physiological functions, but helps to increase resistance against noxious or stressful influences of a physical, chemical, or biological nature and in general has a normalizing effect [94,95]. Its activity appears to be based on whole body

TABLE 25.3 Immunoregulatory Roles of Ginseng [97–103]

Immune component	Ginseng effect
Innate immunity	• Macrophages: improved phagocytic activity, stimulation of nitric oxide generation, stimulation of NK cells and T cells • NK (natural killer) cells: improved natural killing activity • DCs (dendritic cells): modulation of maturation markers
Acquired immunity	• Induced responses and production of a number of immunoglobulins (IgA, IgM, IgG) • Enhanced antibody-dependent cellular cytotoxicity • Stimulation of cell proliferation, particularly T cells
Cytokine production	• Modulation of cytokine (TNF-α and various IL) release linked to components of both innate (macrophages, DCs) and acquired immunity • Stimulation of interferon production
Antimicrobial activity	• Bacteria (both Gram-positive and -negative), influenza virus, HIV-1, and rotavirus

effects, rather than particular organs or systems, which lends support to the traditional view that ginseng is a tonic that can revitalize the functioning of the organism as a whole. In general, it was noted that effects of ginseng were particularly evident "when the resistance of the organism was diminished or was taxed with extra demands" [96]. Especially for people aged over 50, these adaptogenic activities of ginseng support the body in counteracting age-related changes in physiology.

When it comes to immune support, several authors have demonstrated both in *in vitro* and in animal studies that ginseng indeed exerts a number of effects and modulates both humoral and cell-mediated immune function (reviewed in Sato and Miyata [97,98]; summarized in Table 25.3 [97–103]). Furthermore, it has been demonstrated in animals [104,105] that ginseng has adjuvant properties and acts synergistically with aluminum hydroxide, improving the potency of commercially available vaccines against porcine parvovirus and *Erysipelothrix rhusiopathiae* infections [105]. Quan *et al*. [106] also investigated the adjuvant roles of common herbal medicines including ginseng and their effects on early immune responses during influenza virus infection in a mouse model. The results indicated that ginseng plays a role as a mucosal adjuvant against influenza virus as well as an immunomodulator during influenza virus infection.

In line with the strong experimental evidence, the immune supportive benefits of *Panax ginseng* have also been documented in human studies. Aqueous and standardized ginseng extracts were tested in a placebo-controlled double-blind study for immunomodulatory actions [107]. A group of 60 healthy volunteers were divided into three sets of 20 each and were given a placebo, or 100 mg of aqueous ginseng extract, or 100 mg of standardized ginseng extract every 12 hours for 8 weeks. Blood samples drawn from the volunteers revealed an increase in chemotaxis of polymorphonuclear leukocytes, the phagocytic index, and the total number of T3 and T4 lymphocytes after 4 and 8 weeks of ginseng therapy, as compared with the placebo group. The group receiving the standardized extract also increased their T4:T8 ratio and the activity of NK cells. The conclusion of this study was that ginseng extract stimulated the immune system in humans, and that the standardized extract was more effective than the aqueous extract.

A study of 227 healthy volunteers demonstrated that daily administration of 100 mg *Panax ginseng* C.A. Meyer (standardized to 4% total ginsensenosides) for 12 weeks enhanced the efficacy of polyvalent influenza vaccine [108]. The patients who received ginseng had a lower incidence of influenza and colds, higher antibody titers, and higher NK cell activity levels. In a study of 75 patients with acute exacerbation of chronic bronchitis who were treated with antibiotics or antibiotics plus *Panax ginseng* C.A. Meyer (standardized to 4% total ginsensenosides), those administered 200 mg ginseng daily for a total of 9 days showed faster bacterial clearance [109]. Researchers at the University of California, Davis, School of Medicine (in Voelker [110]) randomized 175 patients aged 21–94 years to receive 200 mg *Panax ginseng* or a placebo for 4 months. At the end of the first month, patients received an influenza vaccination. Patients' IgG, IgM, and IgA antibody responses were evaluated at 4, 8, and 12 weeks after vaccination. Compared with patients in the placebo group, those who received ginseng had higher levels of IgA and IgM antibodies after being vaccinated. There was no difference in IgG titers between the groups.

In conclusion, a number of *in vitro*, animal, and human studies show that *Panax ginseng* supports the immune system. This is particularly relevant to the elderly, who are more prone to infections than younger counterparts. Indeed, *Panax ginseng* has a long history of traditional use as a tonic to energize the body, especially during recovery from illness and old age. Especially for people aged over 50, the adaptogenic and immune supportive

activities of ginseng help the body in counteracting age-related changes in physiology [10].

25.5 CONCLUSIONS

The number of persons older than 65 years is expected to rise dramatically in most areas of the world because of advances in average life expectancy. As individuals age, immunosenescence causes an increased susceptibility to infections, which results in greater morbidity and mortality compared with younger adults. Demands on health services will clearly escalate as a result of this demographic revolution and will, at least in part, be linked either to infectious diseases directly or to the related comorbidities [18].

Active aging has been defined by the WHO [111] as the process of optimizing opportunities for health, participation, and security in order to extend healthy life expectancy and quality of life for all people as they age. At the same time, with greater access to health-related information, elderly individuals have become more literate, better educated, and increasingly capable of making their own decisions regarding their health care. Indeed, a trend towards an increased usage of herbal medicines and nutritional and dietary supplements has been reported [112,113], indicating a proactive approach aimed at improving quality of life and preserving health.

While multiple factors determine whether an individual will become sick or not, the immune system remains the first line of defense against all external pathogens and noxious insults. Furthermore, infectious diseases can affect the status of several micronutrients in the body, thus setting up a vicious circle of undernutrition, compromised immunity, and recurrent infection. Therefore, it is of utmost importance to adequately feed the immune system by providing immune-relevant micronutrients, especially for a recognized risk group such as the elderly [5,13]. Ideally, a sufficient and balanced diet should cover overall micronutrient requirements. However, since senior citizens do not obtain adequate amounts of essential vitamins and minerals through the diet, for a number of physiological, psychological, economic, and social reasons, micronutrient supplementation can help close this dietary gap.

In addition to essential micronutrients, herbal remedies can also be beneficial in terms of preserving health, vitality, and immune function. Among them, *Panax ginseng* seems to be a very promising immune-boosting candidate. Traditionally used as an aid during convalescence and as a prophylactic to build resistance, reduce susceptibility to illness, and promote health, vitality, and longevity [10,16,17], its efficacy as an immune supportive agent has been demonstrated in a number of studies.

Finally, preservation of a functionally youthful immune system throughout the years is the best way to preserve health throughout life and to gain longevity accompanied by good quality of life [5]. Based on their individual properties and their synergistic mode of action outlined in this chapter, a combination of micronutrients and *Panax ginseng* may be considered a reasonable association in order to improve the organism's immune system – the cornerstone of health – particularly during aging.

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CHAPTER

26

The Role of Micronutrients in Preventing Infections in the Elderly

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26.1 INTRODUCTION

Multivitamins and minerals are the most commonly used dietary supplements in the United States [1]. The use of these supplements has increased significantly during recent years. In the National Diet and Nutrition Survey of British adults aged 19–64 years, 40% of those surveyed were taking vitamin and mineral supplements [2]. This is largely due to the belief that multivitamin supplements could improve health and prevent certain diseases, among them infections [3]. In only a few cases does the Department of Health recommend supplements for healthy populations, such as the use of folic acid before and during the early stages of pregnancy. Classical vitamin deficiency syndromes are uncommon in Western societies. Subclinical deficiency is more common, where the deficit in a nutrient is not sufficiently severe to cause clinical signs and symptoms, but there may be metabolic effects [4]. However, certain groups, such as elderly people, are at higher risk for suboptimal vitamin status and vitamin deficiency [5]. Many factors can lead to micronutrient deficiency in the elderly, such as poor dentition, social isolation, depression, and dementia. The presence of other diseases such as diabetes and intestinal ischemia cause malabsorption [6,7]. Also, it is well known that in the elderly zinc has low intestinal absorption [8]. Moreover, older people often take many medications that could affect vitamin absorption or metabolism [9].

Infection is among the most common disorders in the elderly. Acute respiratory infections account for more than 50% of all types of infections in this age group, followed by genitourinary, skin, and gastrointestinal infections [10]. Acute respiratory infections last longer and are associated with higher mortality and morbidity [11]. An aged-related decline in immune response is believed to be an important cause of the increased risk of infection.

The number of Americans aged over 65 years was 35 million in 2000 and is expected to reach 69 million by 2030, accounting for approximately one-fifth of the total US population [12]. Infection in such a large section of the population is of considerable importance to public health. It is worth investigating potential modifiable factors, such as micronutrient supplements, that may reduce susceptibility to infection in this population. In this chapter, the effects of micronutrient supplements on infection risks in this age group will be discussed in the light of recent data.

26.2 MICRONUTRIENTS

Micronutrients (trace elements and vitamins) are required in microgram quantities for correct function of the human body. Most micronutrients or their derivatives have central roles in certain biochemical pathways. All the required micronutrients should be available from a balanced diet, which includes fruit and vegetables, dairy products, and meat or pulses. Trace elements can be defined as those elements that (individually) make up no more than 0.01% of the dry weight of the body. Some are non-essential, such as lead, but others are required for normal health, function, and development [13].

Vitamins are conventionally divided into two groups according to their solubility characteristics. The B group

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of vitamins and vitamin C are water-soluble, while vitamins A, D, E, and K are insoluble in water but soluble in lipid or lipid solvents and are thus classified as fat-soluble vitamins. Fat-soluble vitamins are stored in the liver and are not easily excreted, so toxic overload is possible from excessive intake. Toxicity with water-soluble vitamins is less likely. Among the fat-soluble vitamins, A and E are extensively studied in relation to infections and immune markers.

Vitamin A refers to retinol and the carotenoids that are converted to retinol [14]. Preformed vitamin A can be obtained mostly from dietary animal sources (liver, fish liver oil, eggs, and dairy products), whereas carotenoids that can be converted into retinol are obtained from vegetables (dark green leafy vegetables and deep orange fruits). Vitamin A plays an essential role in a large number of physiological functions that encompass vision, growth, reproduction, hematopoiesis, and immunity [15]. The association between vitamin A and immunity was noticed even before its structure was realized in 1931 [16].

Vitamin E consists of a family of eight related compounds, the tocopherols and the tocotrienols. The major chemical forms of vitamin E are the tocopherols – alpha, beta, gamma, and delta. Gamma-tocopherol is the most abundant form in foods, and is usually the form used in supplements [14].

26.3 MICRONUTRIENTS AND IMMUNE FUNCTION

Many factors, including (among others) age, genetic predisposition, smoking, and nutrition status, can affect immune functions. An adequate intake of vitamins and trace elements is required for the efficient function of the immune system [17] and protection from reactive oxygen species. A variety of reactive oxygen species is formed continuously in tissues by endogenous and exogenous mechanisms [18]. These free radicals are immunosuppressive. Antioxidant vitamins and trace elements (vitamins C and E, selenium, copper, and zinc) are free-radical scavenging nutrients that protect cells from damage by pro-oxidants [19,20].

Some evidence suggests that micronutrients, such as zinc, selenium, and β-carotene, play an important role in maintaining a balance between cell-mediated and humoral immunity by regulating patterns of cytokine secretion [21–22]. Studies have also shown that low-dose supplementation of zinc and selenium leads to significant improvement in the humoral response following vaccination in elderly patients [23]. Vitamin A deficiency impairs both innate immunity (mucosal epithelial regeneration) and adaptive immune response to infection, resulting in impaired ability to counteract extracellular pathogens [24]. It is also associated with pathological changes in the respiratory system [25], gastrointestinal tract [26], bladder [27], and ocular tissues [15]. Vitamin D deficiency is correlated with a higher susceptibility to infections due to impaired localized innate immunity and defects in antigen-specific cellular immune response [28].

Most cells of the immune system, with the exception of B cells, express vitamin D receptors. In recent years there have been efforts to understand possible non-calcemic roles of vitamin D, including its role in the immune system. Some results suggest an important role for vitamin D in autoimmune disorders, providing a fertile and interesting area of research that may herald important new therapies [29].

Vitamin B6 is essential for nucleic acid and protein synthesis. It is an essential coenzyme for more than 60 enzymes, many of which are involved in the metabolism of amino acids. Antibodies and cytokines are built up of amino acids, and require vitamin B6 as a coenzyme in their metabolism.

Folate plays a crucial role in nucleic acid and protein synthesis by supplying, in concert with vitamin B6 and B12, one carbon unit. Inadequate folate intake significantly alters the immune response. The major function of vitamin C is to control the redox potential within cells, acting as an antioxidant and scavenger for free radicals. Vitamin E is a powerful antioxidant. Being fat-soluble, it is particularly important in protecting lipids, including cell membranes, from oxidative damage. Selenium is essential for optimum immune response, and influences the innate and acquired immune systems. It plays a key role in the redox regulation and antioxidant function through glutathione peroxidase that removes excess potentially damaging free radicals produced during oxidative stress.

Zinc is required for many enzymes, either structurally or as a co-factor. These include enzymes involved in nucleic acid synthesis. It is essential for highly proliferating cells such as those in the immune system, and so influences both innate and acquired immune functions. Copper is required for the activity of several enzymes, including superoxide dismutase. This enzyme is responsible for the destruction of free radicals. Copper has been shown to have a role in the development and maintenance of the immune system. It is reasonable to conclude that micronutrients can influence the body's defense through various mechanisms, including proliferation of immune system cells, synthesis of proteins such as antibodies, and scavenging of free radicals.

26.4 EFFECT OF MICRONUTRIENT SUPPLEMENTS ON THE AGING IMMUNE SYSTEM

Aging, even in the absence of nutritional deficiencies, is associated with impaired immune response,

particularly in the cell-mediated arm of the system. On the other hand, effects of micronutrient supplements on different surrogate markers of immune response (e.g., antibody titre, delayed-type hypersensitivity response, and cytokine production) have been investigated in this age group, using a variety of study designs. Most of these studies reported an enhancement of at least one surrogate marker. Vitamins A, C, and E, zinc, and selenium supplementation were shown to have beneficial effects on the immune systems [30]. Vitamin A supplementation to preschool children is known to decrease the risks of mortality and morbidity from some forms of infections. These effects are likely to be the result of actions of vitamin A on immunity. Some of the immunodulatory mechanisms of vitamin A have been described [31]. Also, β-carotene in high doses has been described as stimulating delayed-type hypersensitivity [32]. Vitamin C may enhance immune functions such as phagocytosis, neutrophil chemotaxis, and lymphocyte proliferation [33,34]; however, contrasting findings of vitamin C have also been published [35].

Meydani *et al*. [36] studied the effect of vitamin E supplementation on immune response *in vivo* in 88 healthy elderly subjects who were randomized to a placebo group or groups receiving 60, 200, or 800 mg/day of vitamin E for 4 months. The results indicate that a level of vitamin E greater than that currently recommended enhances certain clinically relevant *in vivo* indexes of T cell-mediated functions in healthy elderly persons. No adverse effects were observed with vitamin E supplementation. Another 3-month intervention trial among apparently healthy elderly subjects demonstrated no effect of 100 mg alpha-tocopheryl acetate on the overall immune responsiveness of this population [37]. This finding was consistent with that of Meydani and colleagues.

Combined vitamin supplementation has been shown to enhance the immune response of elderly individuals. Penn *et al*. [38] randomized 30 elderly subjects to receive either placebo or dietary supplementation with physiological doses of vitamin A, C, and E for 28 days. Following vitamin supplementation, cell-mediated immune function improved. In contrast, no significant changes were noted in the immune function of the placebo group. Another study demonstrated improvement of immune function in elderly females after consumption of 1 g of vitamin C and 200 mg of vitamin E for 16 weeks [33]. A study by Wolvers *et al*. [39] demonstrated enhancement of a delayed-type hypersensitivity in elderly subjects (average 57 ± 10 years) who received a mix of micronutrient supplements (vitamins E and C, β-carotene, and zinc) for 10 weeks, compared with a placebo group. Fortes *et al*. [40] found an improvement in the cell-mediated immune response following zinc supplementation (25 mg zinc sulfate). In a more recent study, Gibson *et al*. [41] demonstrated that increased fruit and vegetable intake improved the Pneumovax® II vaccination antibody response in older people, which links an achievable dietary goal with improved immune function [41]. However, vitamin A (800 μg retinol palmitate) had a negative effect on immune indicators in the older population in the same study.

Several studies reported some changes in the immune system following administration of micronutrients. The observed effect of these supplements on immune response does not necessarily imply a beneficial clinical outcome for the subjects receiving these supplements. Studying the clinical effects, such as frequency and severity of infectious, has much greater relevance.

26.5 EFFECTS OF MICRONUTRIENT SUPPLEMENTS ON THE FREQUENCY AND SEVERITY OF INFECTIONS

One of the earliest clinical trials to investigate such clinical effects was conducted in France by Chavance *et al*. in 1989 [42]. This trial enrolled a total of 218 subjects over 60 years of age. The participants received either multivitamin supplements or placebo for 4 months. No significant difference was found between the two groups in the incidence of infections. In fact, the observed incidences were higher in the treatment group than in the placebo group.

Girodon *et al*. [43] reported results of a large trial in which 725 institutionalized elderly patients from 25 geriatric centers in France were separated into four groups and randomized to receive vitamins (β-carotene, ascorbic acid, and vitamin E), minerals (zinc and selenium sulfide), both, or neither. The trial showed an enhanced antibody response to the influenza vaccine and a reduction in the incidence of respiratory infections in the minerals group ($P = 0.06$), but not in the vitamins group. Supplementation with either trace elements or vitamins significantly reduced the incidence of urogenital infections. Survival analysis for the 2 years, to exclude seasonal variation, did not show any differences among the four groups, and any beneficial effects of mineral supplementation may be due to the correction of subclinical deficiencies in those who received such supplementations.

Another study from The Netherlands [44] enrolled a total of 652 non-institutionalized individuals aged 60 years or more. Physiological doses of multivitamin and minerals, 200 mg of vitamin E, both, or placebo, were given for 15 months. Neither daily multivitamin and mineral supplementation at physiological doses nor 200 mg of vitamin E showed a favorable effect on the incidence and severity of acute respiratory tract infections. Instead, adverse effects of vitamin E on illness severity were observed.

A study by Meydani *et al*. [45] reported that supplementation with 200 IU per day of vitamin E for 1 year did not have a statistically significant effect on the incidence

of lower respiratory tract infections in elderly nursing home residents. However, they observed a protective effect of vitamin E supplementation on upper respiratory tract infections, particularly the common cold. A US study in primary care found that multivitamin and multimineral supplements had no significant clinical effects on infections in people aged 65 and over [46].

Barringer and colleagues assessed the effect of a typical one-a-day multivitamin and mineral supplement for 1 year on infection rate among individuals aged 45 years or older. They demonstrated a reduction in incidence of infection. However, in subgroup analyses, persons with diabetes had the largest benefit in infection-related outcomes; this accounted for most of the overall observed effect. Correction of micronutrient deficiencies would be the most likely explanation for their results.

Merchant *et al*. [47] evaluated the association between intake of antioxidants and B vitamins and the risk of community-acquired pneumonia in well-nourished middle-aged and older men. This was a prospective study conducted among 38,378 male health professionals aged 44–79. There were no associations between total intakes of antioxidants or B vitamins and the risk of pneumonia. They concluded that vitamin supplements are unlikely to reduce the risk of pneumonia in well-nourished middle-aged and elderly men.

A meta-analysis of randomized controlled trials found the evidence for multivitamin and mineral supplements on the risk of infections in older people to be weak and conflicting [48]. However, an updated review for this meta-analysis concluded there was no benefit for the use of multivitamins in preventing infections in the elderly [49]. A trial conducted by Avenell *et al*. [50] largely confirms previous research. They found that routine multivitamin and multimineral supplementation in older people (910 men and women aged 65 or over) living at home does not affect self-reported infection-related morbidity. Participants were recruited from six general practices in Grampian, Scotland. The limitation of this study was the low dose of multivitamins and multiminerals used. This trial provided good evidence against the efficiency of multivitamin supplementation in preventing symptoms of acute respiratory infection among healthy elderly subjects. While there is evidence linking micronutrient supplementation to changes in immune responses, their role in reducing the risk of infections in the well-nourished elderly requires careful consideration.

26.6 PROBLEMS WITH MICRONUTRIENT SUPPLEMENTS

There is no standard definition for multivitamins; any product containing two or more vitamins or trace elements can be listed as a multivitamin. The commonly used multivitamin supplement contains at least 10 vitamins and 10 minerals [1]. The potential for adverse effects from excessive lipid-soluble vitamins, such as vitamin A, is well documented [51]. Higher doses of zinc and vitamin A supplements impair cellular immunity and the health of bones among older people with vitamin D deficiency [52,53]. Recent studies have suggested that β-carotene may act as a co-carcinogen [54,55]. Also, supplementation with high doses of β-carotene, five times the nutritional requirement, led to an increased incidence of lung cancers [56]. Bjelakovic *et al*. [57] did a systematic review to analyze the effects of antioxidant supplements (β-carotene, vitamins A, E, and C, and selenium) on all causes of mortality in adults [57]. They included antioxidant supplements at any dose, duration, and route of administration. Then they analyzed the antioxidants administered singly, in combination with other antioxidants, or with other vitamins or trace elements. They found that β-carotene, vitamin A, and vitamin E, given singly or combined with another antioxidant supplement, significantly increased mortality. In another study, vitamin E seemed to worsen the severity of infections [58]. After exclusion of high-bias risk trials, however, vitamin E given singly or combined with other micronutrients significantly increased mortality. This is in agreement with a recent meta-analysis [59]. The possible harm of micronutrient supplements is another factor that requires consideration.

26.7 CONCLUSION

Aging is often associated with a decline in immunocompetence, particularly in T cell-mediated functions. Nutrient deficiencies can contribute to further impairments in immune function in the elderly and thus render them even more vulnerable to acute and chronic infections than they would be if they were nutrient sufficient. Although micronutrients may help normalize certain parameters in the immune system, in elderly subjects suffering from nutritional deficiencies several clinical studies have argued that those changes have no demonstrable clinical benefits in terms of reduction of mortality and morbidity in adequately nourished elderly people. From the above studies it is clear that the healthy elderly population received no benefit from these supplements in terms of prevention of infections, although it is possible that nutritionally deprived individuals may have benefited. However, concerns about the potential side effects of micronutrients should make well-nourished persons cautious about taking such supplements.

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PART IV

FOOD AND SUPPLEMENTS IN CHRONIC HEART DISEASES, OBESITY, AND STROKE

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CHAPTER

27

Dietary Protein and the Risk of Stroke

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27.1 INTRODUCTION

Stroke is the fourth leading cause of death in the United States and one of the leading causes of serious long-term disability [1]. More than 795,000 Americans have a stroke each year, and 6.8 million are living with a history of stroke [1,2]. Women are at higher risk of stroke than men, with a lifetime likelihood of 20% versus 17% [2]. Nearly half of individuals who die of a stroke do so before they reach the hospital [3], and the direct lifetime cost of ischemic stroke is $140,000 per person [3] – almost 30 times the mean annual healthcare expense per person [1].

It is estimated that by 2030 an additional 4 million people in the United States will suffer a stroke, a 22% increase from 2013 [2]. In recognizing the estimated burden of cardiovascular disease, Healthy People 2020, the US's national objective statement developed by the Department of Health and Human Services, has prioritized prevention, detection, and treatment of risk factors for stroke, as well as those for overall cardiovascular health and quality of life, as one of its top goals [4]. The American Heart Association (AHA) and American Stroke Association (ASA) have worked to reduce the burden of stroke with evidence-based approaches [5], including guidelines for reducing cardiovascular disease by diet and other lifestyle practices [6], and ways to improve cardiovascular health at the community level [7].

This chapter focuses on the relation between dietary protein and stroke. The type of protein one consumes is a modifiable behavior that can have a significant impact of one's risk of stroke. The chapter reviews dietary protein sources, daily protein requirements, and the relation between major sources of protein in relation to stroke. Major sources include red and processed meat, poultry, fish, dairy, eggs, nuts, legumes, and grains. A critical review of the literature, including meta-analyses, prospective cohort studies, and clinical trials, is provided to allow for an understanding of our current knowledge and to identify gaps in knowledge. The chapter concludes with recommendations for the prevention of stroke through modification of dietary protein intake.

27.2 SOURCES OF DIETARY PROTEIN AND DAILY REQUIREMENTS

In addition to carbohydrate and fat, protein is one of the three macronutrients that are the main sources of calories in the diet [8]. Compared to fat (9 kcal/g), carbohydrate and protein provide fewer calories per gram (4 kcal/g each). Unlike carbohydrate and fat, however, protein provides amino acids for building and preserving muscle and tissue [8]. It is key for bone, muscle, skin, and cartilage formation, in addition to providing the substrate for enzyme and hormone production [9]. Major sources of animal protein include red meat, poultry, seafood, eggs, and dairy products, while major plant (vegetable) sources include legumes (e.g., soybeans, peas, lentils), nuts, and seeds [9].

The United States Department of Agriculture (USDA) reports that the main source of protein in US adults is animal protein (69% of total protein intake), with meat, poultry, and fish protein constituting the majority of that (42%) and dairy protein constituting a large portion of the remainder (20%) [8]. The highest contributor to plant protein intake is grains (18% of total protein intake) [8]. While plant sources of protein may not contain as much protein on average per serving as animal sources (see Appendix A to this chapter), combining plant protein

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sources, such as bread and peanut butter, or beans and rice, increases the variability of available amino acids so that all essential amino acids may be provided in a single meal [10].

Daily protein requirements vary depending on age, sex, and physical activity [9]. According to the USDA, about 10–35% of daily calories should come from protein in order to maintain health and provide energy for exercise, tissue repair, growth, immunity, and metabolism [9]. Recommendations for adults are 0.8 g protein per kg of body weight per day (equal to 0.36 g protein per pound) and tend to be higher for pregnant or lactating women and growing children [10].

With the exception of nearly 20% of homebound elderly who do not get enough protein [10], most Americans consume more than the recommended amounts [11]. Males 20 years and older consume approximately 102 g/day (82% greater than recommended amounts of 56 g/day for a male weighing 154 lbs [11] and 57% more than that needed for a male who weighs the US average of 180 lbs [12]), and females 20 years and older consume on average 70 g/day (52% greater than the recommended amounts of 46 g/day for a female weighing 126 lbs [11] and 32% more than that needed for a female who weighs the US average of 147 lbs [12]). Protein intake above the recommendations may contribute to excess caloric intake [13]. There is also evidence that high protein intake, particularly animal protein, may accelerate kidney damage in those with existing kidney disease [13,14] and may raise low-density lipoprotein (LDL) cholesterol as a result of the accompanying saturated fat content of animal protein sources [13].

27.3 TOTAL, ANIMAL, AND VEGETABLE PROTEIN

Studies in the late 1990s and early 2000s looked at the relationship between total daily protein intake, including total animal and vegetable protein intake, and stroke risk. For instance, the Nurses' Health Study, which followed 85,764 women from 1980 to 1994, concluded that animal protein was inversely associated with intraparenchymal hemorrhagic stroke risk when comparing highest intake (81.6 g/day) to lowest (42.7 g/day) intake (RR: 0.32, 95% CI: 0.10–1.00, $P = 0.04$) [15]. The Life Span Study of 40,349 Japanese adults followed for 16 years also concluded that animal protein intake was associated with a lower risk of intracerebral hemorrhage, although the risk reduction was not as great as that seen in the Nurses' Health Study (RR: 0.76, 95% CI: 0.58–0.99, $P = 0.03$), and also found a decreased risk of total stroke (RR: 0.88, 95% CI: 0.77–1.00, $P = 0.04$), both when comparing the highest (231 g/day) to the lowest median intake (121 g/day) [16]. These authors followed up with another study of 3731 individuals followed for a mean of 14 years, and found that as total protein intake increased (from 48 to 92 g/day) the risk for cerebral infarction decreased (HR: 0.42, 95% CI: 0.20–0.85, $P = 0.01$), and as animal protein intake increased (from 18 to 54 g/day) the risk for cerebral infarction decreased similarly (HR: 0.45, 95% CI: 0.23–0.89, $P = 0.02$) [17]. In this study, there was no significant association with vegetable protein intake (from 24 to 46 g/day; HR: 1.12, 95% CI: 0.57–2.21) [17].

The Health Professionals Follow-up Study, which included 43,960 men followed from 1986 to 2004, also examined the relationship between total, animal, and vegetable protein and risk of stroke, but found different associations [18]. Total protein was compared from the top (22.5% of energy, approximately 101 g/day) to the bottom (14.6% of energy, approximately 78 g/day) quintile. There was a relative risk for total stroke of 1.14 (95% CI: 0.90–1.43, $P = 0.43$) with no significant association observed for either animal (RR: 1.11, 95% CI: 0.87–1.41, $P = 0.52$) or vegetable (RR: 0.82, 95% CI: 0.60–1.12, $P = 0.17$) protein, although the associations were in opposite directions [18]. The authors suggested that the reason their findings may have been different from those of earlier studies was because they followed US white male health professionals that had typically been exposed to a Western diet and had high intakes of protein overall, with perhaps a smaller range of intake between the top and bottom quintiles [18].

One of the most recent studies, the Swedish Mammography Cohort, which followed 34,670 women for a mean of 10.4 years, examined the association between total, animal, and vegetable protein with the incidence of stroke, and how associations were modified by the presence of hypertension [19]. They observed an inverse relationship between total protein intake and stroke with a RR of 0.74 (95% CI: 0.61–0.91, $P = 0.006$) when comparing the highest (>78.7 g/day) to the lowest (<61.8 g/day) quintiles. For animal protein (comparing >58.0 g/day to <38.3 g/day), the RR for stroke was 0.71 (95% CI: 0.57–0.88, $P = 0.01$). A history of hypertension led to a stronger inverse association between total protein intake and stroke (RR for those with hypertension: 0.56, 95% CI: 0.40–0.78; RR for those without hypertension: 0.86, 95% CI: 0.67–1.10, P for interaction = 0.02). By replacing 5% of energy from total fat or saturated fat with energy from protein, there was a reduction of stroke risk by 13% [19].

Thus, in sum, the majority of prospective cohort studies have found that higher total or animal protein is associated with a reduced risk of stroke, although the benefit may not be seen at particularly high intakes, as observed in the US population [18].

One reason that dietary protein may have an association with stroke risk in certain populations is through its effect on blood pressure. A systematic review, which

included 15 observational studies, 13 biomarker studies, and 20 trials through June 2010, and a meta-analysis, which included 40 trials through April 2011, both recently summarized the evidence on dietary protein and blood pressure [20,21]. The systematic review found that protein, especially plant protein (although data on protein from specific sources were too scarce to draw conclusions), had a small lowering effect on blood pressure [20], while the meta-analysis found both vegetable and animal protein were associated with similar significant reductions in systolic (vegetable: −2.27 mmHg, 95% CI: −3.36, −1.18; animal: −2.54 mmHg, 95% CI: −3.55, −1.53) and diastolic (vegetable: −1.26 mmHg, 95% CI: −2.26, −0.26; animal: −0.95 mmHg, 95% CI: −1.72, −0.19) blood pressure [21]. Other systematic reviews and meta-analyses found small to moderate results with high-protein diets (median: 27% total daily energy intake) on blood pressure reduction (systolic: −0.21, 95% CI: −0.32, −0.09; diastolic: −0.18, 95% CI: −0.29, −0.06) [22]. Notably, blood pressure reduction may in part be due to a decrease in carbohydrate intake which typically accompanies an increase in dietary protein [23].

How an increase in protein is mechanistically associated with a lowering of blood pressure is not clear. It may be due to the lower glycemic response to protein versus carbohydrate, which manifests as a smaller postprandial increase in insulin and glucose, both of which may be linked with blood pressure [21]. In addition, vegetable protein may provide relatively high amounts of non-essential amino acids (e.g., arginine, alanine, glycine, and serine) compared to essential amino acids (e.g., methionine, tryptophan, and lysine) [18]. These non-essential amino acids decrease the release of insulin, while essential amino acids increase it; in addition, stroke risk is associated with higher fasting insulin levels [18]. Furthermore, the non-essential amino acid arginine may increase nitric oxide, a vasodilator, which decreases blood pressure [18], although some studies have shown that the essential tryptophan and tyrosine may also reduce blood pressure [21].

27.4 RED AND PROCESSED MEAT

Meat is the largest source of protein in the US diet [24], and intake continues to increase in the United States, European Union, and developing world [25]. In the US, although there has been a temporal increase in poultry consumption, red meat continues to be the largest contributor to meat intake. Of all meats consumed, red meat accounts for 58% while processed meat accounts for approximately 20% [25]. In a 2003–2004 National Health and Nutrition Examination Survey, the average total meat (sum of red meat, poultry, and fish) intake was 128 g/day [22].

Red meat typically includes unprocessed beef, veal, pork, lamb, and mutton, and processed red meat includes smoked, cured, or salted salami, bacon, sausage, hot dogs, and deli/lunch meats [26–28]. A recent meta-analysis of six prospective cohort studies with 329,495 participants through May 2012 concluded that consuming red or processed meat was significantly and positively associated with an increased risk of total and ischemic stroke but not hemorrhagic stroke [29]. For total stroke, the relative risks (RRs) of each additional serving per day of red, processed, and total meat were 1.11 (95% CI: 1.03–1.20), 1.13 (95% CI: 1.03–1.24), and 1.11 (95% CI: 1.06–1.16), respectively [29]. The RRs for ischemic stroke with each additional serving per day of red, processed, and total meat were 1.13 (95% CI: 1.00–1.27), 1.15 (95% CI: 1.06–1.24), and 1.12 (95% CI: 1.05–1.19), respectively [29]. Thus, both unprocessed and processed meat appears to elevate stroke risk.

A subsequent meta-analysis looked at five prospective cohort studies and 239,251 participants through June 2012 [26]. This analysis confirmed that red and processed meat consumption increased the risk for ischemic stroke [26]. The pooled RRs when comparing the highest category of consumption with the lowest were similar to the risks observed in the earlier study: 1.15 (95% CI: 1.05–1.25) for total meat, 1.09 (95% CI: 1.01–1.18) for red meat, and 1.14 (95% CI: 1.05–1.25) for processed meat. In an additional dose–response analysis, the risk of stroke increased significantly by 10% for total meat (RR: 1.10, 95% CI: 1.05–1.15) and 13% (RR: 1.13, 95% CI: 1.03–1.23) for unprocessed red meat with each 100 g/day increase. Processed meat was associated with an 11% increased risk (RR: 1.11, 95% CI: 1.02–1.20) for each 50 g/day increase [26]. Notably, a typical quarter-pound hamburger weighs approximately 100 g (including 30 g of protein), while three slices of bacon weigh approximately 35 g (including 12 g of protein) [30].

A third meta-analysis also looked at red and processed meat intake, but in addition to stroke it included risk of coronary heart disease (CHD) and diabetes mellitus [31]. Twenty studies were included (17 prospective cohorts and three case–control studies with 1,218,380 individuals). However, the results did not show the same positive association as did the earlier findings on total stroke or stroke subtype; the pooled RR between red meat and total or ischemic stroke was 1.17 (95% CI: 0.40–3.43); the pooled RR for processed meat and total or ischemic stroke was 1.14 (95% CI: 0.94–1.39) (only one study was included in each of these analyses); the pooled RR for two studies on total meat and ischemic stroke was 1.24 (95% CI: 1.08–1.43); for the one study on total meat and hemorrhagic stroke, the RR was 1.64 (95% CI: 0.75–3.60). While the two earlier meta-analyses analyzed six and five studies respectively, this later meta-analysis only analyzed three. The authors had stricter inclusion

criteria for their analysis and included fewer studies in the meta-analysis, noting that there were no two studies that evaluated the same meat and stroke subtype [31].

Two prospective cohort studies published after the meta-analyses, including the Shanghai Women's Health Study and the Shanghai Men's Health Study, followed 74,941 women for 3 years and 61,483 men for 4 years, respectively [32], and evaluated the relation between meat intake and stroke risk in Asian populations. There was no significant association between red meat and ischemic stroke when comparing the highest to the lowest quintile in women (HR: 0.84, 95% CI: 0.55–1.28) or in men (HR: 1.22, 95% CI: 0.69–2.15). Red meat intake, however, was inversely associated with a risk in hemorrhagic stroke in women (HR: 0.57, 95% CI: 0.37–0.87, $P = 0.01$) but not in men (HR: 0.71, 95% CI: 0.43–1.20, $P = 0.32$). The authors noted that a reason for the inverse association between red meat and hemorrhagic stroke in women is not clear; however, women in the highest quintile of red meat intake had higher education and higher intake of vitamin supplements, and, while these confounders were adjusted for, the authors acknowledged there may be residual confounding [32].

In sum, two of three recent meta-analyses concluded that both red and processed meats are associated with an increased risk of ischemic stroke [26,29]. The third showed positive associations with wide confidence intervals and suggests that total meat (the sum of unprocessed and processed meat) is most strongly associated with stroke risk [31].

Multiple mechanisms may explain these findings. Red meat is a source of saturated fat and cholesterol [29], which increase LDL cholesterol and thus stroke risk. The iron in red meat may lead to oxidative stress, which increases the peroxidation of lipids, protein modification, and DNA damage; over time this leads to atherosclerosis [26,29]. Processed meat contains additives, such as nitrite and sodium, which may also contribute to increased blood pressure and vascular stiffness [26,29]. The sum of these nutrients and compounds may collectively be responsible for the association observed between red and processed meat and stroke risk. Interesting, although saturated fat may help protect against hemorrhagic stroke through improved cerebral artery integrity and reduced cerebral arterionecrosis risk [33], other compounds in red and processed meat may outweigh this benefit.

27.5 FISH AND POULTRY

Consumption of poultry has grown in the US over the past 35 years [25]. Poultry now accounts for 32% of the total meat consumption in the US while fish accounts for 10% [25]. Fish consumption, in fact, appears to have changed little over the past century [25].

The relationship between fish intake and stroke risk has long been studied in part because of its early association with reduced heart disease risk [34]. An ecological study using data from 1961–1963, 1979–1981, and 1989–1991 found there were significant inverse correlations between fish intake and mortality from all causes ($P < 0.001$), ischemic heart disease ($P < 0.01$), and stroke ($P < 0.05$) [35]. The Zutphen Study, a 10-year longitudinal cohort study which began in 1960 with 552 men, concluded that participants who consumed more than 20 g/day of fish had a lower stroke risk than those who consumed less than 20 g/day (HR: 0.49, 95% CI: 0.24–1.01) [36].

These early findings have been substantiated in recent systematic reviews and meta-analyses. The first, a meta-analysis of nine cohort studies and 200,575 participants through October 2003, looked at fish intake of one to three times per month and found inverse associations: the pooled RR for total stroke was 0.91 (95% CI: 0.79–1.06), and for ischemic stroke it was 0.69 (95% CI: 0.48–0.99) [37]. A dose–response meta-analysis with 15 prospective cohort studies and 383,838 individuals through May 2011 concluded that there was a weak but still significant inverse association between fish (lean and fatty combined) consumption and stroke risk (RR: 0.88, 95% CI: 0.81–0.96) [38]. For every increase of three servings per week of fish, stroke risk decreased by 6% (RR: 0.94, 95% CI: 0.89–0.99) [39].

A more recent meta-analysis summarized the results of fish consumption and stroke in 19 cohort studies from 16 prospective studies with 402,127 individuals through April 2012 [39]. There was a significant inverse trend between fish consumption and the incidence of total stroke. Compared to those who did not consume fish, those who consumed fish one to three times per month had a RR of 0.97 (95% CI: 0.87–1.08); those who ate fish one time per week had a RR of 0.86 (95% CI: 0.80–0.93); those who ate it two to four times per week had a RR of 0.91 (95% CI: 0.85–0.98); and those who ate it more than five times per week had a RR of 0.87 (95% CI: 0.79–0.96). There were significant and slightly stronger inverse associations between fish intake and risk of ischemic stroke: compared to those who ate fish less than once per month, those who ate it one to three times per month had a RR of 0.96 (95% CI: 0.84–1.11); those who ate it once per week had a RR of 0.82 (95% CI 0.73–0.93); those who ate it two to four times per week had a RR of 0.89 (95% CI: 0.81–0.97); and those who ate it more than five times per week had a RR of 0.83 (95% CI: 0.75–0.92) [39].

By contrast to the above meta-analyses, a 37-year follow-up study of the Boyd Orr cohort, which followed pre-World War II British families, found that higher intake of fish in children was associated with a higher stroke risk in adulthood, with an adjusted rate ratio between the highest (44.5 g/day) and lowest (1.8 g/day) quartiles of fish intake of 2.01 (95% CI: 1.09–3.69, $P = 0.01$) [40].

The authors suggested that fish intake in early life may influence stroke risk (and especially hemorrhagic stroke risk) differently than in later life, perhaps by altering membrane concentrations of arachidonic acid [40].

It is clear that the type of fish consumed also has an effect on stroke risk. Oily fish (e.g., kippers, herring, pilchards, tuna, sardines, salmon, and mackerel) consumption has been associated with a lower risk of stroke in several studies [41–43]. No association has been found with lean, non-fatty, or white fish (e.g., cod, haddock, plaice, sole, halibut, hake, and fish fingers) in other studies [42,43]. Fried fish and fish sandwiches have been associated with a 44% increased ischemic stroke risk (95% CI: 12%–85%, $P = 0.003$) [44] while tuna, along with broiled or baked fish, one to four times per week, compared to less than one time per month, has been associated with a 25% reduced risk of total stroke risk (95% CI: 2%–43%) and a 27% reduced risk of ischemic stroke (95% CI: 2%–45%) [44]. No significant association was identified in one study of shellfish and stroke risk [41].

By contrast to fish, poultry has been less well-characterized in relation to stroke risk. In the same prospective Chinese cohort study that looked at red meat and stroke risk [32], investigators found that the HR between poultry intake and ischemic stroke risk for women, comparing the highest to the lowest quintile (19.9 g/day and 11.9 g/day, respectively), was 1.04 (95% CI: 0.69–1.56), while for men (highest quintile, 22.3 g/day; lowest quintile, 11.9 g/day) the HR was also not statistically significant (0.92, 95% CI: 0.54–1.57). The risk of hemorrhagic stroke was 1.20 (95% CI: 0.79–1.80) in women and 0.89 (95% CI: 0.56–1.40) in men [32].

In the Nurses' Health Study and the Health Professionals Follow-up Study, two prospective cohorts which followed 84,010 women for 26 years and 43,150 men for 22 years, higher poultry consumption was associated with a lower stroke risk [45]. Total stroke risk reduction was significant in women (RR: 0.82, 95% CI: 0.71–0.94, $P < 0.01$) and in men and women combined (RR: 0.87, 95% CI: 0.78–0.97, $P = 0.02$) when comparing highest intake (0.54 servings/day in women, 0.63 servings/day in men) to lowest (0.14 servings/day in both). Ischemic stroke risk reduction was significant in women (RR: 0.78, 95% CI: 0.64–0.95, $P = 0.02$) [45]. In pooled analyses, one serving per day of poultry compared to one serving per day of red meat was associated with a 27% decreased stroke risk (95% CI: 12%–39%) [45].

Thus, in sum, with regards to the "white" meats (poultry and fish), increased intake of fatty or oily fish, and possibly poultry, appears to be inversely related to stroke risk. The mechanisms underlying the relationship between fish intake and a reduced stroke risk are likely due to the antithrombotic activity of the long-chain omega-3 polyunsaturated fatty acids [39], which may also decrease stroke risk by reducing triglyceride concentrations, lowering blood pressure, and improving endothelial function [38,46]. Long-chain omega-3 fatty acids found in fish were associated with a reduced risk for ischemic (pooled RR: 0.93, 95% CI: 0.87–0.99) and hemorrhagic (pooled RR: 0.81, 95% CI: 0.70–0.94) stroke in a meta-analysis of cerebrovascular disease risk [47]. Whether other nutrients found in fish – such as vitamins A, D, and B12, ferritin, iodine, selenium, and zinc – play a role is unclear, and these nutrients are not unique to fish [48]. Mechanisms with poultry are less clear. It may be that poultry contains as yet unrecognized nutrients, or that it is eaten in place of less healthy alternatives such as processed and unprocessed red meat, or that it is a marker for other healthful lifestyle habits [32].

27.6 DAIRY AND EGGS

Dairy and eggs are commonly consumed protein sources in the US diet, with approximately half of adults consuming up to a serving of dairy per day and about one in 10 consuming four or more servings per day [49]. In 2012, an estimated 249 eggs were consumed per person, equal to approximately four to five eggs per week per person [24]. Few studies, however, have evaluated these foods in relation to stroke risk.

A meta-analysis was conducted in 2010, with 17 prospective studies and 611,430 participants, on dairy products, including milk and other high- and low-fat dairy products and the risk of coronary heart disease (CHD), stroke, and total mortality [50]. There was a modest inverse association between milk consumption and overall cardiovascular disease (CVD) risk with a pooled RR of 0.94 (95% CI: 0.89–0.99) per 200 ml/day, although milk consumption was not associated independently with risk of CHD (RR: 1.00, 95% CI: 0.96–1.04), stroke (RR: 0.87, 95% CI: 0.72–1.05), or total mortality (RR: 0.99, 95% CI: 0.95–1.03) [50]. A prospective case–control study nested into two populations, however, concluded that dairy or milk fat intake was inversely related to first-event stroke risk in women (OR: 0.41, 95% CI: 0.24–0.69) but not men (OR: 0.88, 95% CI: 0.63–1.2) [51].

More recently, the Swedish Mammography Cohort, a large prospective study following 74,961 women over 10 years, examined the consumption of low-fat dairy foods, CVD, and cancer [52]. This study concluded that low-fat dairy intake was inversely associated with stroke risk [52]: the highest quintile (four servings per day) compared to the lowest (none) was associated with a RR of 0.88 (95% CI: 0.80–0.97) for total stroke and 0.87 (95% CI: 0.78–0.98) for cerebral infarction. Total dairy (RR: 0.91, 95% CI: 0.80–1.03), full-fat dairy (RR: 0.94, 95% CI: 0.83–1.07), milk (RR: 0.90, 95% CI: 0.82–1.00), sour milk/yogurt (RR: 0.98, 95% CI: 0.90–1.08), cheese (RR: 0.91, 95% CI: 0.81–1.01), and cream (RR: 1.00, 95% CI: 0.89–1.12)

showed no significant association with stroke risk [52]. A pooled analysis of the Nurses' Health Study and the Health Professionals Follow-up Study reported that low-fat dairy and whole-fat dairy, when replacing one serving per day of red meat, were associated with an 11% (95% CI: 5%–17%) and 10% (95% CI: 4%–16%) lower stroke risk, respectively [45]. Furthermore, dietary calcium from dairy products was associated with a reduced stroke and ischemic risk in middle-aged Japanese when comparing the highest (116 mg/day) to the lowest (0 mg/day) intake (HR: 0.69, 95% CI: 0.56–0.85, $P = 0.007$; HR: 0.69, 95% CI: 0.52–0.93, $P = 0.05$, respectively) [53].

With eggs, a meta-analysis of prospective cohort studies was conducted in 2012 using 8 articles with 17 reports (9 for coronary heart disease and 8 for stroke) and 210,404 stroke participants over 4,148,095 person-years [54]. It concluded that an increase of one egg per day was not associated with increased risk of CHD (RR: 0.99, 95% CI: 0.85–1.15, $P = 0.88$) or stroke (RR: 0.91, 95% CI: 0.81–1.02, $P = 0.10$) [54]. There was, however, a decreased risk for hemorrhagic stroke with egg consumption (RR: 0.75, 95% CI: 0.57–0.99, $P = 0.04$) [54]. The authors suggested that the cholesterol from eggs, as with the saturated fat noted above [33], may decrease the risk of hemorrhagic stroke [54].

The Harvard Egg Study, which was not included in the meta-analysis, used data from the Health Professionals Follow-up Study (37,851 men) and the Nurses' Health Study (80,082 women) and concluded that there was no relation between egg consumption of more than six eggs per week and risk of total (RR: 0.90, 95% CI: 0.70–1.10) or ischemic stroke (RR: 0.90, 95% CI: 0.70–1.10) [55]. Hemorrhagic stroke risk was not reported.

Thus, it appears that low-fat dairy and eggs may provide modest reduction in risk of stroke, but that there are limited data, especially for eggs, and confidence in findings is thus modest. Mechanisms explaining the potential association between low-fat dairy intake and stroke risk may be due to the calcium and potassium in dairy, which have an antihypertensive effect [50]. Calcium, potassium, and magnesium may also reduce insulin resistance and platelet aggregation [51]. In addition, B vitamins may reduce risk through their beneficial effect on plasma homocysteine, antioxidant defenses, and endothelial function [33]. Blood pressure reduction may result from peptides in fermented milk and vitamin D [52]. In addition to the role of fat and cholesterol on hemorrhagic stroke risk, noted above, the protein and vitamin D found in eggs may lower cardiovascular risk [54].

27.7 LEGUMES, NUTS, AND GRAINS

Plants are a key source of protein in the developing world, and for those following plant-based dietary patterns in the developed world [56]. Major plant protein sources include grains, beans, peas, and other legumes [57]. Soybean products, including tofu and soymilk, are a key plant source of protein in Asian countries [58]. Soybeans provide all essential amino acids, are higher in protein than other legumes, and are also a rich source of fiber, fatty acids, and isoflavones [59].

While grains and food made from grains provide 50% of the protein consumed by humans worldwide [60], in the US the average intake of whole grains is less than a serving per day, and only 10% consume the recommended three servings per day [24,60]. Glutens, found chiefly in grains such as wheat, barley, spelt, and rye [61], are a major source of vegetable protein in the diet [62].

A systematic review in 2012 identified only two studies of soy intake and stroke risk [63]. One of the studies, a case–control (374 cases and 464 controls) conducted from July 2007 until July 2008, found a significant and substantial decrease in ischemic stroke risk with intake of soybeans (OR: 0.20, 95% CI: 0.09–0.48), soymilk (OR: 0.18, 95% CI: 0.06–0.51), tofu (OR: 0.56, 95% CI: 0.36–0.98), and total soy foods (OR: 0.23, 95% CI: 0.14–0.39) [64]. The second study, with 40,462 individuals followed over 12.5 years, examined the association between soy and isoflavone consumption and the risk of cerebral infarction (CI) [65]. In this study, soy consumed 5 or more days per week compared to 2 or fewer days per week was inversely associated with CI in women (HR: 0.64, 95% CI: 0.43–0.95, $P = 0.04$), but not in men (HR: 0.95, 95% CI: 0.72–1.26, $P = 0.41$) [64,65]. The inverse association was more pronounced in postmenopausal women, suggesting that the isoflavones in soy, which are similar to estrogens, may bind to the estrogen receptor and perhaps protect against atherosclerosis [65]. In premenopausal women, isoflavone may be less beneficial due to the circulating estradiol [65].

The Nurses' Health Study and the Health Professionals Follow-up Study, which followed 84,010 women for 26 years and 43,150 men for 22 years, reported no association between legumes and total stroke risk, but reported a significant association between legumes and ischemic stroke risk (RR: 1.45, 95% CI: 1.06–2.0, $P = 0.02$) [45]. Etiology for this positive association is not clear, but may be related to other ingredients cooked with legumes, such as salt or fat.

Peanuts, including peanut butter, are the most consumed nut in the US [66]. The Physicians' Health Study, a randomized trial including 21,078 individuals followed for 21 years, compared the highest intake (seven or more servings per week) to the lowest intake (none) of nuts and observed that there was no significant association between nut intake and total stroke (HR: 1.07, 95% CI: 0.79–1.46), ischemic stroke (HR: 0.93, 95% CI: 0.65–1.34), or hemorrhagic stroke (HR: 1.84, 95% CI: 0.95–3.57) [67]. However, the Nurses' Health Study and the Health Professionals Follow-up Study found that

the highest quintile of nut consumption (0.34 servings/day) compared to the lowest (zero servings/day) was associated with a lower total stroke risk (RR: 0.86, 95% CI: 0.75–0.98, $P = 0.05$) [45].

The Nurses' Health Study also examined whole grains and ischemic stroke in 75,521 women over a 12-year period and found an inverse association comparing the highest (median intake 2.7 serving/day) to the lowest (median intake 0.13 servings/day) quintile, with a RR of 0.69 (95% CI: 0.50–0.98, $P = 0.04$ for trend) [68]. In contrast, the Atherosclerosis Risk in Communities (ARIC) Study followed 11,940 individuals over 11 years and found no association [69]. The ARIC authors suggest that misclassification of whole- versus refined-grained consumption in the 66-item food-frequency questionnaire, which did not differentiate these grains in the food list, may explain the null association [69].

In sum, increased soy intake is associated with a reduction in stroke risk [64,65], nuts may be protective but there are limited data, and there are similarly limited data on whole grains and stroke risk. Mechanisms explaining the relationship between soy intake and stroke risk may be due to the presence of antioxidants such as the phytoestrogenic isoflavones (including genistin, daidzin, and formon) which may inhibit the formation of oxidized lipoproteins and protect against atherosclerosis [65]. Peanuts, along with almonds, pistachios, pecans, and macadamia nuts, are low in saturated fat and high in mono- and polyunsaturated fats – factors that may help lower LDL cholesterol [70]. Nuts and peanut butter may also inhibit lipoprotein oxidation, reduce inflammation, decrease insulin resistance, and improve endothelial function [70]. Whole grains reduce serum cholesterol [60,71] and lower blood pressure through increased insulin sensitivity and improved endothelial function [71,72]. By contrast, even after fortification of refined grains, there appeared to be no cardiovascular benefits to their consumption when compared to whole grains [71]. Refined grains may contribute to hyperglycemia and hyperlipidemia and reduced insulin sensitivity, increasing the risk for stroke [73].

27.8 DIETARY PATTERNS

Much research over the past 20 years has evaluated the relation between dietary patterns, rather than individual foods or nutrients, in relation to health and disease. Evidence suggests that the interplay (i.e., synergy or antagonisms) between dietary components from varying foods within a dietary pattern is important [74]. Attention, for example, has focused on the traditional Mediterranean diet as a particularly healthful diet [74–77]. This diet incorporates a high ratio of monounsaturated fats to saturated fats; a high intake of vegetables, fruits, legumes, nuts, and cereals; a moderate-to-high intake of fish; a low intake of dairy and meat; and a moderate intake of alcohol (mostly wine during meals) [77,78]. As such, the total protein content does not vary much from low-adherers of the diet to high-adherers, but there is an increased consumption of more healthful proteins, including fish, whole grains, legumes, and nuts among individuals who closely follow the diet [78]. The importance of this diet to stroke and coronary heart disease prevention has been seen in the Nurses' Health Study [78], the EPIC Study [77], the Washington Heights/Hamilton Heights Columbia Aging Project (WHICAP) imaging sub-study [79], the *EPICOR* study [80], and the PREDIMED randomized control trial [74], and supported in a 2010 meta-analysis of seven prospective studies [76].

Evidence also supports other dietary patterns, such as the Dietary Approach to Stop Hypertension (DASH) diet [80–83] and vegetarian diets [81,84,85] for the control of blood pressure, which in turn may prevent stroke. The DASH diet, similar to the Mediterranean diet (although it does not emphasize intake of olive oil and wine) includes fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts; it too is rich in potassium, magnesium, calcium, dietary fiber, and protein, and low in fat, cholesterol, red meats, sweets, and sugary beverages [81]. The Nurses' Health Study concluded that adhering to the DASH diet was associated with a decreased risk for stroke when comparing the highest to the lowest quintile of adherence (RR: 0.82, 95% CI: 0.71–0.94, $P = 0.002$) [82]. While the American Dietetic Association supports a vegetarian diet to help reduce cardiovascular disease [86], a very low-fat vegan diet may hypothetically increase the risk of stroke as the diet may decrease IGF-I activity, which helps sustain the metabolic and structural integrity of the cerebral vasculature, and thus may in turn raise ischemic and hemorrhage stroke risk [85].

A low-carbohydrate, high-protein diet (with an average of 62.9 g/protein per day, including 42.3 g/animal protein per day) increased the risk of subarachnoid hemorrhage (RR per two-unit increase in diet score: 1.17; 1.00–1.38) for women with animal protein intake above the median, and decreased risk for those with animal protein intake below the median (RR per two-unit increase: 0.90; 0.76–1.07) [87], suggesting, as with studies of total animal or vegetable protein discussed above, that there may be a threshold above which dietary protein's risks may outweigh its benefits for cardiovascular health.

27.9 PROTEIN SUPPLEMENTATION POST-STROKE

While most of the clinical and epidemiological literature has focused on the role of dietary protein in the

prevention of stroke, several studies have also looked at the role of protein in the acute treatment of stroke. One study reported that 16% of stroke patients were protein-deficient on admission to the hospital, 26.4% after 1 week of admission, and 49% prior to admission to a rehabilitation facility [88]. In a randomized control trial of 48 patients with sub-acute stroke, there was a significant improvement in cognitive function as measured by the Mini-Mental State Examination (MMSE) with protein supplementation [89]. The supplemented group (mean protein intake: 67 g/day), compared to the control group (mean protein intake: 39 g/day), increased their recovery of cognitive function after 21 days (MMSE + 0.6 ± 0.4 score, $P = 0.01$ from baseline) [89]. A similar 30-day study of 17 sub-acute ischemic stroke patients reported a positive association between protein intake and neurologic and cognitive retrieval [90]. The randomized controlled trial FOOD (Feed Or Ordinary Diet) had 4023 patients receive either a normal hospital diet plus an oral protein and energy supplement (equivalent to about 62.5 g/l in protein per day) or a control (normal hospital diet), but did not see improvement in the group with added protein [91].

Thus, protein supplementation post-stroke appears beneficial in several studies, although data are limited and which type of protein provides the greatest benefit is not clear. Mechanisms contributing to improved cognitive function may be related to reductions in expansion of brain infarction and increases in neuron survival and function, or the formation of neurotrophins and interleukins, which are important for cognitive function [89,90].

27.10 CONCLUSION

Stroke is the fourth leading cause of death, one of the leading causes of long-term disability [1], and is expected to increase 22% over the next two decades [3]. It is therefore imperative to focus on prevention efforts. Dietary change can play a key role in these efforts, and data exist on the way in which dietary protein choices impact stroke risk. Importantly, many of the associations observed between dietary protein and stroke prevention have also been seen with dietary protein and coronary heart disease or cancer prevention [27,28,31]. For instance, red and processed meats have been associated with cancer, CVD, and premature death, and therefore minimizing or eliminating their intake reduces disease risk [27,28]. Fish intake has been recommended by the American Heart Association for the prevention of heart disease, and epidemiological data links high soy, legume, and cereal grain intake with heart health as well [59,60,64,65].

To summarize the evidence to date on dietary protein and risk of stroke:

1. Higher amounts of total dietary animal and vegetable protein are associated with a reduced risk of stroke.
2. Red and processed meats are associated with an increased risk of stroke.
3. Intake of fatty and oily fish, and perhaps poultry, is inversely related to stroke risk.
4. Low-fat dairy intake is associated with a lower stroke risk.
5. The relation between egg intake and stroke risk remains uncertain.
6. Soy intake may help reduce stroke risk.
7. Frequent nut intake has been associated with reduced stroke risk.
8. The literature on whole-grain intake and stroke risk is limited and with mixed results, but whole-grains have been shown to decrease blood pressure, a key risk factor for stroke.
9. Dietary patterns, and particularly Mediterranean-style diets, may reduce stroke risk.
10. Protein supplementation following a stroke appears promising for cognitive recovery, though data are limited.

The evidence on dietary protein and risk of stroke is largely consistent with US dietary recommendations. For instance, MyPlate.gov recommends building a healthy plate by cutting back on foods high in solid fats, sugars, and salt; limiting meats and poultry to small portions of lean or low-fat selections; varying protein food choices to include beans and seafood rich in omega-3 fatty acids; making half the plate fruits and vegetables; switching to skim or 1% milk; making at least half the grains whole-grains; limiting processed meats due to high sodium; and choosing unsalted nuts for a lower sodium intake [92]. The Centers for Disease Control and Prevention recommend choosing an overall healthy eating plan that provides protein and other nutrients, including lower-fat proteins that are leaner cuts with fat trimmed; removing skin from turkey and chicken; substituting pinto or black beans for meat in chili or tacos; choosing low-fat or fat-free milk, yogurt, and cheese; and choosing egg whites or pasteurized egg-white products [93].

Despite abundant evidence on the relation between dietary protein and stroke risk, gaps in knowledge remain and provide opportunities for further research. Such gaps include:

1. The degree to which the association between dietary protein and stroke risk is modified by comorbid conditions, such as obesity, diabetes, hypertension, and hypercholesterolemia

2. How stroke risk is impacted by childhood dietary protein intake
3. How specific legumes, other than soy and peanuts, impact stroke risk
4. The role of low-fat diets in relation to hemorrhagic stroke risk
5. How different protein sources impact cognitive recovery following a stroke.

Despite these gaps, much has been accomplished in the past several decades in understanding the relation between dietary protein and stroke. While further research is needed, efforts should continue to improve dietary healthfulness; following US dietary guidelines is a step in the right direction. Through such efforts, the individual and public health burden of stroke may be reduced.

APPENDICES

Appendix A: Protein Content of Select Major Protein Sources

	Product	Serving size	Energy (kcal)	Protein (g)	Total fat (g)	Saturated fat (g)	Carbohydrate (g)	Fiber (g)
Unprocessed red meat	Hamburger (95% lean)	3 ounces	139	22	5	2.3	0	0
	Porterhouse steak	3 ounces	181	24	8.8	3.4	0	0
Processed red meat	Bacon (pan fried)	3 slices	161	11.7	12.1	4.1	0.6	0
	Beef bologna	1 slice	90	3.3	7.8	3.1	1.3	0
Fish	Salmon (Atlantic)	3 ounces	155	21.6	12.5	2	0	0
	Yellow fin tuna	3 ounces	110	24.8	<1	<1	0	0
Poultry	Duck w/o skin	3 ounces	151	24.75	5	1.1	0	
	Turkey w/o skin	3 ounces	135	24.7	3.3	1	0	0
	Chicken breast	3 ounces	122	24	3	0.7	0	0
Dairy	Milk (1%)	1 cup	105	8.5	2.4	1.5	12.2	0
	Milk (buttermilk)	1 cup	137	10	5	3	13	0
	Cheese (cheddar)	1 ounce	114	7	9.4	6	0.4	0
Eggs	Egg (poached)	1 large	72	6.3	4.7	1.6	0.4	0
	Egg yolk	1 large	55	2.7	4.5	1.6	0.6	0
	Egg white	1 large	17	3.6	0.06	0	0.2	0
Nuts	Peanuts	1 ounce	161	7.3	14	1.9	4.6	2.4
	Walnuts	1 ounce	175	6.8	16.7	1	2.8	1.9
Grains	Wheat flour (whole grain)	1 cup	408	15.8	3	0.5	86.4	12.8
	Wheat flour (white, enriched)	1 cup	455	12.9	1.2	0.2	95.4	3.4
	Oat bran	1 cup	88	7	1.9	<1	25	5.7
	Wheat bran (crude)	1 cup	125	9	2.5	<1	37.4	24.8
	Rice bran	1 cup	373	15.8	24.6	5	58.6	24.8
	White rice	1 cup	169	3.5	0.3	0.07	36.7	1.7
	Brown rice	1 cup	218	4.5	1.6	0.3	46	3.5
	Durum wheat	1 cup	651	26.3	4.7	0.9	137	
Beans	Black beans	1/2 cup	113	8	<1	<1	20	8
	Lentils	1 cup	230	18	<1	<1	40	15.6
	Soybeans (cooked)	1 cup	254	22.2	11.5	1.3	20	7.6
	Soybeans (dry roasted)	1 cup	419	36.8	20	2.9	30.4	7.5

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Appendix B: Literature Search

An electronic search primarily using PubMed through October 2013, supplemented with a review of websites hosted by the US federal agencies (e.g., Centers for Disease Control and Prevention, Healthy People 2020, and United States Department of Agriculture), was conducted to identify relevant studies on dietary protein and stroke. Search terms included protein, dietary protein, total protein, animal protein, vegetable protein, plant protein, red meat, processed meat, fish, poultry, dairy, eggs, legumes, nuts, grains, dietary patterns, DASH diet study, DASH diet and proteins, DASH diet and stroke, meta-analysis stroke and protein, diet and aging, diet and aging and stroke and protein, epidemiology of stroke and protein, statistics, daily protein intake, total protein intake, stroke. Reference lists of articles retrieved were searched for additional articles.

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CHAPTER

28

Care for Stroke Patients with Eating Difficulties

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28.1 INTRODUCTION

The phenomenon of eating difficulties after stroke is multifaceted and complex, and can be considered from at least two perspectives: the biomedical/professional perspective, and the insider perspective. Despite the fact that thousands of people have to cope with eating difficulties as one of the consequences of a stroke, knowledge is scarce on how these difficulties affect the individual. In a recent qualitative study, when describing younger stroke-survivors' experiences of living at home with such eating difficulties, one participant gave the following description:

> It was the first food that I ate since the stroke [more than 1 year had passed]. I was very cautious and worried that it would not pass the throat like I had been experiencing. But, I ate it, and it was really pleasant. This was the first food, I mean "real" food, not the mashed one. It was delicious. I recall that I woke up during the nighttime, I couldn't sleep. I was so excited that I had been able to eat. I felt that I could conquer the whole world. Eating normal food and feeling appetite was a sign for me and my family that everything would turn out the best.
>
> Klinke *et al*. [1].

In this chapter we will provide insights into both perspectives. The biomedical perspective will be represented by a holistic, nursing perspective as well as the specific dysphagia treatment perspective. While undernutrition, which is the ultimate biomedical consequence of eating difficulties, has been studied since the 1940s, research on all other aspects of eating difficulties has only been developed recently. There is still a lack of consensus among researchers on the definition of eating difficulties. To clarify the components on which we want to focus, we will use the definition given by Klinke *et al*.: "any activity and emotional requirement and relations, which alone or in combination interfere with the process of preparing food, transferring food into the mouth, chewing and swallowing" [2]. Below we will further elaborate on the different aspects of eating difficulties, how they can be identified, and what treatment and support should be provided by healthcare professionals.

Eating difficulties, undernutrition, and dehydration are prevalent problems for stroke patients. In a recent study, Crary *et al*. [3] identified undernutrition in 32% of patients admitted to hospital for stroke, while 53% showed evidence of dehydration and 37% had difficulties with swallowing (dysphagia). Eating difficulties following stroke have been identified in 36–84% of cases [2]. Among stroke patients suffering undernutrition, nutritional status has been reported to deteriorate during a hospital stay, with elderly patients especially at risk [3–5], emphasizing the need for systematic nutritional care to prevent further change for the worse. For patients, undernutrition causes a comprised ability to recover from stroke, as well as increased mortality [6]. For society, longer hospital stays and medical complications related to undernutrition cause considerable costs [7]. Most patients at risk of undernutrition are impaired by

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unintentional weight loss, low BMI, or eating difficulties such as dysphagia [8]. Recently, easy-to-use tools to identify patients at risk of undernutrition, as well as to train swallowing function, have been developed [9,10]. However, two recent systematic reviews have concluded that knowledge regarding the effects of the different components of nutritional care (for example, dysphagia therapy and nutritional and fluid supplementation) on patients' functional outcomes is insufficient [11,12].

28.2 EATING DIFFICULTIES

As stated above, eating difficulties are common after a stroke and comprise multiple and complex aspects. Those aspects include mealtime problems with handling food on the plate and transporting it to the mouth (ingestion); problems with chewing and swallowing (deglutition), and lack of energy to complete a meal; difficulty maintaining upright posture; and visual, perceptual, and attention deficits; as well as contextual factors related to eating as a social activity [2,13,14]. Dysphagia is of particular concern because the prognosis is more grave for dysphagic stroke patients than for non-dysphagic patients. Thus, eating difficulties are multifaceted, and so are the interventions that constitute effective and safe care for those patients. To be able to plan for relevant and effective interventions, structured and documented assessments of patients' eating difficulties and nutritional risk are needed. Nowadays, in Western society, with meals and food choices being important aspects of quality of life, healthcare professionals need not only to be knowledgeable about evidence-based methods for nutritional care but also to include patients' experience and preferences when they design the rehabilitation plan.

The experience of living with eating difficulties after a stroke has not been investigated to a great extent. However, findings from a few qualitative studies indicate that during the acute phase of stroke the experience is entirely negative, with feelings of fear of choking, discomfort in the mouth, and shame and of being dependent on others at mealtimes [15]. After 3 months, stroke patients that still have eating difficulties have described their struggle in taking control of the eating situation to be able to eat safely and properly [16].

28.3 DYSPHAGIA

Dysphagia – that is, impaired or unsafe oropharyngeal transit of food or liquids – is seen in about half of stroke patients during the acute phase, and in about 10% of patients 14 days post-stroke [17,18]. In such cases, unilateral and bilateral cerebral hemispheric infarctions are seen more often than brainstem events. However, dysphagia is more likely to occur when stroke involves the brainstem, and in this case the prognosis is serious and will often have a fatal outcome [19,20]. Dysphagia may cause two types of severe problems for stroke patients: decrease in the efficacy of deglutition, leading to undernutrition and dehydration; and a decrease in swallowing safety, leading to aspiration pneumonia – a life-threatening condition [21]. Early screening for dysphagia at hospital admission is one aspect of evidence-based stroke care [22,23].

The incidence of aspiration, including silent aspiration, varies from 22% to 42%, as assessed by videofluoroscopy [24]. In many patients, neither their clinical history, such as a cough or impaired gag reflex, nor changes in voice or the neurological evaluation can predict the presence of silent aspiration [25]. Aspiration following stroke occurs more frequently in those with brainstem lesions [26]. However, patients with lesions in the posterior region and with a history of pneumonia (reported by up to 32%) are at greater risk of impaired pharyngeal safety, which signifies that videofluoroscopic examination is mandatory in these patients [27]. Unfortunately, neither videofluoroscopy nor fiberoptic endoscopy can serve as a perfect "gold standard" for the detection of aspiration, because each yields both false-negative and false-positive results. It has been claimed that dental status and good oral hygiene are of great importance in order to avoid the risk of aspiration [28,29].

Dysphagia comprises sensory and motor dysfunction of several different cranial nerves (CNs). It is evident from videofluorographic studies of swallowing that stroke patients have some degree of sensory loss in the pharynx [30]. Identification of patients with dysphagia is therefore the first vital step in their appropriate management. The primary goals of dysphagia therapy should be to establish optimal nutrition, and to eliminate or reduce the risk of developing complications. It seems appropriate to categorize dysphagia into its preoral, oral, pharyngeal, and esophageal forms [31]. *Preoral dysphagia*, or the ingestion phase of eating as described above, includes all kinds of difficulties in conveying food and liquids from the plate to the mouth. *Oral dysphagia*, or the deglutition phase of eating, can be due to (for example) orofacial paresis, palsy of the tongue, dryness of the mucosal membranes, reduced oral sensation, or absence of the swallowing reflex in the anterior faucal arcs. *Pharyngeal dysphagia* is due to sensory failure, weakness, or palsy of the pharyngeal swallowing muscles and hence the inability to protect the laryngeal entrance. *Esophageal dysphagia* is often separated into two types: (1) constant dysphagia with retention of food due to a benign or malignant stricture, or to achalasia cardiae; and (2) intermittent dysphagia in patients with, for example, a hiatus hernia. In stroke patients, dysphagia often takes both oral and pharyngeal forms simultaneously.

28.3.1 Oropharyngeal Neurophysiology

Chewing and swallowing are dependent on several motor and sensory CNs integrated into a coordinated oropharyngeal function, and the complex neurophysiology is described briefly below. The swallowing process can be described in two phases: the oropharyngeal and esophageal phases. The first phase of swallowing is of short duration (0.6–1.0 seconds) and is remarkably constant in all humans, despite the extraordinary complexity involving not only pharyngeal and laryngeal muscles (CN IX, X), but also muscles in the oral cavity such as the tongue (CN XII) and suprahyoid muscles (CN V, VII, XII). In the esophageal phase (CN X), the outer longitudinal muscle contracts when the upper esophageal sphincter opens, and the inner circular muscles contract, initiating a peristaltic wave with a water transit time of <7 seconds [31,32]. The inner and outer muscles of the upper third of the esophagus are striated muscles and the lower two-thirds are smooth muscles.

The motor part of the six different CNs, with striated muscles that are involved in oropharyngeal swallowing, is represented in the precentral motor area in the brain cortex. The importance of the oral function is best illustrated by the relatively large area in the cortex that is occupied by the oropharyngeal cavity (Figure 28.1) [33]. The enormous network of the extrapyramidal tracts from the cortex via the basal ganglia and reticular formation and ending in the motor nucleus in the brainstem or in the spinal tract is a prerequisite for brain plasticity. Of similar importance for plasticity are the afferent sensory pathways, besides their direct connection with the cortical postcentral sensory area, that have indirect connections via the reticular formation. The swallowing reflex is an autonomous function that consists mainly of the interactions between the nucleus tractus solitarius (NTS), nucleus vagus, and nucleus amiguus, each of which is closely connected with the reticular formation as well as with cortico-nuclear pathways.

The cranial facial nerve has two peripheral branches: an upper branch from the forebrain to the eye-closure muscles, and a lower branch to the nasolabial muscles and to the buccal and orbicularis oris muscles. The classical (and prevailing) view has been that the upper branch has a central bicortical representation and therefore is not clinically affected by unilateral cortical lesions, whereas the lower branch has only unilateral cortical representation, causing a nasolabial smooth down and a dip in the angle of the lip on the contralateral side in stroke patients with a central facial paresis. This view is not consistent with findings in a recent study by Hägg and Tibbling [34] on facial function in dysphagic patients after stroke. In this research study, 74% of the patients had subclinical orofacial motor dysfunction in three or four facial quadrants, and 52% had pathology in all four quadrants with simultaneous 100% pathology in the lower quadrant contralateral to the cortical lesion as assessed with a facial activity test (FAT) [35]. However, whether subclinical orofacial motor dysfunction can be present in stroke-afflicted patients without dysphagia is unknown. Six CNs are involved in swallowing: V, VII, IX, X, XI, and XII. Of particular interest in this context is the motor supply to the facial nerve (CN VII).

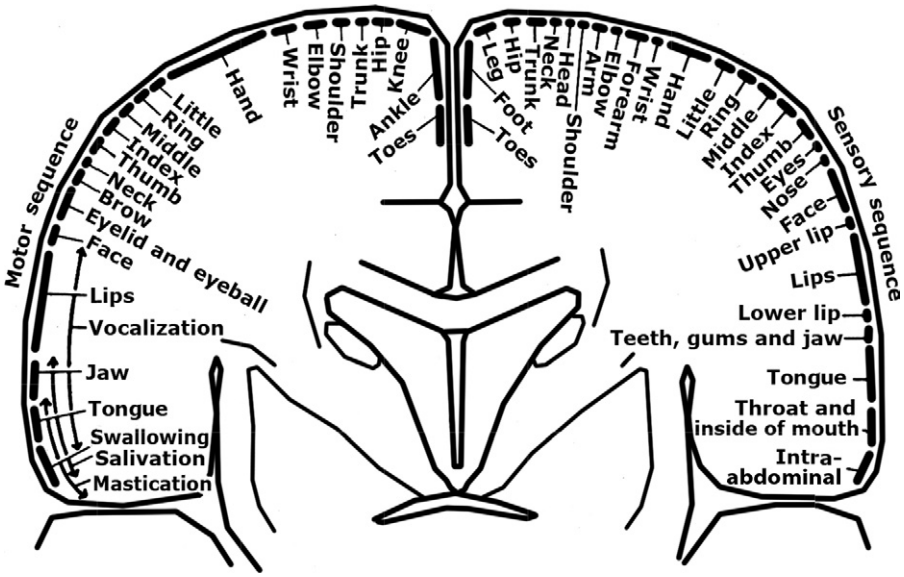

FIGURE 28.1 Illustration showing the vast representation of the oropharyngeal cavity in the cortex. *Illustrations by Mary Hägg®, from the Rasmussen and Penfield diagram of sensory and motor sequences in cerebral cortex of man as determined by electrical stimulation [33].*

The oral cavity and pharynx are anatomically separate yet functionally integrated regions of the head and neck. These two regions are involved in the complex motor responses that include feeding, chewing, swallowing, speech, and respiration. The oral and pharyngeal phases are closely interrelated, and the distinction between them is often unclear. In the oropharyngeal region, several CNs are involved in the sensory–motor reflex arc, which is activated by sensory stimulation via the afferent pathway and the impulses are transmitted to the medullary NTS. Some impulses reach the cerebral motor cortex, and return via the efferent motor pathways necessary for triggering the swallowing reflex. Perioral, submental, and lingual striated muscles can be controlled by the medullary CPG (central pattern generator) beyond the cortical drive [36]. Food/saliva in the mouth and the cortical drive to the tongue and the floor of the mouth are necessary for voluntarily induced swallowing, whereas triggering of spontaneous swallowing does not require any cortical drive [37]. However, a reflex mechanism does play a role in both types of swallowing. Not only are these CNs of importance for normal swallowing function; body and head posture and appropriate breathing are also of significance. Studies evaluating swallowing disorders in stroke patients have found that a majority recover, reflecting the enormous neuronal plasticity of the central nervous system. Recovery of swallowing is associated with increased pharyngeal representation in the unaffected hemisphere [38,39], and is highly dependent upon the frequency, intensity, and duration of sensory stimulus applied [40,41].

28.3.2 The Oral Phase

The oral phase of swallowing is mainly voluntary and highly variable in duration, depending upon, for example, taste, hunger, and motivation, but it also includes reflexive components integrated with feeding and chewing [42]. It is primarily related to oral preparation, including the activating of the jaw-closing muscles of the mandible (CN V), chewing and stabilizing the mandible while activating the movements of the tongue (CN XII), and propelling the bolus backwards toward the pharynx. To raise the tongue, especially for a solid bolus, the suprahyoid muscles in the floor of the mouth (CN V, XII) are particularly important. Similarly, the orbicularis oris (CN VII) and buccinator muscles (CN VII) close the mouth to prevent food from escaping forwards [43]. Their contraction and muscle tone act as a valve mechanism [30].

28.3.3 The Pharyngeal Phase

The pharyngeal phase is considered to be a reflex response. When a bolus is propelled from the oral cavity to the base of the tongue, then to the upper third of the epiglottis, to the pillars of the fauces and to the walls of the pharynx, the tactile-, mechano-, chemo-, and thermoreceptors provide information essential for bolus identification and to trigger swallowing. All sensory input through the afferent fibers running within the maxillary branch of the trigeminal nerve (CN V), the glossopharyngeal nerve (CN IX), and the vagus nerve (CN X), especially its superior laryngeal branch, reach the brainstem and end in the NTS. The NTS receives the main sensory fibers, not only from the oropharyngeal and laryngeal regions, but also from cortical descending inputs. Some sensory inputs that initiate swallowing are transmitted to the region of the caudal-lateral sensorimotor cortex, which facilitates initiation of the swallowing [31,42].

Once swallowing is initiated, the cascade of muscle activation and events occurs in rapid overlapping succession. The main events are the transport of food to the pharyngeal-esophageal segments by the movements of the tongue, submental/suprahyoid muscles, and pharyngeal constrictor muscles, and the relaxation and opening of the crico-pharyngeal sphincter muscle (UES). During food transport, the airway is protected and closed by several laryngeal muscles, the larynx is drawn up, and the epiglottis is tilted backwards to cover the laryngeal entrance. All events, from the cranial to the esophageal phase, are controlled mainly by the CPG of the brainstem [31,42].

Thus, swallowing is a complex sensorimotor behavior involving the coordinated contraction and inhibition of the musculature located around the mouth and at the tongue, larynx, pharynx, and esophagus bilaterally. To conclude, during a swallow, different levels of the central nervous system from the cerebral cortex to the medulla oblongata are involved and many of the striated muscles innervated by the CNs are excited and/or inhibited sequentially for the execution of the passage of a bolus from the mouth to the stomach [31,41].

28.4 GENERAL CARE AND NURSING INTERVENTIONS FOR PATIENTS WITH EATING DIFFICULTIES

The harmful effects of undernutrition are well recognized, yet the importance of nutritional care as an essential element of stroke care appears to have been too little regarded [12]. The distribution of roles and responsibilities for the provision of nutritional care has been described as obscure and confusing [44]. Perry and McLaren found significant gains when testing multidisciplinary nutrition support guidelines that included the screening of nutritional status and swallow function, timely referrals, mealtime management, and assistance and monitoring of patients during meals [45]. In another

recent study, Perry *et al*. [12] performed a systematic review with the aim of identifying nursing interventions intended to improve nutritional status and related outcomes of stroke patients, and to examine the outcomes of identified nursing interventions on nutrition-related outcomes. The researchers identified the following categories of nutritional support activities/interventions described in the literature as important aspects of stroke patients' nutritional care:

- Screening, assessment, and referral
- Mealtime management
- Modification of mealtime activities and the dining environment
- Eating assistance and feeding skills
- Training and monitoring
- Oral care
- Nutritional education.

We will further elaborate on these aspects of care below.

28.4.1 Screening, Assessment, and Referral

A variety of factors may impact on stroke patients' ability to eat. Such factors include, for example, dysphagia, poor oral health, pain or discomfort in the mouth, nausea, anorexia, taste changes, gastrointestinal problems such as constipation, drowsiness, depression, confusion, or embarrassment. To capture the whole picture of a stroke patient's eating difficulties, comprehensive assessments of eating function, including dysphagia and nutrition status, must be conducted as soon as possible after hospital admission to ensure patient safety. Based on the findings, a patient's individualized care should be planned, performed, and evaluated, with the goals being to provide relevant nutrition for the individual, to prevent risks related to dysphagia, and to make the patient and family members feel secure and well-informed.

There is lack of consensus on how to identify patients who have some degree of dysphagia and who are thus in need of referral to a speech-language therapist. After performing a systematic review of methods to be used by nurses in their screening for eating difficulties, Westergren [14] suggested three steps to identify these problems: (1) screening for dysphagia using the Standardized Bedside Swallowing assessment (SSA) instrument, and applying pulse oxymetry simultaneously to SSA to possibly add to the accuracy of aspiration detection; (2) observation of the patient's eating, including ingestion, deglutition, and energy; and (3) conducting a patient interview about his or her experience of eating [14]. In a more recent review, however, Daniels *et al*. [46] concluded that a water-swallowing assessment method (for example, SSA) is feasible to manage, but that the most valid protocol remains to be determined [46].

The Minimal Eating Observation and Nutrition Form – Version II (MEONF-II) has been developed and tested by Westergren and colleagues for stroke patients as well as for other hospital inpatients, with the aim of identifying patients needing preventive intervention as well as those needing nutritional treatment [9,47,48]. The instrument has been found to be an easy-to-use, relatively quick and sensitive screening tool for such assessments, also providing important information on what components of eating are impaired. The instrument is constructed by the following six dimensions: unintentional weight loss, BMI, eating problems, swallowing function, energy/appetite, and clinical signs indicating risk for undernutrition.

28.4.2 Mealtime Management

Important aspects of mealtime management for stroke patients with eating difficulties are ensuring the delivery of appropriate meals, providing monitoring and assistance with eating (where needed), and the positioning of patients for safe eating. Appropriate meals mean meals that are adjusted for individual patients' nutritional needs as well as the need for a modified consistency diet. Nurses are responsible for the supervision of feeding, especially for patients with dysphagia, and for monitoring food and fluid intake. Another aspect of mealtime management is the provision of enteral tube feeding (ETF).

28.4.3 Modification of Mealtime Activities and the Dining Environment

The mealtime environment has not been prioritized in research on interventions to prevent undernutrition and its related low quality of life. However, modification of the mealtime situation and providing a calm environment with minimal distraction has been shown to enhance the mealtime experience to allow patients to enjoy and be able to eat their food. In the UK, the Protected Mealtimes model (PM) has been implemented in many hospitals, with the aim of allowing patients to eat their meals without disruption and enabling staff to focus on providing assistance to those patients unable to eat independently [49]. Key principles of PM are: (1) focusing on the meal and the patient; (2) making sure that the patient is ready to eat; (3) making sure that the environment encourages eating; (4) providing assistance with eating; (5) observation and monitoring; and (6) making sure that the patient is eating. Implementation of the PM model has been studied in research projects with varying results, emphasizing the importance for leaders to recognize that, for PM to work, a change of ward culture might be needed. Such change requires active leadership and

a strategy for change [50,51]. To encourage optimal mealtimes for stroke patients, nursing staff need to consider the following:

- The side of the body that has been affected – is it better to set out food from a particular side?
- Any visual deficits as a consequence of the stroke – if so, consider rotating the plate during the meal for the patient to see all the food.
- Stroke patients may need a long time to self-feed.
- Self-feeding should be encouraged – always ask if the patient wants assistance; never assume.

28.4.4 Eating Assistance and Feeding Skills

Providing eating assistance and feeding, either orally or by means of ETF, are everyday duties for nursing staff, although these are activities demanding skills and an ethical stance with respect for the patient's integrity and preferences. How skilled nurses act when providing such assistance has not been studied to a great extent. Examples of basic prerequisites for safe, assisted eating or feeding are: positioning the patient and ensuring maintenance of optimal positioning; ensuring that sensory aids such as hearing aid, glasses, and dentures are in place before meals; and encouraging the patient to eat. For safe feeding of patients with eating difficulties, nurses' feeding skills entail optimal feeder positioning, providing verbal or tactile prompts, matching food consistency and bolus size to the patient's requirements, and providing emotional support. One important aspect of eating assistance and feeding patients with eating difficulties is the monitoring of chest status for possible aspiration [12]. Oral feeding of older, frail patients is a time-consuming nursing activity, and nursing staff in geriatric and elderly care have been reported to request feeding via percutaneous endoscopic gastrostomy (PEG) in order to facilitate care [52]. Indications for ENT and suggestions for choice of route, such as a nasogastric tube or PEG, as well as effects on patients' nutritional status, are included in most national guidelines for stroke care, and will not be further elaborated here.

From the insider perspective, experience of having to live with assistance when eating has been described as a complex activity based on coordinated attention between the patient and the helper. The constituents of assisted feeding were, for example, the experience of paralysis as a condition of life, individuals' efforts for realization of their own values around meals, and balanced use of meal-time devices [53]. Experience of living with PEG has been described as "a burden of treatment" composed by aspects of physical restrictions on mobility, serious technical problems, and gastrointestinal symptoms [54].

28.4.5 Training and Monitoring

People with eating difficulties after a stroke have articulated that they feel abandoned by healthcare providers in their need to learn a new way of eating, and left to cope with their impairment on their own [55]. For patients suffering from dysphagia, swallow training is provided, usually by speech-language therapists. However, methods for swallow training exist that are well suited for nurses to manage.

28.4.5.1 Swallow Training

Currently available therapy modalities are (1) compensatory procedures and (2) active exercises combined with swallowing of food or liquid, so-called direct therapy, or indirect procedures combined with swallows of only saliva by patients who aspirate [30]. Most of these therapies are managed by speech-language therapists. Both therapy modalities are described below.

- Compensatory procedures control, improve or change the flow of food and can eliminate the patient's symptoms, but do not necessarily change the pathophysiology. The procedures also include postural techniques to improve sensory input, modified diet volume and consistency, as well as intraoral prosthetics (i.e., artificial substitutes for missing, altered, or deformed oral structures).
- Active exercises are designed to improve the range of motion of oral and pharyngeal structures of lips, jaw, tongue, tongue base, larynx, and vocal folds, to improve sensory input (thermal–taste–tactile stimulation), and to take voluntary control over the timing/coordination of selected oropharyngeal movements through swallow maneuvers and respiration.

28.4.5.2 Other Modalities

Other treatment modalities are orofacial regulation therapy, exercise with a palatal plate [35,36] or an oral screen called IQoro, DPNS (deep pharyngeal neuromuscular stimulation), FMEP (facial muscular exercise program), and Vitalstim (neuromuscular electrical stimulation) [56].

Orofacial Regulation Therapy

The orofacial regulation therapy developed by Castillo Morales is based on two hypotheses and comprises three levels: (1) manual body, and (2) orofacial regulation, in combination with (3) a palatal plate [57].

The first hypothesis of Morales is that body and orofacial regulation have an impact on dysphagia. The hypothesis is based on the interdependence of the orofacial complex (orofacial muscles, mandible, and oropharynx), breathing, head control, and body posture

at deglutition – called the first pattern way of motion [57]. To reach optimal results in the treatment of swallowing, it is necessary to recognize the head, neck, and body as a functional entity [57,58]. Normal overall function depends on a complicated interplay of sensory and motor functions involving a large number of muscle groups that must achieve a proper balance. The goal of the therapy, therefore, is to secure that balance. The hyoid bone is directly connected to the skull, the mandible, and the shoulder girdle by minor muscle chains, and indirectly to the pelvis through the large muscles (Figure 28.2). This is why the hyoid bone always has to adjust to the body posture.

The second hypothesis by Morales [57] is that a palatal plate and orofacial regulation have an impact on swallowing dysfunctions based on the sensory–motor reflex arc. The reflex is activated by sensory stimulation through the afferent path returning as an impulse in the efferent motor path. Five CNs in the mouth are involved in that reflex arc: the second pattern way of motion (Figure 28.2).

Body Regulation Body regulation (Figure 28.3) is restricted to the shoulder–neck–head region. It aims at achieving optimal head control, and equilibrium of the infrahyoidal (CN XII) and suprahyoidal muscles (CN VII, V, XII), in order to facilitate swallowing [35]. Body regulation includes seven procedures; each procedure is performed three times in 15 minutes. The therapist sits behind the patient, who is resting in the supine position with a pillow under the knees. The patient's muscles are stretched under pressure and vibration, and then quickly released to evoke contraction. A muscle with low tonus demands short intermittent vibration, while a muscle with high tonus demands long vibration under firm pressure. The same applies to orofacial regulation therapy [35].

Orofacial Regulation Orofacial regulation therapy includes 14 different procedures, which are summarized in Figure 28.4.

Body regulation and orofacial regulation therapy can also be used on people who are unconscious. For a detailed description, read Hägg and Larsson [35].

Training with a Palatal Plate The palatal plate has to be inserted two or three times daily, for 10–30 minutes before eating [35]. The plate, made of thin acrylic material

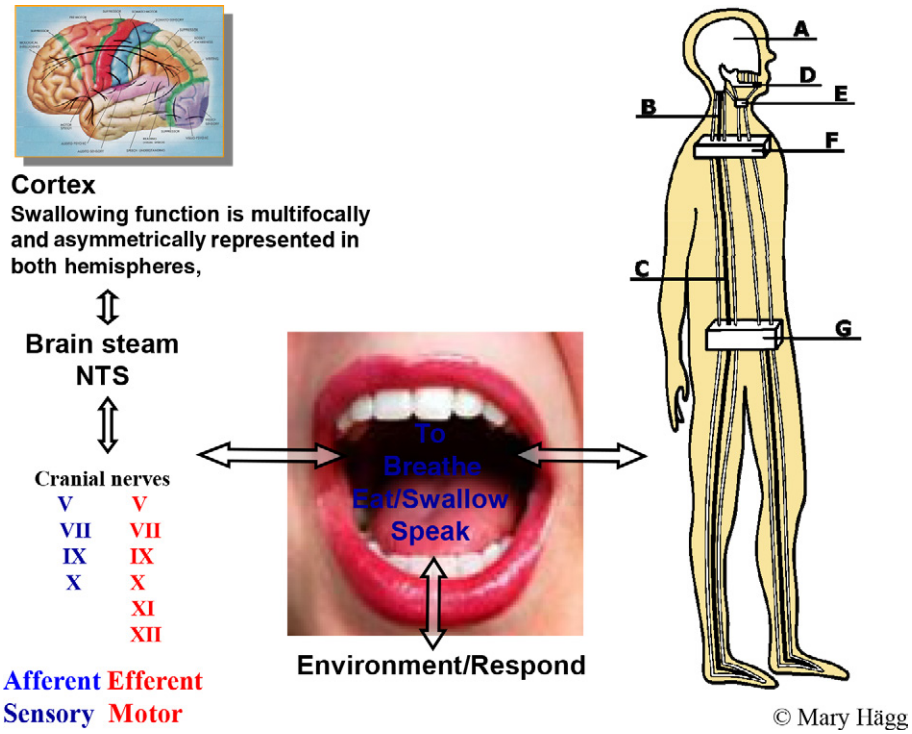

FIGURE 28.2 Mouth functions – breathing, eating, swallowing, and speaking – are dependent on both the entire sensory-motor reflex arc, and a body in balance. The functions are also affected by motor function, sensory function, anatomy, postural control, general health, environment, and customer care. The lower jaw and the hyoid bone constantly have to adjust to the body posture because the hyoid bone and the mandible are directly connected to the skull and to the shoulder girdle by minor muscle chains, and indirectly connected to the pelvis through the large muscles. A, skull; B, neck vertebral column; C, vertebral column; D, mandible; E, hyoid bone; F, shoulder girdle; G, pelvic girdle. *Illustration by Mary Hägg®.*

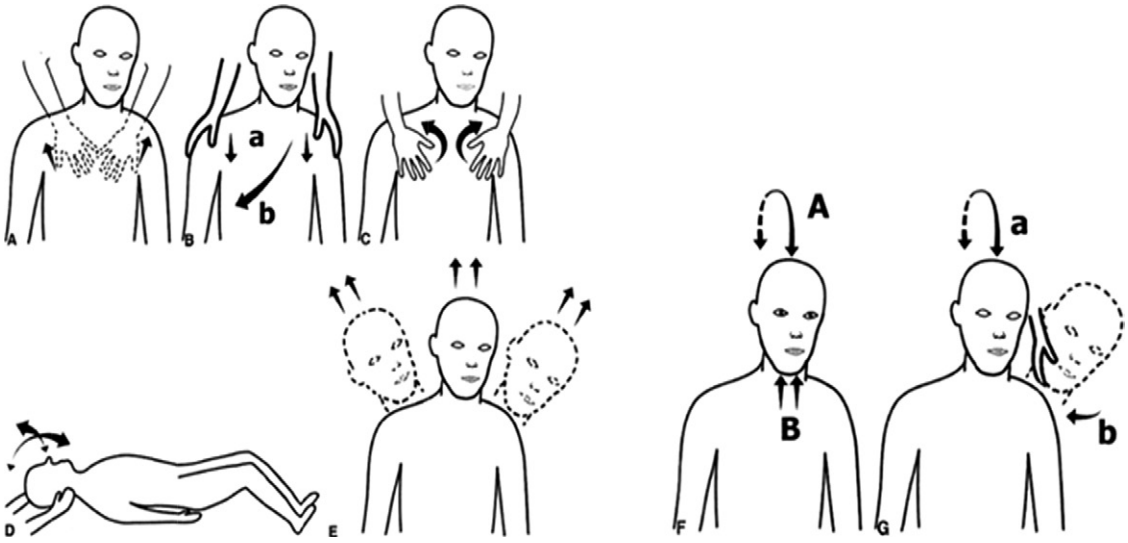

FIGURE 28.3 Seven procedures in body regulation therapy [35]. *Illustration by Mary Hägg®.*

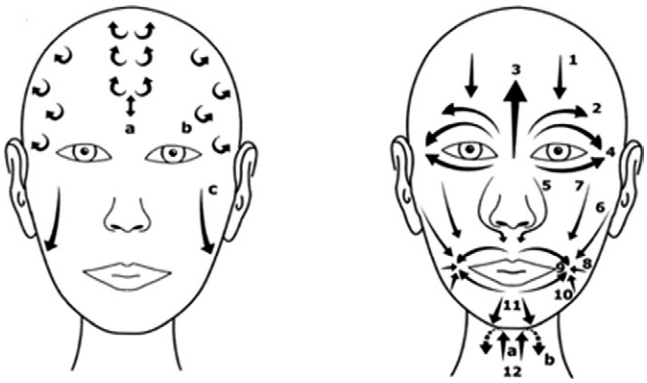

FIGURE 28.4 Orofacial regulation therapy includes 14 different procedures [35]. *Illustration by Mary Hägg®.*

FIGURE 28.5 The oral screen IQoro consists of a predental shield with a handle.

with spring retention elements, covers the entire palatal region. Four vestibular small acrylic plates ("bumpers") with knobs made of stainless steel act as stimulators for the upper lip and the buccinator mechanism [59]. For stimulation of the tip of the tongue, a mobile cube of stainless steel is attached to a dentoalveolar arch placed behind the incisors and in line with the canine teeth. For tongue-base stimulation, a velum arch provided with three small pointed convexities in the middle and to the sides is placed close to the A-line that is the border between the soft and hard palates. The patients are also encouraged actively to exercise the upper lip, the tip of the tongue, the tongue base, and the cheek, making at least three movements against each stimulator each time.

Training with the Oral Screen IQoro

IQoro is a unique, standardized medical device made of acrylic and supplied with a handle (Figure 28.5). It has to be inserted predentally behind closed lips three times daily for 30 seconds (1.5 minutes of total training time per day) before eating [60,61]. Each exercise session consists of horizontal, gradually increasing pulling maneuvers three times, for 5–10 seconds (Figure 28.6). For training, the patient pulls the handle and tries to withhold the screen with the lips. When the patient is unable to hold the oral screen, relatives or nursing staff are instructed to assist with the traction. The training period is set to at least 5 weeks.

Daily training with IQoro stimulates the brain and strengthens the entire "eating tract," from mouth to stomach. Treatment is mainly conducted over a period of 5–13 weeks, with lasting results. Studies prove that the method has resulted in significant improvements. More than 97% of the stroke patients in one study improved their swallowing capacity and, of these, 63% regained normal swallowing [60]. In another study, 33% of palatal

plate patients and 71% of IQoro patients regained normalized swallowing capacity, improvements that were sustainable over time [61].

IQoro has been shown to trigger the entire natural chain of neuromuscular activity, initiating a swallow (Figure 28.7). The buccinator mechanism involves m. orbicularis oris, m. buccinator, m. constrictor pharyngeus superior, m. constrictor pharyngeus middle, m. constrictor pharyngeus inferior, and m. cricopharyngeus. The oral screen that is placed predentally stimulates the sensory input by touching the intraoral membranes (CN V). When pulling the oral screen and pressing the lips (CN VII), the entire buccinator mechanism will be activated (CN VII, IX, X), and tonus and contraction of the bottom of the mouth will increase (CN V, XII) and act as a valve mechanism, thereby enhancing the intra-oral negative pressure. That in turn activates tongue retraction (CN XII), which in turn stimulates the sensory part of the anterior faucial arcs (CN IX), the soft palate (CN V, VII, X), and the intraoral mucous membranes (CN V), as well as the stylohyoid and the digastric posterior [61].

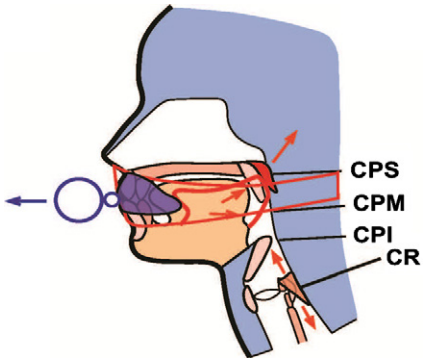

FIGURE 28.6 **IQoro training.** The buccinator mechanism (marked with a line) involves m. orbicularis oris, m. buccinator, m. constrictor pharyngeus superior (CPS), m. constrictor pharyngeus middle (CPM), m. constrictor pharyngeus inferior (CPI), and m. cricopharyngeus (CR) = upper esophageal sphincter. The OS is placed predentally and stimulates the sensory input by touching the intra-oral membranes (V). When pulling the oral screen and pressing the lips (VII) the entire buccinator mechanism will be activated (VII, IX, X), and tonus and contraction of the bottom of the mouth increase (V, XII) and act as a valve mechanism, thereby enhancing the intra-oral negative pressure. That in turn activates tongue retraction (XII), which in turn stimulates the sensory part of the anterior faucial arcs (IX), the soft palate (V, VII, X), and the intra-oral mucous membranes (V). Finally, the stylohyoid and the digastric posterior muscles (VII) are activated. *Illustration by Mary Hägg®.*

28.4.6 Oral Care

After a stroke, a person's oral health may be affected by difficulties with speech, chewing, and swallowing, as well as by poor tongue function and paralysis of the arm/hand. It is well known that oral health affects the older person's general health and risk of heart and lung diseases, as well as quality of life [62,63], though oral health is still often an overlooked aspect of stroke care [64]. A growing number of people still have their natural teeth at an advanced age, and an increasing proportion of those who do not have natural teeth have had implant-supported tooth replacement. Patients who cannot perform oral care themselves become dependent on

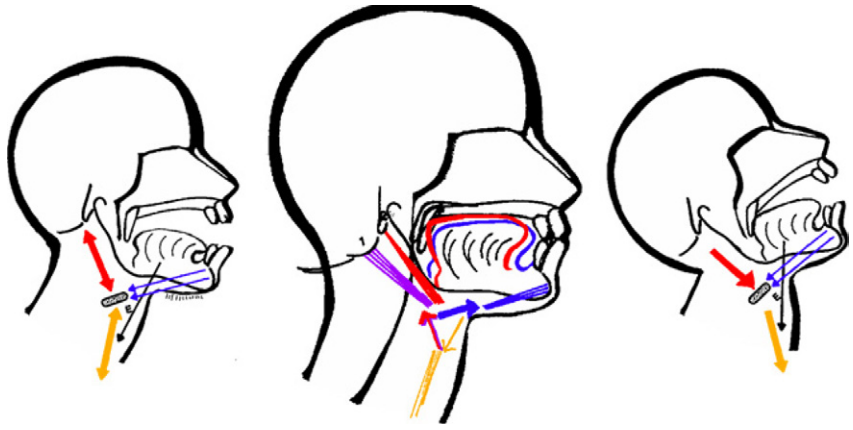

FIGURE 28.7 Body and IQoro therapy are aimed to achieve optimal equilibrium/balance of the infrahyoidal (n XII hypoglossus) and suprahyoidal muscles (n VII facialis, n V trigeminus, n XII hypoglossus), and to stimulate the swallowing reflex. **Middle figure:** A normal swallow is prepared by pulling the hyoid bone backwards/upwards (red arrow) at the same moment as the tongue retracts. The swallow reflex is triggered when the hyoid bone is pulled forward/upward (blue arrow) at the same moment, during apnea, when epiglottis folds over the laryngeal inlet and tongue forward movement is initiated. The chewing muscles are active throughout the swallow. **Left figure:** If the chewing muscles are too weak, the patient cannot lift the mandible or close the lips, which complicates swallowing. At the same time, the lower muscle groups of the tongue pull the hyoid bone downward, which further complicates swallowing. **Right figure:** When the head falls backwards, because of impaired head control, the mouth opens spontaneously and the equilibrium of the hyoid bone is eliminated completely, resulting in swallowing difficulties. The same thing happens when tooth grinding. *Illustration by Mary Hägg®.*

nursing staff and their knowledge regarding oral health and their ambition to prioritize good oral health [65]. Thus, patients suffering from acute stroke need early screening for oral health problems, and continuous and skilled oral care. Patients and family members also need instructions for further oral care after discharge from hospital.

As suggested by the British Society of Gerontology, the goal for oral health in continuious care should be to guarantee that patients/residents will have an opportunity for good oral health [66]. To fulfill that goal, the following requirements are stated:

- All qualified nurses will have a basic knowledge and understanding of the importance of oral health and disease.
- Oral assessments will be used to identify oral status and oral hygiene needs.
- There will be a clear referral procedure for routine and emergency dental advice and treatment.
- Oral hygiene equipment appropriate to a patient's needs will be available.
- Specific oral hygiene aids recommended by the dental team will be available.
- Patients will have access to privacy for oral hygiene.
- Information about oral care will be available for patients and staff.

28.4.7 Nutritional Education

Registered nurses' educational roles include training patients with dysphagia, instructing other nursing staff in safe feeding strategies, and teaching family members feeding skills. Education about healthy eating after stroke, as well as about nutrition supplements and ETF, are usually managed by dietitians. A wide variety of web-based educational programs are available for persons who have suffered a stroke; for example, the US National Stroke Association's *Recovery after Stroke* includes fact sheets about healthy eating (available from www.stroke.org).

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CHAPTER

29

Homocysteine, B Vitamins, and Cardiovascular Risk

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29.1 HOMOCYSTEINE METABOLISM

Homocysteine ($C_4H_9NO_2S$, or 2-amino-4-sulfanylbutanoic acid) is an essential amino acid with a molar mass of 135.18 g/mol which is formed during the conversion of methionine to cysteine. Methionine is converted into S-adenosylmethionine, which then loses a methyl moiety and becomes S-adenosyl-homocysteine, which finally hydrolyzes into homocysteine and adenosine. Homocysteine is metabolized via two pathways. The first one is remethylation, where homocysteine is reconverted into methionine. In this pathway, homocysteine acquires a methyl group either from the conversion of 5-methyltetrahydrofolate into hydrofolate (in which vitamin B12 is an important coenzyme) or from the conversion of betaine into N,N-dimethylglycine. The second pathway is transulfuration, where homocysteine is converted into cysteine. In this pathway homocysteine is attached to a serine moiety and forms cystathionine with the help of cystathionine β-synthase (CBS) and vitamin B6, which serve as an enzyme and coenzyme, respectively [1] (Figure 29.1).

Plasma homocysteine levels increase with aging, and are typically higher in men than women [2,3]. The reference range of plasma homocysteine may vary with the technique used. Reference values by age are as follows: 4.6–8.1 μmol/l for age <30 years; 6.3–11.2 μmol/l (males) and 4.5–7.9 μmol/l (females) for age 30–59 years; 5.8–11.9 μmol/l for age >59 years. Hyperhomocysteinemia has been arbitrarily defined as moderate (15–30 μmol/l), intermediate (30–100 μmol/l), and severe (>100–μmol/l) [4]. The reference value of urine homocysteine (measured by a 24-hour urine collection) ranges between 0 and 9 μmol per gram of urine creatinine [4].

29.2 VITAMINS B6, B9, AND B12

29.2.1 Vitamin B9

Folic acid ($C_{19}H_{19}N_7O_6$ or (2S)-2-[(4-{[(2-amino-4-hydroxypteridin-6-yl) methyl] amino} phenyl) formamido] pentanedioic acid, or folate, or vitamin B9, or pteroyl-L-glutamic acid) consists of the aromatic pteridine ring linked to para-aminobenzoic acid and glutamate. Folic acid, which is not biologically active *per se*, is converted to dihydrofolic acid in the liver, which in turn is converted to tetrahydrofolate. The human species cannot synthesize folic acid, and relies on diet for its supply. The main biological effect of folic acid is in DNA synthesis and methylation, hence promoting cell division and growth. It also serves as a co-factor in several biological pathways [5].

The term "folic" is derived from the Latin word *folium*, which means "leaf." Leafy vegetables such as spinach, asparagus, turnip greens, and lettuce contain large quantities of folic acid and constitute its main source. Other sources of folate include beans, peas and lentils, liver, kidney, and certain fruits such as orange, pineapple, and grapefruit. The dietary folate equivalent of folic acid is defined as 1 μg of dietary folate or 0.6 μg of folic acid supplement. Although most adults do not consume an adequate quantity of folic acid [6], deficiency is rather uncommon due to programs of folic acid fortification

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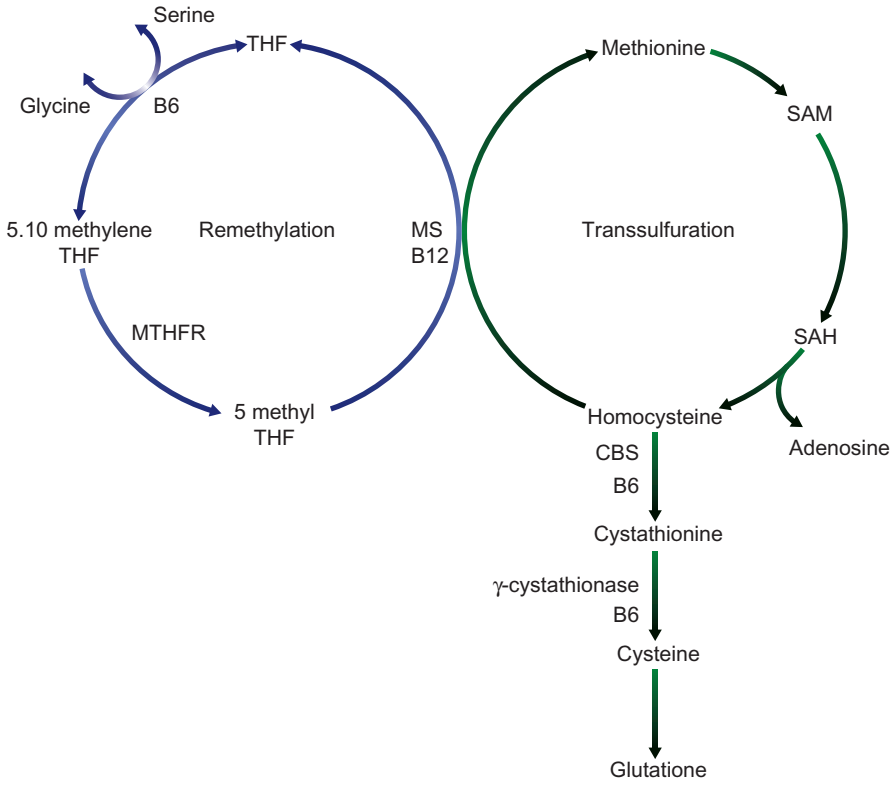

FIGURE 29.1 **Homocysteine metabolism.** THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; MS, methionine synthetase; SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine; CBS, cystathionine-synthetase.

of cereals and grains. Folic acid deficiency is associated with, among others, neural tube defects in embryos, and macrocytic anemia. The metabolism of folic acid constitutes a target for a large number of drugs. Such drugs include trimethoprim, sulfonamides, methotrexate, pyrimethamine, valproic acid, and others.

The serum concentration of folic acid largely reflects its short-term balance rather than its tissue stores. In this context, it was shown that a few days of decreased folate intake will reduce serum folate concentration despite the presence of normal tissue stores. A more reliable marker of tissue folate stores is its folate concentration in the red cell, which does not present the fluctuations of folic acid concentration in the serum; however, this examination is more expensive than the serum concentration measurement. As a screening approach, it was suggested that serum folate should be measured initially; if its levels are >9.1 nmol/l, then deficiency can be practically ruled out. On the other hand, levels <4.5 nmol/l are diagnostic of folic acid deficiency. For levels between 4.5 and 9.1 nmol/l, red-cell folate concentration could be measured [7].

29.2.2 Vitamin B12

Vitamin B12 (C63H88CoN14O14P, or α-(5,6-dimethylbenzimidazolyl) cobamidcyanide, or cobalamin) is structurally based on a corrin ring, which resembles the porphyrin ring found in heme, which contains cobalt. Vitamin B12 cannot be produced by animals or plants, but only by bacteria and archaea such as *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus*, *Propionibacterium*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcus*, and *Xanthomonas* [8]. It can be found in several forms, called vitamers, such as cyanocobalamin, hydroxycobalamin, lethylcobalamin and adenosylcobalamin, and in most dairy products, fish, meat, poultry, milk, and eggs. The adult dietary reference intake is 1.5–3 μg daily. Intestinal absorption of vitamin B12 may be compromised by several drugs, including ethanol, aminosalicylic acid, antibiotics, colchicine, colestipol, cholestyramine, H2 antagonists like ranitidine, metformin, proton-pump inhibitors like omeprazole, and zidovudine.

The reference values for vitamin B12 depend on the laboratory method used to assess it (i.e., chemiluminescence or radioassay). In practice, levels >221 pmol/l are considered normal, 148–221 pmol/l borderline, and <148 pmol/l low. A number of limitations should also be taken into consideration when interpreting vitamin B12 levels; for example, serum vitamin B12 levels may show a large intraindividual variation (>23% or >100 pmol/l)

[9]. For these reasons, and especially for patients with borderline vitamin B12 levels, the measurement of homocysteine and methylmalonic acid is more sensitive and reliable for the diagnosis of vitamin B12 deficiency [10,11]. Similarly, the measurement of these metabolites may be helpful to clarify whether folic acid or vitamin B12 is deficient when their levels are equivocal [12]. Serum homocysteine and serum and urine methylmalonic acid levels are increased in vitamin B12 deficiency. On the contrary, only homocysteine is elevated in folate deficiency, given that folic acid is not involved in the metabolic pathway of methylmalonic acid [11,13,14].

29.2.3 Vitamin B6

Vitamin B6 may be found in different forms (i.e., pyridoxine, pyridoxal, and pyridoxamine). It serves as a cofactor in several reactions of amino acid metabolism, such as transamination, deamination, decarboxylation, and gluconeogenesis. It is widely distributed in foods, particularly in meat, wholegrain products, and fruits. Vitamin B6 deficiency is rather rare due to its abundance in foods, and the mechanism of its absorption in jejunum and ileum (passive diffusion) which allows absorption of more vitamin B6 than is needed. The recommended daily dose is 1.3–1.7 mg.

29.3 CAUSES OF HYPERHOMOCYSTEINEMIA

Normal reference values for homocysteine are between 5 and 15 μmol/l. Several conditions are associated with hyperhomocysteinemia: the most frequent genetic defect is a mutation of the methyl-tetrahydrofolate reductase (MTHFR) enzyme, which leads to a 677C→T substitution and a thermolabile variant of the enzyme with reduced activity; this defect is associated with only mild hyperhomocysteinemia in its homozygous state [15]. In population-based studies, the frequency of heterozygocity of this enzymatic defect ranges between 5% and 14%. On the other hand, homozygous deficiency of cystathionine β-synthase (CBS) is associated with severe hyperhomocysteinemia up to 40-fold higher than normal [16,17]. The D919G mutation in the methionine synthase gene presents with hyperhomocysteinemia (200–400 μmol/l), mental retardation, skeletal malformations, and premature atherosclerosis [18].

Homocysteine levels increase with increasing age, probably due to decrease in the activity of CBS [19]. Homocysteine is also higher in males than in premenopausal females; this difference is also present in postmenopausal women, albeit slighter [20]. Diet, and particularly the amount of methionine intake, is also directly associated with homocysteine levels. Hence, homocysteine levels are low in persons with low intake of animal proteins; on the contrary, homocysteine levels are inversely related to vitamin intake and tend to be lower in persons with a diet rich in fruits and vegetables [21], and increased in patients with deficiency of folic acid, B6, and B12 [22,23]. Alcohol increases serum homocysteine, possibly due to interference with the metabolism of folic acid. Several drugs cause hyperhomocysteinemia, such as cyclosporine, methotrexate, fibrates, and L-dopa [24]. Serum homocysteine is directly associated with renal function, and patients with end-stage renal disease present with significant hyperhomocysteinemia [25]. Other causes of hyperhomocysteinemia include leukemia [26], psoriasis [27], sickle cell anemia [28], polycythemia vera, and idiopathic thrombocytosis [29].

29.4 ATHEROTHROMBOTIC EFFECT OF HOMOCYSTEINE

Homocysteine is associated with atherogenic and prothrombotic effects. Homocysteine-induced histopathologic findings include thickness of the intima layer, disruption of the elastic lamina, smooth muscle hypertrophy, platelet aggregation, and the white thrombus formation [30–33]. These changes may be mediated by several pathophysiologic mechanisms: upregulation of the monocyte chemoattractant protein-1 and interleukin-8 expression and secretion, and subsequent leukocyte recruitment [34]; binding of thiolactone (a homocysteine metabolite) with low density lipoprotein (LDL) cholesterol to form aggregates, which are phagocytozed by macrophages in tunica intima that in turn enrich the atherosclerotic plaques with lipid [35]; smooth muscle cell proliferation and increased collagen production [36,37]; attenuation of endothelial tissue plasminogen activator binding sites; increased blood viscosity; protein C inhibition; factors VIIa and V activation; increased fibrinopeptide A and prothrombin fragments 1 and 2; decreased endothelial antithrombotic activity [38]; increased oxidative stress [39]; platelet aggregation [40]; and downregulation of the dimethylarginine dimethylaminohydrolase, which results in inhibition of the nitric oxide synthase and impaired production of nitric oxide and impaired endothelium-dependent vasodilation [40].

29.5 ASSOCIATION OF HOMOCYSTEINE WITH INCREASED CARDIOVASCULAR RISK

A few decades ago, McCully suggested that increased homocysteine levels are associated with vascular disease, after observing advanced arterial lesions in two children with homocysteinuria (due to inborn errors of methionine metabolism) who died following ischemic stroke

[30]. Soon after this observation, many epidemiological studies associated hyperhomocysteinemia with increased risk for vascular events such as myocardial infarction, stroke, carotid artery stenosis, venous thromboembolism, and adverse outcomes after angioplasty [41–43].

In 1999, the Perth Carotid Ultrasound Disease Assessment Study (CUDAS) associated serum homocysteine with carotid intima media thickness (IMT) in an asymptomatic population. After adjustment for age, sex, and other conventional risk factors, subjects in the highest versus the lowest quartile of homocysteine had an odds ratio of 2.60 for increased IMT and 1.76 for carotid artery plaque [44].

Another study, performed on 133 asymptomatic patients, correlated homocysteine levels with the progression of atherosclerosis within an 84-month period using an electron-beam computed tomography (EBT) calcium score. Individuals with elevated homocysteine levels (≥12 μmol/l) showed a mean increase in coronary calcium progression of 35% per year, compared to 17% per year in patients with homocysteine levels <12 μmol/l ($P = 0.0008$) [45].

A meta-analysis concluded that elevation of plasma homocysteine levels by 25% was associated with about 10% higher risk of cardiovascular events and 20% higher risk of stroke. In this context, hyperhomocysteinemia could be responsible for about 10% of total cardiovascular risk, and additional risk factors (smoking, arterial hypertension, diabetes mellitus, and dyslipidemia) may act synergistically and increase overall cardiovascular risk. It was estimated that an increase in homocysteine levels of 5 mmol/l conveys an increase in cardiovascular risk similar to an increase in cholesterol levels of 20 mmol/l. Also, a 5-μmol/l increase in homocysteine levels was associated with a relative risk of 1.5 (95% CI: 1.3–1.9) for ischemic stroke, and 1.6 (95% CI: 1.4–1.7) and 1.8 (95% CI: 1.3–1.9) for coronary heart disease in males and females, respectively [46]. Similarly, meta-analysis of the Homocysteine Studies Collaboration showed that a 3-μmol/l decrease in serum homocysteine was linked with a 24% and 16% reduction in the incidence of ischemic stroke and coronary heart disease, respectively, whereas a 25% reduction of homocysteine levels reduces the aforementioned risks by 19% and 11%, respectively [47]. Based on these findings, hyperhomocysteinemia was considered as an independent cardiovascular risk factor; the next challenge was to identify safe and effective ways to modify this risk factor by reducing its levels.

29.6 VITAMIN B SUPPLEMENTATION TO DECREASE HYPERHOMOCYSTEINEMIA

Folic acid and vitamins B12 and B6 are the main determinants of homocysteine levels. Numerous interventional studies investigated the role of B vitamin supplementation to reduce homocysteine [21,48–53]. Supplementation with folate, vitamin B12, vitamin B6, or a combination of all three was shown to reduce homocysteine levels within 6 weeks by 42%, 5%, 12%, and 50%, respectively. The decrease of homocysteine levels with folate supplementation was not significantly different than the combination of the three vitamins [54]. In the PACIFIC (Prevention with A Combined Inhibitor and Folic Acid In Coronary Heart Disease) study, 723 patients were assigned to folic acid (2 or 0.2 mg/day) or placebo. The decrease in serum homocysteine was significantly greater at the higher dose (16% vs 11%, 1.8 vs 1.2 μmol/l, respectively) [42].

In 2005, a meta-analysis of the Homocysteine Lowering Trialists' Collaboration included 25 randomized trials and 2596 patients to test the efficacy of B vitamins in reducing homocysteine levels [55]. It was shown that homocysteine levels decreased by 13% (95% CI: 10–16) and 25% (95% CI: 22–28) after 8 months' supplementation with 0.2 mg and 5 mg of folic acid, respectively. Vitamin B12 offered a further 5% reduction in homocysteine levels, whereas vitamin B6 had no significant additional effect. The beneficial effect of folic acid on homocysteine levels was further confirmed by the finding that mean homocysteine concentration and prevalence of hyperhomocysteinemia (defined as levels above 13 μmol/l) decreased in the USA from 10.1 to 9.4 μmol/l and from 18.7% to 9.8%, respectively, after the implementation of mandatory fortification of cereals with folic acid in 1998 [56].

29.7 SAFETY OF VITAMIN B SUPPLEMENTATION

Early epidemiological studies identified a reciprocal association between B vitamin supplementation and colorectal cancer [57]. Surprisingly, three randomized trials not only failed to confirm this finding but also implied a positive association between cancer and folic acid supplementation: in the Aspirin and Folic Acid Polyp Prevention Study (AFPPS), colorectal adenomas and prostate cancer incidence increased during 7 years of treatment with folic acid [58]. Another study suggested that a transient increase in colorectal cancer incidence in Canada and the USA between 1996 and 1998 could be causally linked to the 1996–1998 introduction of folic acid fortification [59]. However, a recent individual-participant-data meta-analysis on 49,621 individuals from 13 trials failed to show an association of folic acid supplementation and cancer: during a mean follow-up of 5.2 years, folic acid supplementation had no significant effect on overall cancer incidence (relative risk (RR): 1.06, 95% CI: 0.99–1.13) of large intestine, prostate, lung, breast or any other specific site [60].

In another meta-analysis on individual participant data of 37,485 individuals at increased risk of cardiovascular disease from eight large, randomized, placebo-controlled trials of folic acid supplementation, there was no significant effect on the risk ratios (95% confidence intervals) for overall cancer incidence (1.05 [0.98–1.13]), cancer mortality (1.00 [0.85–1.18]), or all-cause mortality (1.02 [0.97–1.08]) after a median follow-up of 5 years [61].

29.8 EFFECT OF VITAMIN B SUPPLEMENTATION ON CARDIOVASCULAR SURROGATE MARKERS

When evidence came available that B vitamins decrease homocysteine levels, several intervention trials were designed and initiated to show whether supplementation of folic acid improves cardiovascular surrogate markers such as carotid intima media thickness (IMT) and flow-mediated dilation (FMD).

In a study in patients with increased cardiovascular risk, supplementation of 5 mg folic acid daily over an 18-month period significantly reduced carotid IMT (0.961 ± 0.092 to 0.933 ± 0.077 mm) compared to significant IMT progression in the placebo group (0.964 ± 0.099 to 0.984 ± 0.094 mm) [62]. Similarly, Till *et al*. [63] concluded that supplementation with folic acid, B12, and B6 over a 1-year period resulted in significant reduction of IMT (1.50 ± 0.44 to 1.42 ± 0.48 mm) in patients at risk for cerebral ischemia (IMT ≥1 mm), whereas IMT increased in placebo-controlled patients (1.47 ± 0.57 mm to 1.54 ± 0.71 mm) [63]. Similar results were reported by Marcucci *et al*. [64] in patients post-renal transplantation, after 6 months of B vitamin supplementation. Vianna *et al*. [65] demonstrated significant IMT reduction in hyperhomocysteinemic patients with end-stage renal disease after 2 years of intermittent (10 mg, three times a week) folic acid supplementation.

On the contrary, in a sub-study of the VITATOPS (Vitamins To Prevent Stroke) trial, the post-treatment IMT (0.84 ± 0.17 mm vitamins versus 0.83 ± 0.18 mm placebo, $P = 0.74$) and FMD (median of 4.0%, IQR 0.9–7.2 in the vitamins group versus 3.0%, IQR 0.6–6.6 in the placebo group, $P = 0.48$) did not differ significantly between groups after a mean treatment period of 3.9 ± 0.9 years [66]. Also, in the ASFAST trial, supplementation with high-dose folic acid for a median duration of 3.6 years was not associated with a significant change of IMT in 315 patients with chronic renal failure compared to controls [67]. In another study, there was no significant change of IMT in patients with coronary artery disease treated with a high dose of folic acid (5 mg) for 3 years, compared to controls [68].

29.9 EFFECT OF VITAMIN B SUPPLEMENTATION ON CARDIOVASCULAR CLINICAL OUTCOMES

In addition to the aforementioned studies which used surrogate markers as endpoints to evaluate the effect of B vitamin supplementation, a number of randomized controlled trials were also designed and performed to assess their effect on hard clinical cardiovascular outcomes such as mortality, stroke, and coronary heart events [69]. These trials had different designs with regard to the population studied (fortified or not), the dose of B vitamins, concomitant diseases (stroke or coronary heart disease or end-stage renal disease), and the duration of treatment.

In the CHAOS-2 (Second Cambridge Anti-Oxidant Heart Study) trial, which was published in 2002, the investigators studied 1882 patients with coronary heart disease for a median of 1.7 years. The trial was terminated prematurely. After a daily dose of 5 mg folic acid, homocysteine levels decreased from 11.2 ± 6.9 μmol/l to 9.7 ± 5.3 μmol/l in the active treatment group; however, there was no reduction in the composite endpoint of non-fatal myocardial infarction, cardiovascular death, or unplanned revascularization (RR: 0.97, 95% CI: 0.72–1.29) [70].

The Vitamin Intervention for Stroke Prevention (VISP) trial was a double-blind, randomized controlled trial which was performed between September 1996 and May 2003 in 3680 patients with non-disabling stroke in 56 centers in Canada, the USA, and Scotland. Patients were randomized to receive once-daily doses of a high-dose formulation of B vitamins containing 25 mg pyridoxine, 0.4 mg cobalamin, and 2.5 mg folic acid, or a low-dose formulation containing 200 μg pyridoxine, 6 μg cobalamin, and 20 μg folic acid for 2 years. Homocysteine levels decreased by approximately 2 μmol/l in the high-dose group compared to the low-dose group. However, like the CHAOS-2 trial, VISP was terminated prematurely because the chance of showing any difference between the treatment groups in the remaining follow-up period was close to nil. There was no statistical difference in stroke recurrence rate at the end of the follow-up period (9.2% in the high-dose group, 8.8% in the low-dose group) [71]. The small difference in homocysteine levels between the two groups of the trial is likely be due to grain fortification with folate since the inception of the VISP trial, which may have reduced the statistical power of the trial [72,73]. The lower than expected rates of recurrent strokes in both groups and the relatively short follow-up period also may have limited its statistical power to identify a modest, but clinically important, cardiovascular effect of B vitamins [72,73].

The NORVIT (Norwegian Vitamin) trial randomized patients with recent myocardial infarction to one of the following four daily treatments for a mean duration of 40 months: 0.8 mg folic acid, 0.4 mg vitamin B12, and 40 mg vitamin B6; 0.8 mg folic acid and 0.4 mg vitamin B12; 40 mg vitamin B6; or placebo. There was no difference regarding the primary endpoint of composite recurrent myocardial infarction, stroke, and sudden death attributed to coronary artery disease (RR: 1.08, 95% CI: 0.93–1.25, $P < 0.31$) despite a 27% homocysteine reduction in patients receiving folic acid plus vitamin B12. The negative results of the trial should not be attributed to reduced compliance or power of the study; although slightly underpowered (compared to the original design of the study), the NORVIT had still an 80% power to detect an 18% reduction of primary endpoints. An explanation for the negative results of the trial could be that mean baseline homocysteine levels were within normal range; however, even patients in the upper quintile of baseline homocysteine (≥19.7 μmol/l) showed no benefit [74].

The HOPE-2 (Heart Outcome Prevention Evaluation-2) trial was published simultaneously with the NORVIT trial, and also reported negative results. The trial included 5522 patients with a history of vascular disease or diabetes who were randomized to receive daily treatment for 5 years with 2.5 mg folic acid, 50 mg vitamin B6 and 1 mg vitamin B12, or with placebo. Of the patients, 72.1% came from countries with mandatory fortification of cereals with folic acid. The study failed to identify a beneficial effect of B vitamins on primary outcome events and cardiovascular mortality. However, a significant 24% reduction of stroke risk (but not transient ischemic attacks) was demonstrated in the active treatment group (RR: 0.75; 95% CI: 0.59–0.97) [75].

The HOST (Homocysteinemia in Kidney and End Stage Renal Disease) trial was the first trial to investigate the role of B vitamin supplementation in patients with advanced chronic kidney disease or end-stage renal disease. The trial included 2056 participants who were enrolled between 2001 and 2006 in 36 centers in the USA and followed up for 32 months; they were randomized to receive a daily capsule containing 40 mg folic acid, 100 mg vitamin B6 and 2 mg vitamin B12, or a placebo. Despite a 25.8% reduction in homocysteine in the active treatment group, there was no benefit on primary (all-cause mortality) or secondary endpoints (stroke or myocardial infarction). As the investigators suggested, the negative outcome of the trial could perhaps be attributed to the fact that post-treatment homocysteine reached normal levels only in about one-third of patients, despite the administration of the highest vitamin doses among all homocysteine-lowering studies [76].

The WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study) randomized 5442 women in the USA with a history of cardiovascular disease or more than three cardiovascular risk factors to receive 2.5 mg folic acid, 50 mg vitamin B6 and 1 mg vitamin B12, or a placebo, for a median of 7.3 years. The trial failed to show a significant difference between the two treatment arms in myocardial infarction, stroke, coronary revascularization, or cardiovascular mortality, despite an 18.5% decrease in homocysteine levels. Similar to the VISP trial, the implementation of mandatory fortification of cereals with folic acid might may have underpowered the study [77].

In the WENBIT (Western Norway B Vitamin Intervention Trial) trial, 3096 patients undergoing coronary angiography were randomized to one of the four following treatments for a median duration of 38 months: daily oral supplementation with 0.8 mg folic acid, 0.4 mg vitamin B12 and 40 mg vitamin B6; folic acid plus vitamin B12; vitamin B6 alone; or with placebo. The trial was prematurely terminated early after the concerns raised by the NORVIT trial. The WENBIT trial failed to demonstrate a beneficial effect of B vitamins on total mortality or cardiovascular events. Of course, the trial was less powered than originally planned due to lower event rates and shorter follow-up. However, it still had an 80% power to detect a 24% reduction in the risk of composite of all-cause death, non-fatal acute myocardial infarction, acute hospitalization for unstable angina pectoris, and non-fatal thromboembolic stroke [78]. A possible explanation for the negative outcome of the trial could perhaps be that only 9.6% of participants were hyperhomocysteinemic [79]. Hence, despite a 30% homocysteine reduction in the treatment group, for most patients homocysteine was reduced to a lower value from a higher but still normal value [78].

In the SU.FOL.OM3 (Supplementation with Folate, Vitamins B6 and B12, and/or Omega-3 Fatty Acids Randomized Trial), 2501 patients with a history of myocardial infarction, unstable angina, or ischemic stroke were randomized to 560 mg folate, 3 mg vitamin B6, 20 mg vitamin B12, or placebo for a mean of 56 months. The trial showed that there was no significant difference on major vascular events (hazard ratio: 0.90; 95% CI: 0.66–1.23) [80].

In the VITATOPS (Vitamins to Prevent Stroke) trial, 8164 patients with recent stroke or transient ischemic attack (within the past 7 months) from 123 medical centers in 20 countries were randomized to 2 mg folic acid, 25 mg vitamin B6, 0.5 mg vitamin B12, or placebo, for a mean of 41 months. There was no difference between the two treatment arms in the composite outcome of stroke, myocardial infarction, or vascular death (RR: 0.91; 95% CI: 0.82–1.00; $P = 0.05$; absolute risk reduction 1.56%, −0.01 to 3.16). However, the trial was limited by incomplete adherence to trial drugs and incomplete follow-up. Also, the median duration of adherence to treatment and follow-up was 2.8 years and 3.4 years, which might not

have been long enough to identify a long-term benefit of B vitamins [81].

In the SEARCH (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) trial, 12,064 patients with myocardial infarction were randomized to 2 mg folic acid, 1 mg vitamin B12, or placebo for a median of 80 months. There was no significant difference between the two treatment arms in any endpoint considered (major coronary event [coronary death, myocardial infarction, or coronary revascularization], fatal or non-fatal stroke, or non-coronary revascularization) [82].

In the Linxian trial, 3318 men and women were randomized to 0.8 mg folic acid, 6 mg vitamin B6, and 18 mg vitamin B12 for a median of 72 months. There was no significant difference in mortality between the two arms (RR: 0.93; 95% CI: 0.75–1.16) [83].

In the FAVORIT (Folic Acid for Vascular Outcome Reduction in Transplantation) trial, 4110 stable kidney transplant recipients were randomized to a high dose or low dose of folic acid, vitamin B6, and vitamin B12 for a median of 48 months. High-dose treatment did not reduce the rates of the primary outcome (hazards ratio [95% CI: 0.99 (0.84–1.17]), secondary outcomes of all-cause mortality (1.04 [0.86–1.26]), or dialysis-dependent kidney failure (1.15 [0.93–1.43]) compared to the low-dose group [84].

In another study, 650 patients with end-stage renal disease on hemodialysis were randomized to two post-dialysis treatments: 5 mg folic acid, 50 g vitamin B12, and 20 mg vitamin B6, or 0.2 mg folic acid, 4 g vitamin B12, and 1.0 mg vitamin B6, given three times per week for 2 years. There was no significant difference in overall mortality (hazard ratio: 1.13; 95% CI: 0.85–1.50) or in cardiovascular events (hazard ratio: 0.80, 95% CI: 0.60–1.07) [85].

29.10 META-ANALYSES OF LARGE RANDOMIZED CLINICAL TRIALS OF VITAMIN B SUPPLEMENTATION

In a recent meta-analysis of 14 randomized controlled trials (published before 2012) and 54,913 participants, B vitamin supplementation was shown to significantly reduce stroke events (RR: 0.93; 95% CI: 0.86–1.00; $P = 0.04$), especially in patients with specific characteristics (such as those living in countries without cereal folate fortification, or with chronic kidney disease) who received appropriate intervention measures [86].

Another meta-analysis including 17 trials and 39,107 patients with pre-existing cardio-cerebrovascular or renal disease failed to show any significant difference between the intervention and control groups. The overall relative risks (95% confidence intervals) of outcomes for patients treated with B vitamins supplementation compared with controls were 1.01 (0.97–1.05) for cardiovascular events, 1.01 (0.94–1.07) for coronary heart disease, 0.94 (0.85–1.04) for stroke, and 1.00 (0.95–1.05) for overall mortality. When low-quality trials and trials in grain fortification countries were excluded, the overall results did not change [87].

In another meta-analysis on individual participant data of 37,485 individuals at increased risk of cardiovascular disease from eight large, randomized, placebo-controlled trials of folic acid supplementation, there was no significant effect on vascular outcomes after a median follow-up of 5 years, with risk ratios (95% confidence intervals) of 1.01 (0.97–1.05) for major vascular events, 1.03 (0.97–1.10) for major coronary events, and 0.96 (0.87–1.06) for stroke.

29.11 VITAMIN B SUPPLEMENTATION AND CARDIOVASCULAR RISK: WHAT WENT WRONG?

Several reasons may explain the conflicting/negative results of the trials: heterogeneity of populations (e.g., non-disabling cerebral infarction in VISP, recent myocardial infarction in NORVIT, diabetes mellitus or vascular disease in HOPE-2), short to moderate duration (20–88 months), and inclusion of patients with normal homocysteine levels. Moreover, folic acid induces the remethylation of homocysteine to methionine with increasing S-adenosyl methionine, which leads to increasing ADMA levels that may inhibit eNOS. In addition, enhancing the methylation pathway affects expression of several proatherogenic genes [88,89].

29.12 SCREENING FOR HYPERHOMOCYSTEINEMIA: IS IT NECESSARY?

Observational studies have established an association between homocysteine levels and cerebrovascular, peripheral vascular, coronary artery, and venous thromboembolic disease. However, interventional trials with B vitamin supplementation have failed to identify a potential role for reduction of homocysteine in the prevention of vascular disease. Therefore, even if hyperhomocysteinemia was to be diagnosed through a screening program, it is still doubtful whether it could be used for planning preventive strategies in the individual patient.

29.13 CURRENT STATUS

The American Stroke Association guidelines for stroke prevention suggest that the use of pyridoxine (vitamin

B6), cobalamin (vitamin B12), and folic acid might be considered for prevention of ischemic stroke in patients with hyperhomocysteinemia, but its effectiveness is not well established [90].

At present, more than 60 countries have introduced mandatory flour and grain fortification with folic acid to prevent neural tube defects. In the USA, grain products have been fortified with folic acid since 1998 in accordance with an FDA regulation. This was designed so that the typical daily folate intake would increase by about 100 µg, whereas the risk of a daily intake >1000 µg (which is the FDA's safe upper limit of daily intake) would be minimal. The prevalence of hyperhomocysteinemia (defined as serum homocysteine above 13 µmol/l) decreased from 18.7% before fortification to 9.8% after fortification [73,91,92].

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CHAPTER

30

Changes in Postprandial Blood Pressure in the Elderly

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30.1 INTRODUCTION

A number of physiologic modifications can occur during the digestive process before, during, and after eating. Many hormones are released into the bloodstream, thereby activating the sympathetic and parasympathetic systems. Blood vessels are stimulated in different manners based on their location in the body. In the middle of this "storm," arterial blood pressure must remain stable. In healthy people, blood pressure levels normalize in microseconds following food stimulus. However, many people do not have stable blood pressure under normal conditions. In such cases, sometimes after eating, parts of the integrated neurologic and endocrine systems can fail, and blood pressure levels do not remain at the previous level (i.e., prior to ingesting food). Such individuals might have postprandial hypotension (PPH).

PPH is a common clinically relevant disorder in elderly patients, and has been associated with falls, syncope, coronary events, stroke, and all-cause mortality in long-term follow-ups [1–3]. PPH has been defined as a 20-mmHg decrease in systolic blood pressure (SBP), or a 10-mmHg decrease in diastolic levels, or a 5-mmHg reduction in mean arterial blood pressure within 2 hours after a meal. The mechanism of PPH is not fully understood. Inadequate sympathetic nervous system compensation from the splanchnic vasodilation induced by meals, baroreflex function impairment, reduced vasoconstriction in skin vessels, insulin-induced vasodilation beyond the necessary, and gastrointestinal peptides with systemic actions may be involved in PPH [4].

On the other hand, aging is associated with several biological changes in the digestive tract, including changes in chewing, salivary flow, and gastric acidity, as well as functional disturbances in the liver and pancreas. These changes in both the endocrine and exocrine systems are barely perceptible in the gastrointestinal tract, because the secretory and absorptive ability of digestive tract cells is so high that clinically detectable manifestations occur only after normal function is reduced by at least 10%. Because the digestive system is also considered an endocrine system, elderly individuals' ability to secrete gastrointestinal hormones (GIH) needs to be properly evaluated. It is important to separate the changes resulting from aging itself from changes that arise from the diseases that affect individuals in this age group.

30.2 GASTROINTESTINAL HORMONES COULD BE CONTRIBUTING TO PPH

The gastrointestinal tract (GI) is the largest endocrine organ in the body, and its hormones were the first to be discovered [5]. These hormones are released by stomach and intestinal mucosae, and many of them exert actions outside of the intestinal tract, such as within the cardiovascular system. Some gastrointestinal hormones (GIH) could play a role in PPH because of their actions in systemic hemodynamics.

Gastrointestinal hormones are distributed throughout the digestive tract, but mainly in the small intestine. The small intestine is considered to be the largest endocrine organ in the human body. Although its products are classified as hormones, they do not always function as substances that target cells at distant locations after they are released into the bloodstream. In fact, these peptides

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are often considered to be paracrine or autocrine, and may also serve as neurotransmitters. Below, we list some major GIH with vasoactive properties.

30.2.1 Vasoactive Intestinal Polypeptide

Vasoactive intestinal polypeptide (VIP) is a powerful vasodilator that increases intestinal blood flow and promotes relaxation in the smooth muscles of the vessels and in the secretions of digestive epithelial cells. Significant concentrations of VIP are present in the gastrointestinal tract, heart, lungs, thyroid, kidney, urinary bladder, genital organs, and brain. The vasodilatory effect of VIP in different vascular tissues or species also may be caused by increases in nitric oxide, cyclic GMP, and other signaling agents. VIP belongs to a family of intestinal peptides that also includes glucagon and secretin. Combined with nitric oxide (NO), it is a non-adrenergic and non-cholinergic component of nervous transmission in the intestines. When this peptide is infused directly into the coronary circulation in humans, it reduces coronary vascular resistance by 46% compared to pretreatment values [6]. Recent studies have shown that VIP is able to increase coronary flow and ventricular contractile strength, and helps to reduce mean arterial blood pressure. On an equimolar basis, this peptide has a vasodilatory action that is 50- to 100-fold more potent than that of acetylcholine [7]. VIP is released in response to nerve stimulation, cholinergic agonists, serotonin, dopamine agonists, prostaglandins PGE and PGD, and growth factors [7].

30.2.2 Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide (CGRP) is a neuropeptide that is produced by enteroendocrine cells in the small intestine. It is released in response to glucose and gastric acid secretion, and can cause marked vasodilation of the vessels in the stomach, splanchnic region, and peripheral circulation. Its action appears to be through the release of NO. It is part of the peptide family that includes calcitonin, amylin, and adrenomedullin [8].

30.2.3 Neuropeptide Y

Neuropeptide Y (NPY) is synthesized and secreted by pancreatic cells in response to impulses from neurons in the central and peripheral nervous systems. It inhibits glucose-stimulated insulin secretion, and is present in the myenteric and submucosal nervous plexuses in the digestive tract. Increases in NPY levels are observed after sympathetic stimulation. Intravascular administration of NPY is associated with marked vasoconstriction of the splanchnic circulation; however, this effect is not affected by adrenergic blockers. In healthy individuals the infusion of progressively higher NPY doses results in increased SAP, but myocardial perfusion, cardiac output, and pulmonary artery pressure remain unchanged [9].

30.2.4 Other Hormones

The majority of hormones secreted during the digestive process can also indirectly alter hemodynamics by affecting the absorption of liquids and electrolytes, thereby modifying volemia and responses to sympathetic and parasympathetic stimulations. Some of these hormones are involved in regulating the release of other GIH and affect the speed of gastric emptying and intestinal motility by increasing or decreasing the absorption time for liquids and nutrients. Amylin, galanin, gastrin, ghrelin, somatostatin, glucagon, glucagon-like peptide-1, and glucagon-like peptide-2 are some of the hormones that are secreted after food ingestion.

In particular, insulin can contribute to important cardiocirculatory changes following food intake. Insulin, a hormone secreted by beta pancreatic cells, helps to metabolize glucose and inhibit glycogenolysis and gluconeogenesis. It also increases the transport of glucose into fat and muscle tissue, increases the glycolysis in these tissues, and stimulates glycogen synthesis. Insulin exhibits vasodilatory properties through the endothelial production of NO [10]. In addition to this vasodilatory action, insulin has the capacity to influence sodium retention by acting directly on the kidney's proximal convoluted tubule [11]. The impaired vascular actions of insulin are also related to obesity, systemic inflammatory status, and metabolic syndrome.

Recent studies of glucagon-like peptide-1 (GLP-1) have shown that it can act similarly to insulin as a microvascular vasodilator in skeletal muscles, and GLP-1 appears to regulate myocardial perfusion [12,13].

30.3 MODIFICATIONS IN AUTONOMIC NERVOUS SYSTEM

Another important system control in regulating postprandial blood pressure is the autonomic nervous system. The integrity of this system is essential to controlling BP levels after eating a meal. Normally, the aging process delays the nervous responses after any stimulation, including the stimulus promoted by eating foods.

To maintain blood pressure, a variety of hemodynamic changes are necessary, including increases in the heart rate, stroke volume, and cardiac output. These responses are partially mediated by a compensatory sympathetic activation, as shown by the increase in plasma norepinephrine and muscle sympathetic nerve activity after food intake in normal subjects. The failure of these compensatory mechanisms appears to be

pivotal in the development of PPH, which explains the greater prevalence of this condition in subjects with autonomic impairment.

In response to food intake, splanchnic vessels dilate and the blood flow in the mesenteric artery increases; to maintain the blood pressure levels, other vascular beds must show vasoconstriction. Skin and striated muscles are the preferential targets to increase vessel resistance. The gastric distension plays an important role in activating a non-baroreceptor that induces the pressor effect on muscle system nervous stimulation.

30.4 TYPES OF FOOD AND PPH

Because of the aging effect on the endocrine and autonomic systems, normotensive elderly individuals can experience different blood pressure changes after eating compared to young people. For example, mean arterial blood pressure (MAP) values decreased in elderly people after eating a meal rich in lipid and carbohydrate contents, and this decrease could be related to a decrease in total peripheral vascular resistance. At rest, the splanchnic circulation comprises 25–30% of the total vascular conductance; thus, changes in splanchnic vascular conductance have a significant influence on blood pressure. The decrease in splanchnic resistance could be secondary to the vasodilatation of splanchnic vessels after ingesting foods. Concomitantly, an increase in cardiac output, stroke volume, and heart rate can be observed; however, these hemodynamic variable changes in elderly people could not maintain blood pressure levels at the pre-meal baseline values. Conversely, in young people MAP can be maintained without significant reductions after eating lipids and carbohydrates, but there are also increases in CI, SI, and HR. For young people, the increases in these variables are sufficient to maintain BP at the baseline levels [14].

Regarding the ingestion of protein meals, mean blood pressure and peripheral resistance do not appear to change from the pre-meal values in normotensive elderly and young people. Simultaneously, no changes in CI, SI, and HR can be observed in either group. We had already observed the same hemodynamic behavior in hypertensive elderly patients after eating PM [15]. Some specific foods can induce changes in cardiac function in normotensive or hypertensive elderly individuals. Jansen *et al*. [4] reported that the depressor effect on BP is greater after the ingestion of carbohydrates, particularly glucose, than after the ingestion of fat, protein, or water; however, the authors did not verify the components of the cardiac function, such as peripheral resistance and CI. Conversely, Hoost and colleagues reported observing increases in CI and HR in young people after a meal with a high protein meal content. The authors

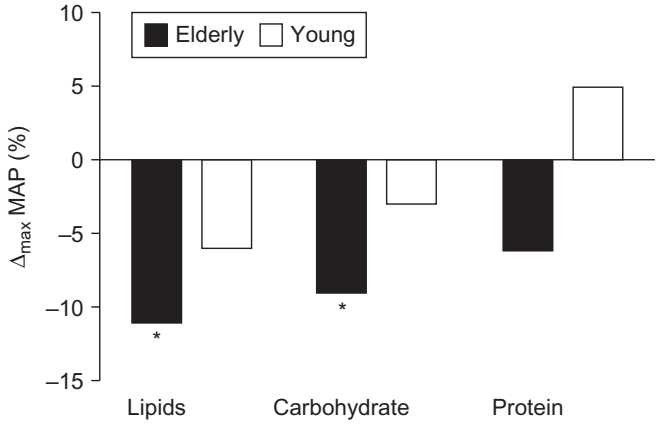

FIGURE 30.1 Maximal percentage changes (%) in MAP in relation to the basal levels after ingestion of meals with lipid (LM), carbohydrates (CM), and protein (PM). MAP, mean arterial pressure. *P < 0.05. *Reproduced from Ferreira-Filho* et al. *[14],* Blood Pressure Monitoring *2012;17:110–5.*

used radionuclide cardiography and plethysmography to calculate the values of these variables [16]. However, we have observed that protein ingestion does not appear to induce significant changes in MAP after food intake. Figure 30.1 shows the maximum change in BP and represents the highest percentage of MAP fluctuation in normotensive elderly and young individuals during the 60 minutes when each meal was consumed [14].

In the vast majority of cases studied, PPH is associated with patients with autonomic failure; however, patients with dopamine beta hydroxylase deficiencies (i.e., with their sympathetic nervous system intact and without the capacity to synthesize norepinephrine) do not present with PPH. This suggests that other transmitters or co-transmitters may block the hypotensive response after meals. Dopamine's vasoconstrictive effects on the splanchnic circulation represent an important way of maintaining systolic arterial pressure after eating. Dopamine antagonists such as metoclopramide reduce SAP, which indicates the presence of a dopaminergic pressor effect. Practical recommendations for controlling PPH have included division of meals and small daily portions to avoid major drops in SAP, but the caloric content and the type of food offered at each meal may be critical in episodes of PPH. Furthermore, splitting of meals could prolong hypotensive episodes.

Hemodynamic changes in PPH are different from those reported in what is called the "dumping syndrome" (DS). In DS, an observed drop in plasma volume and a large increase in the flow through the superior mesenteric artery along with an increase in sympathetic activity results in tachycardia, sweating, and other signs

of a sympathetic aid response to maintain systemic pressure levels [17]. In elderly individuals specifically, there are no reports in the literature comparing the differences in both the hemodynamic and clinical presentations of these two syndromes.

30.5 TREATMENT OPTIONS

A few non-pharmacological and pharmacological interventions have been advocated to prevent the development of PPH. In mild cases, reduced meal sizes or carbohydrate/lipid contents may be useful. However, carbohydrate reduction is difficult because carbohydrates represent 45–65% of the normal Western diet. However, increasing the protein content of each meal could be beneficial for some patients. In the end stage of chronic kidney disease, increased protein ingestion must be carefully monitored. Severe cases are challenging to treat; these patients can be symptomatic even while supine, and have a higher risk of syncope if they stand up after meals. Hence, a pharmacological intervention is often necessary.

The ingestion of water can cause changes in some systemic hemodynamic variables. In contrast to individuals who consume proteins, carbohydrates, and lipids, which reduce TPR and increase CO, individuals drinking water exhibit what is called the gastropressor response. This response consists of systemic vasoconstriction and, consequently, an increase in SAP. Moreover, the HR does not increase. Some authors attribute these effects to gastric distension with increased sympathetic activity, or to increased vagal activity on the heart [18]. Other studies suggest that the hypo-osmolar environment of the digestive tract after the ingestion of water could be responsible for the resulting gastropressor effect.

Three different pharmacological approaches have been used to improve PPH. One approach has been to increase the baseline sympathetic nervous system with 3,4-DL-threodihydroxyphenylserine before meal ingestion. Another solution has been to block the release of gastrointestinal and pancreatic hormones with octreotide. A third approach has been to antagonize the effect of vasodilators, such as adenosine, with caffeine. Although these drugs appear to ameliorate PPH, their use is limited by aspects of their clinical pharmacology, modes of application, and adverse effects.

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CHAPTER

31

Diet Modification After Acute Coronary Events

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31.1 INTRODUCTION

Many patients who have suffered a heart attack now survive, but are at high risk of having a repeat event. The targeted treatment of heart attack survivors to prevent future heart attacks and premature death is referred to as secondary prevention, and there is an extensive literature and guidelines that describe the multiple aspects involved in good secondary prevention [1–7]. In the medical literature heart attacks are more usually described as an acute coronary syndrome (ACS). This chapter examines the importance of diet in patients who have suffered an acute coronary event. We review the current guidelines on diet after an ACS, and the evidence for dietary change.

31.2 DIETARY RECOMMENDATIONS FOR PATIENTS FOLLOWING ACUTE CORONARY SYNDROMES

Most health organizations recommend some form of diet modification after a patient suffers an acute coronary event. These recommendations range from providing guidance on specific nutrients, to guidance on changes in eating patterns, to more specific guidance on suggested diets; however, most guidelines aim to achieve similar overall goals through diet modification (see Table 31.1).

31.2.1 Dietary Fats and Lipid Profile Management

Achieving an optimal lipid profile is a main goal of guidelines on cardiovascular disease secondary prevention [1–3,5–9]. Modification of diet is generally accepted as a means of achieving an optimal lipid profile. Central to dietary guidelines of all organizations is the reduction in consumption of saturated fatty acids (SFAs). Most organizations recommend restricting consumption of SFAs to less than 10% of total daily energy intake. Some organizations, such as the American Heart Association (AHA) and the National Heart Foundation of Australia, recommend a total of less than 7% [1,3,7]. Most of these organizations recommend a reduction in SFA intake through replacement of SFAs with poly- or monounsaturated fatty acids. Foods that are generally described as being rich dietary sources of poly- and monounsaturated fatty acids include certain vegetable oils, such as extra virgin olive, canola, soybean, and linseed oils, as well oily fish [1,4,7]. In addition, all organizations recommend limiting consumption of trans fat as far as possible – to, for example, less than 1% of total energy intake. Trans fats are formed from the hydrogenation of monounsaturated or polyunsaturated fat [4]. This process is commonly used in the preparation of margarine and vegetable shortening. Food products prepared with these ingredients will contain trans fats [10]. Several guidelines also specifically point out that food rich in polyunsaturated fatty acids (PUFAs) should be consumed, rather than added to the diet through supplementation [2,6,8]. The role of PUFAs and fish oils will be discussed in more detail later in this chapter.

31.2.2 Dietary Sodium and Blood Pressure

Blood pressure control is of major importance in both the primary and secondary prevention of cardiovascular disease [1,3,6,11], and as such is featured in most

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TABLE 31.1 Overview of Dietary Guidance Contained in Coronary Heart Disease Secondary Prevention Guidelines

Health organization	Energy intake	Saturated fatty acids (SFAs)	Type of oil	Fruits and vegetables	Sodium	Fiber	Alcohol
American Heart Association [1,3]		<7% of total energy intake <1% tFA <300 mg cholesterol	Fish twice per week	Diet rich in vegetables and fruits	≤2400 mg of sodium/day Target <1500 mg/day <6 g/day salt		Moderation
National Heart Foundation of Australia [7]		<7% SFA <1% tFA total energy intake	If EPA >2 g ALA daily	Eat mainly plant-based foods	≤4 g/day 1550 mg sodium		Males ≤2 Females ≤1 Standard drinks/day
European Society of Cardiology [2]	DEER to maintain or obtain BMI <25 kg/m^2	<10% SFA <1% tFA total energy intake	Increase through diet not supplements Fish twice a week, one being oily fish	200 g fruit (2–3 servings) 200 g vegetables (2–3 servings)	<5 g salt/day	30–45 g fiber/day from wholegrain products, fruits and vegetables	2 glasses (20 g/day of alcohol) 1 glass (10 mg) females
British Heart Foundation on behalf of National Institute for Health and Care Excellence [5,8]	Total fat <30% of daily energy intake	<10% SFA total energy intake	No fish oil supplements to prevent MI ≥2 portions (cooked 140 g), one oily per week	Mediterranean style 5 × 80-g portions of fruit and vegetables			<21 units/week men; 14 units/week women Avoid binge drinking (>3 drinks in 1–2 hours)
Joint British Societies [4]	Total fat <30% of daily energy intake	≤10% total fat intake Replace	Regular intake of fish >2 servings/week	≥5 portions/day	<100 mmol/l day <6 g salt <2400 mg sodium/day		<21 units/week men; 14 units/week women
World Health Organization [6]	Total fat <30% of daily energy intake	<10% total energy intake		≥400 g fruits and vegetables daily	<5 g salt <90 mmol sodium		

guidelines. High salt and sodium diets have been related to increasing blood pressure [12]. Salt consists of sodium chloride and contains 400 mg of sodium per gram of salt. Guidelines recommend the upper level of dietary salt intake to be 6 g (approximately 1 teaspoon) per day, with most recommending less than 5 g and one recommending less than 4 g. This equates to 2300 mg or 100 mmol sodium.

31.2.3 Fruits and Vegetables

All guidelines generally recommend increasing the consumption of fruits and vegetables in patients with coronary heart disease (CHD). Some guidelines advise that approximately 400 g of fruits and vegetables are to be consumed daily. This is equivalent to four to six servings, where a serving is equal to half a cup of cooked vegetables or 1 cup of salad or 1 medium-sized piece of fruit [13]. In some guidelines the Mediterranean diet, which is characterized by high intake of fruits and vegetables [5], is specifically recommended.

31.2.4 Alcohol Consumption

Most organizations make reference to alcohol consumption. High alcohol consumption is related to increased blood pressure [14]. Most guidelines recommend that a moderate intake of alcohol is consistent with a healthy diet. In general, moderate alcohol consumption is described as not more than two standard drinks for males and one standard drink for females per day. A standard drink contains approximately 10 grams of alcohol – for example, a standard drink of wine is approximately 150 milliliters [2]. The volume varies depending on the type of alcoholic beverage. One guideline also makes recommendations to avoid binge drinking [5].

31.2.5 Other Components of Diet

Weight loss is important in the management of a number of important risk factors for cardiovascular disease, especially in management of diabetes and high blood pressure. A reduction in overall energy consumption as

well as an increase in physical activity are generally encouraged in most secondary prevention guidelines to achieve the goal of weight loss [1–3,5–7]. Energy consumption is noted in some of the dietary guidelines we reviewed but not all, probably because clear agreement on how weight reduction can or should be achieved through diet modifications is difficult. We noted that guidelines tend to avoid specific recommendations on how to reduce energy consumption through diet, but rather focus on improving dietary patterns to make them heart healthy.

It is also of note that increased intake of dietary fiber is only described in one of the guidelines. There is some observational evidence of the importance of dietary fiber in reducing the risk of cardiovascular disease in primary prevention, but less specific studies that examine the role of dietary fiber in reducing risk after patients have suffered an acute coronary syndrome. Some of this is detailed later in this chapter.

Table 31.1 provides an overview of dietary guidance contained in coronary heart disease secondary prevention guidelines.

31.3 WHAT IS THE EVIDENCE THAT DIET MODIFICATION IMPROVES OUTCOMES?

Changing your diet is difficult, and, while patients who have had a heart attack may be motivated to change, many patients still have difficulty changing their behavior despite wanting to. Surveys of patients who have suffered an acute coronary syndrome indicate that many are unable to change behaviors. Among 18,809 patients from 41 countries enrolled in the Organization to Assess Strategies in Acute Ischemic Syndromes (OASIS) 5 randomized clinical trial, only 29.9% reported adhering to diet and exercise advice 30 days after their acute coronary event [15]. In this section we discuss some of the evidence and the major trials of diet modification in patients that have suffered an ACS.

A number of observational studies have found there are benefits of dietary modification for the secondary prevention of cardiovascular events post-myocardial infarction. In the analysis of the OASIS 5 trial described above, there was a significant decrease in risk for overall cardiovascular events at 6 months after the index acute coronary event for those that complied with both diet and exercise (54% reduction; odds ratio: 0.46; 95% confidence interval: 0.38–0.57). Conversely, there was a 3.8-fold (95% CI: 2.5–5.9) increase in the risk of cardiovascular events in patients that persisted in smoking and did not adhere to diet or to exercise, compared to those that were never-smokers and adherent to both diet and exercise modification (Figure 31.1).

Improved diet quality after myocardial infarction has also been associated with lower rates of all-cause mortality. In an analysis of 2258 women from the Nurses' Health Study and 1840 men from the Health Professionals Follow-up Study, diet quality was assessed using the Alternative Health Eating Index (AHEI-2010). A higher AHEI-2010 score was associated with a lower all-cause mortality (pooled hazard ratio: 0.71; 95% CI: 0.56–0.91) and cardiovascular mortality (pooled HR: 0.60; 95% CI: 0.41–0.86) [16].

In separate analyses of 6137 men and 11,278 women from the Health Professional Follow-up Study and the Nurses' Health Study, intake of a Mediterranean-style dietary pattern was also associated with lower mortality [17].

31.3.1 Changing Dietary Patterns

There are relatively few randomized controlled trials of diet modification after myocardial infarction. Trials of diet modification are challenging to conduct because, unlike taking a simple pill, modification of diet is very complex. Here we discuss some of these.

31.3.2 The Mediterranean Diet

What is popularized as a Mediterranean-style diet or Mediterranean-style cooking is based on typical foods of the countries of southern Italy, Spain, Portugal, and Greece. It is generally characterized by consumption of olive oil, legumes, fruits and vegetables, and fish.

31.3.3 The Lyon Diet Heart Study

The Lyon Diet Heart Study assessed the effectiveness of a Mediterranean-type diet on recurrence of cardiovascular events after myocardial infarction (MI) (Table 31.2) [18,19]. This was a trial of 605 patients aged less than 70 years, from Lyon, France, who, after their first myocardial infarction, were randomized to receiving dietary guidance or no guidance. The intervention group was counseled in a 1-hour long session to consume a diet containing more bread, more root and green vegetables, more fish, and daily fruit. Poultry replaced red meat, and butter and cream was replaced by margarine. Rapeseed and olive oils were recommended for salads and food preparation. The oil and margarine was supplied by the study. Wine, consumed in moderation at meal times, was also allowed [18].

A comparative analysis of the nutrient content of the diets in the intervention group versus controls found that the intervention group consumed a lower number of total daily calories than the control group. The intervention group consumed 30% of calories from fat, with 8% from saturated fat, compared to 34% total and 12%

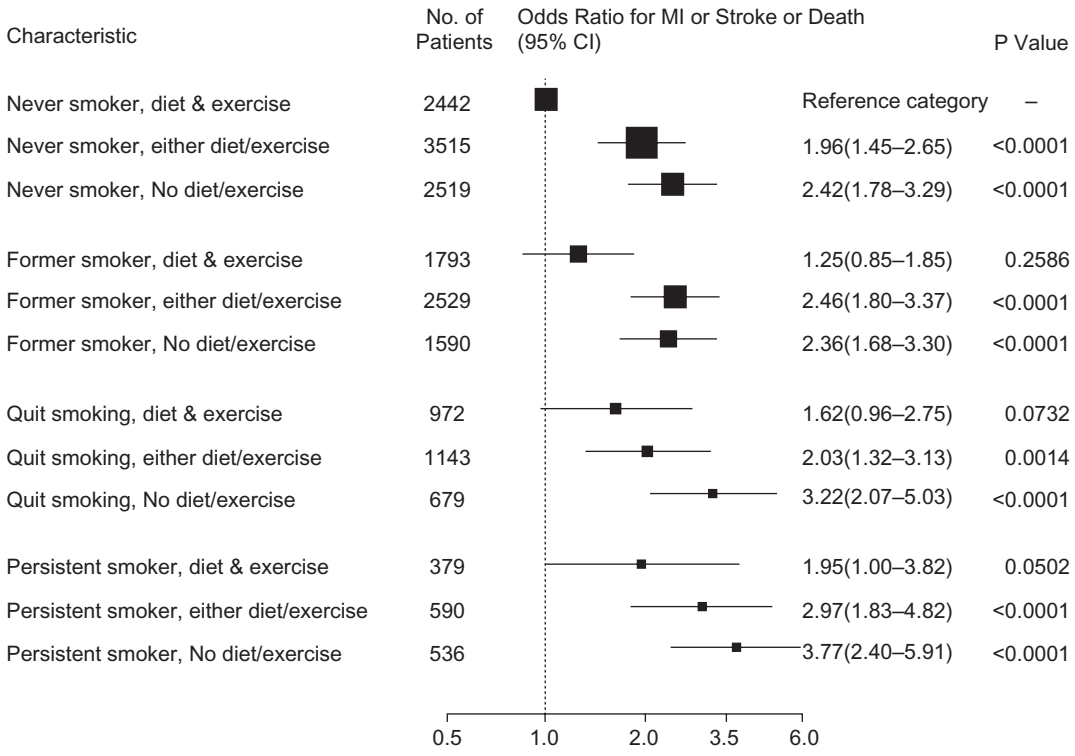

FIGURE 31.1 Risk of repeat cardiovascular events with progressive behavioral changes. All models adjusted for age, sex, region, history of hypertension, diabetes, prior MI, BMI, and creatinine. *Reproduced from Chow et al. [15] with permission.*

TABLE 31.2 Overview of Dietary Pattern Modification Trials

Trials	No. of participants	Location	Study population	Follow-up period	Intervention focus	Outcomes
Lyon Heart Diet [18,19]	605	France	Post-MI, <70 years	46 months	Mediterranean diet	Cardiac deaths 0.35 RR Total mortality 0.44 After 27 months, 73% decrease in cardiac death and non-fatal MI, with 70% decrease in CV mortality Maintained at 46 months
The Global Secondary Prevention Strategies to Limit Event Recurrence (GOSPEL) Study [20]	3241	Italy	Post-MI, 87% male, mean age 57.9 years	3 years	Comprehensive CR (supervised exercise, diet, and RF counseling)	CV mortality plus non-fatal MI and stroke 33%, cardiac death plus non-fatal MI 36%, non-fatal MI 48%
Dietary Approaches to Stop Hypertension (DASH) Diet [22,24]	459	USA	Adults; 133 with hypertension	8 weeks	Fruits and vegetables; combination diet containing fruits and vegetables, low-fat dairy, and reduced saturated and total fat	In hypertensive participants, combo diet reduced SBP by 11.4 and DBP by 5.5 more than control Normotensive = 3.5 reduction in SBP and 2.1 in DBP

saturated fat in the control group. The daily cholesterol intake in the intervention group was 203 mg, which was substantially lower than the 312 mg in the control group. Alcohol consumption was similar across both groups. The intervention group consumed more fiber than the control group, at 18.6 versus 15.5 g.

After 27 months of follow-up the intervention group was found to have a significant decrease of 73% in the combined endpoints of cardiac death and non-fatal MI. These benefits were maintained after 46 months' follow-up. Surprisingly, there was no difference in body mass index (BMI), blood pressure, or lipid levels. One

interpretation of these findings was that the type of fatty acid consumed is more important than the amount of total fat for decreasing events related to coronary disease.

Adherence to the Mediterranean diet, as demonstrated through diet score, was significantly inversely associated with both systolic and diastolic blood pressure. Intakes of olive oil, fruits, and vegetables were significantly inversely associated, whereas intakes of cereals, meat and meat products, and alcohol were shown to have a detrimental effect on blood pressure.

The Mediterranean diet has been reported to be protective in several chronic health conditions. In 2010 this literature was reviewed, and the authors summarized that the Mediterranean diet was associated with a significant reduction in overall mortality (relative risk [RR] = 0.92; 95% CI: 0.90–0.94), cardiovascular incidence or mortality (RR = 0.90; 95% CI: 0.87–0.93), cancer incidence or mortality (RR = 0.94; 95% CI: 0.92–0.96), and neurodegenerative diseases (RR = 0.87; 95% CI: 0.81–0.94) [21].

31.3.3.1 The Global Secondary Prevention Strategies to Limit Event Recurrence (GOSPEL) Study

The GOSPEL Study, conducted in 2001, examined a 3-year multifactorial continued educational and behavioral program compared to usual care (Table 31.2) [20]. Participants (n = 3241, mean age 57.9, SD 9.2) were post-MI patients recruited from 78 Italian cardiac rehabilitation centers and thus had all completed a standard 4-week cardiac rehabilitation program prior to entry. Participants were mostly under the age of 70 years (91.6%) and commenced the program on average 60.4 days (SD 20.2) after their acute MI.

The intervention was performed by a multidisciplinary cardiac rehabilitation team, including a nurse, physiotherapist, cardiologist, and (if needed) a psychologist and occupational therapist. Intervention sessions were monthly for the first 6 months and every 6 months until the 3-year mark. Each session consisted of supervised exercise, lifestyle and risk factor counseling, and reinforcement of preventive activities. These were aimed at individualizing risk factor and lifestyle management and pharmacological treatments based on current guidelines, and included the targets of smoking cessation, Mediterranean diet, physical activity, and biological risk factors. With respect to diet, at baseline overall 26.1% of patients had Mediterranean-like dietary habits (score >19.0). This score rose by 18% in the intervention compared to 14.1% in the control group at 6 months (i.e., 3.9% higher in intervention, $P < 0.001$), and this difference was maintained throughout the study. Physical activity, stress management, and biological risk scores improved overall, but were better in intervention compared with control.

The intervention did not significantly decrease the primary outcome (composite of CV mortality, non-fatal MI/stroke, CV hospitalization and revascularization), but did improve the secondary endpoint of CV mortality, non-fatal MI, and stroke (33%, 95% CI: 0.47–0.95; $P = 0.02$). Non-fatal MI was reduced by 48% (95% CI: 0.31–0.86; $P = 0.01$). No significant decrease in total mortality, sudden death, and total stroke was observed.

31.3.4 Other Dietary Pattern Studies

The Dietary Approaches to Stop Hypertension (DASH) diet (Table 31.2) is rich in fruits, vegetables, and low-fat dairy foods, and has reduced amounts of saturated fat, total fats, and cholesterol [22]. This diet has featured and/or been endorsed by a number of guidelines [1,3]. However, in our review of the literature it is not a diet that has been explicitly examined for its benefits in patients following an acute coronary syndrome. It has, however, been evaluated in a number of studies for its blood pressure-lowering effects [11,22–24].

For example, in a study conducted from 1994 to 1996 of 459 adults not taking antihypertensives, Appel *et al*. [22] found a significant decrease ($P < 0.001$) in both systolic and diastolic blood pressures after 8 weeks. Participants were randomized to either the DASH or a control diet. The control diet reflected the average nutrient consumption of Americans, including mineral consumption at the 25th percentile and average fiber levels. The DASH diet contained more fruits and vegetables, vitamins and minerals at levels close to the 75th percentile of the American consumption along with high amounts of fiber and protein. The sodium content of both diets was similar, at daily intake of approximately 3 g. In hypertensive participants (n = 133) the DASH diet reduced systolic blood pressure (SBP) by 11.4 mmHg and diastolic blood pressure (DBP) by 5.5 mmHg ($P < 0.001$ for each) more than the control diet. A reduction was also observed in normotensive patients with a 3.5 mmHg ($P < 0.001$) reduction in SBP and 2.1 mmHg (0.003) DBP recorded.

In another study conducted in the United States, the effect of the DASH diet and reduced intake of dietary sodium was evaluated [24]. A total of 412 healthy participants were randomly assigned to either the DASH diet or control, and consumed foods with high, intermediate, and low levels of sodium for a 1-month period in random order. After a 2-week run-in period where all participants ate a high sodium control diet, all participants were then randomly assigned to follow one of two diets. The control diet consisted of what many people in the USA might eat, whereas the DASH diet as described above was rich in fruits, vegetables, and low-fat foods. Within the two diet options, participants were randomly assigned to each of the three sodium levels for 30 consecutive days in a crossover design.

The primary outcome was SBP at the end of each 30-day period. The DASH diet, as compared with the

control diet, resulted in a significantly lower ($P < 0.001$) SBP at every sodium level. The secondary outcome for DBP was significantly lower at the high and intermediate sodium levels.

In comparison to the high sodium control diet, the low sodium DASH diet observed larger reductions in both systolic and diastolic blood pressures compared with the DASH diet alone or a reduction in sodium alone.

The effects of sodium reduction were greater in participants with hypertension than normotensive participants. Combination of DASH diet and low sodium intake lowered SBP more in hypertensive patients (11.5 mmHg, $P < 0.001$) and more in women (10.5 mmHg, $P < 0.001$) [24].

Table 31.2 provides an overview of dietary pattern modification trials.

31.4 ENRICHING DIETS WITH SPECIFIC NUTRIENTS

31.4.1 Studies Examining the Role of Fish and Fish Oils

Omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have been identified as potentially protective in cardiovascular disease. They are found in fish and also in fish oil, and can be provided as supplements [25]. An early observation that led to identification of its potential protective effect was the observation that coronary heart disease rates were low in Eskimo populations that had a diet high in fish [26]. Several secondary prevention trials have evaluated the benefit of ω-3 fatty acids on cardiovascular endpoints.

An early demonstration of the potential protective role of fish in the prevention of coronary disease was observed in the Diet and Reinfarction Trial (DART) [27] conducted in 1989 (Table 31.3). This trial assessed the role of fat, fiber, and fish consumption on myocardial reinfarction rates in 2033 men who had recovered from a myocardial infarction. In a randomized controlled trial with a factorial design, participants were randomly assigned to receive or not to receive advice on each of three dietary factors: reduction in fat with increase in ratio of polyunsaturated to saturated fat, increase in fatty fish, and increase in cereal fiber. Participants randomized to "fish advice" were encouraged to consume two portions of fatty fish a week or to take fish oil supplements. The "fiber advice" group was encouraged to eat six slices of wholemeal bread per day or equivalent cereal fiber from mixed sources. The third group received advice to reduce total fat intake and increase the ratio of polyunsaturated fatty acid (PUFA) to SFA.

At 2-year follow-up, the reported fatty fish intake was almost six times higher in those given fish advice (35 g/day) compared to those not receiving advice (9 g) [27], and the "fish advice" group had a 29% reduction in all-cause mortality (RR = 0.71; 95% CI: 0.54–0.92; $P < 0.05$). The effect was not altered by adjustment for potential confounders. The benefit was observed early and maintained for 2 years (Figure 31.2). A 32% reduction in coronary heart disease mortality was also observed. There were no significant interactions between the three dietary advices [27].

The DART population was followed up in 2000, [34] after 21,147 person-years. It found the fish advice group reported still eating more fish, including more fatty fish, compared to the no fish advice group, though the difference between groups was reduced compared to the 2-year follow-up. They also found that the fish advice group was more likely to take fish oil supplements. However, the difference in all-cause mortality rates between the two was not maintained.

Probably one of the most well-known trials in this area is the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto (GISSI)-Prevenzione Study. This study recruited 11,324 patients within 3 months of having an acute myocardial infarction between October 1993 and September 1995, and randomly assigned them to supplements of ω-3 polyunsaturated fatty acids (PUFAs) (1 g daily, $n = 2836$), vitamin E (300 mg daily, $n = 2830$), both ($n = 2830$), or none (control, $n = 2828$) for 3.5 years. Treatment with ω-3 PUFA, but not vitamin E, significantly lowered the risk of the primary endpoint (death, non-fatal myocardial infarction, stroke) (10%, 95% CI: 1–18). The GISSI Prevenzione trial [28] also found a significant 17% decrease in cardiovascular death. Interestingly, this benefit was observed as early as 4 months. In addition it found no effect of comorbidities, lifestyle habits, or interventions on the observed benefit of fish consumption [35].

In a closer look at the clinical outcomes, this trial found early protection and a decreased risk of sudden cardiac death in patients. It was observed that total mortality was significantly lowered after 3 months of treatment (RR: 0.59) [36]. There was also significant reduction in sudden cardiac death (RR: 0.67; 95% CI: 0.49–0.92), cardiac death (RR: 0.72; 95% CI: 0.57–0.91), and coronary death (RR: 0.75; 95% CI: 0.59–0.96) [35].

The largest randomized controlled trial to date of marine ω-3 fatty acids was the Japan Eicosapentaenoic Lipid Intervention Study (JELIS) [29] of 18,645 participants. This study was not a secondary prevention study, and is thus different from the above two. It was conducted in patients that were hypercholesterolemic on statin therapy, but without evidence of coronary artery disease. Also of note was that this study was solely among Japanese, and the baseline consumption of fish was likely to be high.

TABLE 31.3 Summary of Trials Examining the Role of Fish and Fish Oils

Trial	No. of participants	Study population	Follow-up period	Intervention focus	Outcomes
CONFIRM					
GISSI–Prevenzione [28]	11,324	Post-MI (within 3 months)	3.5 years	Four groups: 1 g ω3-PUFA + 2 g DHA; vitamin E; combination of the two; control	15% decrease in all-cause mortality, non-fatal MI CV death 30% reduction
Diet and Reinfarction Trial [27]	2033	Post-MI males	2 years	Combination of advice on three diet topics: **1.** Reduce fat intake to 30% of daily energy consumption **2.** Oily fish consumption of 200–400 g weekly **3.** Fiber intake 18 g daily	29% all-cause mortality in participants advised to eat fish
Japanese EPA Lipid Intervention Study [29]	18,645	Cholesterol 6.5 mmol/l or greater	Mean follow-up 4.6 years	1800 mg EPA daily with statin, or statin only	19% RR in major coronary events
The Indian Experiment of Infarct Survival [30]	360	Post-MI within 18 hours	1 year	Three groups: **1.** Receiving EPA 1.08 g/day **2.** Receiving 2.9 g ALA/day **3.** Control group with placebo	At 28 days, cardiac events were significantly lower in the treatment groups At 1 year, total cardiac deaths and non-fatal infarction were significantly lower in the fish oil group compared to the placebo group
FAIL TO CONFIRM					
SU.FOL.OM3 [31,32]	2501, 1863 with CHD	45–80 years old, history of MI, unstable angina, ischemic stroke 12 months prior to randomization	Mean follow-up 4.2 years	Dietary supplement of B group vitamins and ω-3 FAs	No significant effect on hard events or revascularization
ALPHA-OMEGA	4837	MI survivors, 78% men, 60–80 years	40 months	Margarines 1–4 for 40 months, supplemented as follows: **1.** EPA and DHA (400 mg) **2.** EPA and DHA (400 mg) with ALA (2 g/day) **3.** ALA alone (2 g/day) **4.** Placebo/standard margarine	Trial finished early due to no significant reduction in events
OMEGA [33]	3851	MI survivors (MI 3–14 days prior)	12 months	Omega-3 acid ethyl esters-90 (1 g/day for 1 year)	No effect of EPA and DHA on sudden cardiac death, total mortality, major cardiac events, or revascularization

In this study, all patients received general dietary guidance, 10 mg of pravastatin, or 5 mg of simvastatin. In addition, the EPA group received two 300-mg capsules containing EPA ethylester (EPA-E) three times a day – a total daily dose of 1800 mg.

Noting that this was a primary prevention study, the overall event rate in this study was low. This study found a 19% risk reduction in the risk of major coronary events. Unstable angina and non-fatal coronary events were significantly decreased. However, sudden cardiac death and coronary death did not differ between the control and intervention groups.

In further sub-analyses [37], compared to patients with normal triglyceride and HDL cholesterol levels, those with abnormal levels had a significantly higher CAD hazard ratio. In this higher vascular risk group,

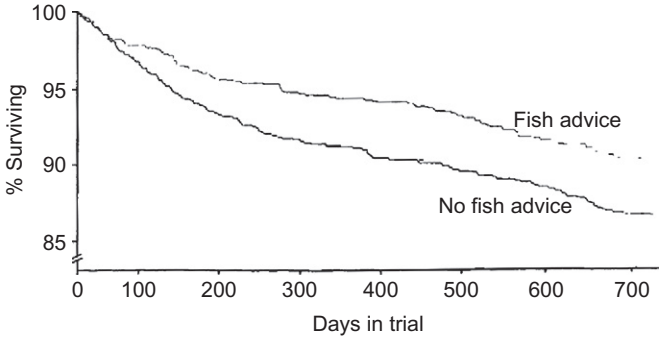

FIGURE 31.2 Survival in participants receiving fish advice versus no fish advice. *Reproduced from Burr et al. [27] with permission.*

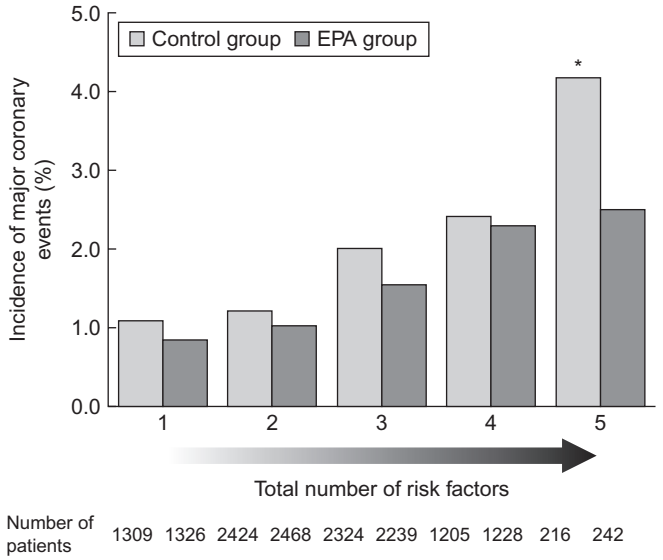

FIGURE 31.3 Incidence of major coronary events in EPA versus control group as a function in groups stratified by the number of cardiovascular risk factors. *$P<0.05$. *Reproduced from Saito et al. [37] with permission.*

the intervention suppressed the risk of CAD by 53% (HR: 0.47; 95% CI: 0.23–0.98; $P = 0.043$) (Figure 31.3) [37].

The use of EPA solely has been postulated as the reason for the success of this intervention. This trial showed higher levels of blood EPA, not DHA, which were found to be associated with a lower incidence of major coronary events [38]. A significant reduction in the risk of coronary events was observed when the ratio of EPA to arachidonic acid (AA) (EPA/AA) was >0.75. At this ratio it has been suggested that platelet aggregation is inhibited and cardiac ischemic injury is reduced [38].

In the Indian Experiment of Infarct Survival trial [30], patients with suspected and confirmed MI received fish oil, mustard oil, or placebo supplements. A total of 360 patients participated, with 122 receiving EPA 1.08 g/day, 120 receiving 2.9 g ALA per day, and a control group of 118 participants receiving placebo capsules. Interestingly, the treatment groups could be rated as higher risk than those receiving the placebo. There were significantly higher numbers of current smokers in both treatment groups, and a higher rate of previous MI and angina in the fish oil group. However, by day 7 all patients had ceased smoking.

After 28 days, cardiac events were significantly lower in the treatment groups compared to the control group. After 1 year, total cardiac deaths and non-fatal infarction were significantly lower in the fish oil group compared to the placebo group. In the mustard oil group, the only reported significant reduction was for total events at the 1-year point.

31.4.1.1 Failure to Confirm

Some recent studies have failed to confirm that ω-3 fatty acids reduce mortality. It can be argued, however, that significant limitations to study design would produce these expected results.

The SU.FOL.OM3 trial [31] evaluated the effects of daily dietary supplementation with B vitamins and ω-3 fatty acids for the secondary prevention of cardiovascular disease. Participants were randomly assigned to receive B vitamins alone, ω-3 fatty acids alone, both active treatments, or placebo.

The EPA and DHA supplement was less than in previous trials (600 mg/day). After a mean follow-up of 4.2 years among the 1863 participants with coronary heart disease, 163 coronary revascularizations were performed, and 95 patients experienced a hard coronary event. The event rate was lower than expected. Neither treatment with ω-3 PUFA nor treatment with B vitamins was associated with any significant effect on the occurrence of hard coronary events. Allocation to ω-3 PUFA was not associated with any significant effect on coronary revascularization. However, treatment with B vitamins was associated with a statistically significant 52% increase in the risk of coronary revascularization [31]. They found allocation to ω-3 fatty acids increased plasma concentrations of ω-3 fatty acids by 37% compared with placebo, but had no significant effect on major vascular events [32].

In the ALPHA-OMEGA trial, participants with a previous history of MI were randomized to consume one of four margarines [39]. Two of the margarines were eicosapentaenoic acid (EPA)- and docosahexaenoic acid (DHA)-based, one with additional α-linolenic acid (ALA), and one margarine ALA only. These two margarines provided a targeted daily EPA and DHA intake of 400 mg (again, lower than the previous trials). A standard margarine was used as a placebo. After 40 months of follow-up, interim analysis was conducted; due to no significant results, the study was finished early.

The participants consumed additional intakes of 226 mg of EPA combined with 150 g of DHA, 1.9 g of ALA, or both. Neither EPA-DHA nor ALA reduced the primary endpoint. There were a number of limitations that could provide some explanation for the negative result. First, the average length of time between MI and enrolment in the study was 3.7 years, compared to the GISSI Prevenzione trial where it was 25 days [40]. Also, the dose of EPA and DHA used was low, at about 400 mg daily. The margarine used for all treatment and control groups itself contained over 6000 mg of ω-6 FA linoleic acid. Given the potential benefits of linoleic acid in lowering the risk of cardiovascular disease, this could be an important confounding factor.

The OMEGA trial [33] examined the effect of EPA and DHA (855 mg/day) on sudden death of 3851 MI survivors. Incidence of sudden death and mortality were unexpectedly low in the population studied, thus severely compromising study power. It seems likely that a sample size of 20,000 would be required to detect a 30% reduction in sudden death, and this study power was based upon event rates in the earlier GISSI Prevenzione Study. Follow-up was shorter in the OMEGA trial.

In addition to the lack of power in this study, other potential reasons for the lack of a positive result in this trial were the high use of current secondary prevention drug therapies. In the GISSI Prevenzione trial, a very small number of participants were taking statins or beta-blockers. This is compared to both the ALPHA-OMEGA and OMEGA trials, where all patients were on optimum medical therapy [25].

Table 31.3 summarizes trials examining the role of fish and fish oils.

The limitations discussed previously, including low dose ω-3 fatty acid use, the lag between intervention and when MI occurred, length of follow-up, and sample size, probably explain why these recently published studies have failed to replicate results reported in previous studies [40].

The recent ORIGIN trial [41], in a *primary prevention* setting of 12,536 patients with dysglycemia, found daily supplementation of 1 g of ω-3 fatty acids did not reduce the rate of cardiovascular events in patients at high risk. Similarly, the Italian Risk and Prevention Study collaborative group, also a primary prevention study, in the setting of 12,513 patients with multiple cardiovascular risk factors but not myocardial infarction, showed no beneficial effect of 1 g of ω-3 fatty acids compared to control (which was olive oil) regarding death from cardiovascular causes, or admission to hospital for cardiovascular causes [42].

With these conflicting results, it is difficult to conclude whether fatty fish or fish oil supplements can reduce cardiovascular events. The postulated mechanism through reduction in sudden cardiac death has been raised as a possible reason for the null findings in primary prevention studies, in which the rate of sudden cardiac deaths is very low. Indeed, one trial has even reported an unexplained increased risk of cardiac death in men in a primary prevention setting [43]. They found that men with angina who were advised to eat oily fish, and particularly those taking fish oil supplements, had a higher risk of cardiac death. Whether the benefits of eating fish can be fully reproduced by fish oil supplements has also not yet been established.

A number of issues remain unaddressed in this area. It is unclear whether enriching diets in fish, and providing fish oil supplements, are the same. A dose–response has not been established in this area, so it is unclear whether greater doses of fish or fish oil have more benefit, or if there is a minimum amount. Some call for the need to confirm the initial trials, and the GISSI Prevenzione trial, in the context of current treatments and in particular with clinical trial methods such as double-blinding. Which components or sources of PUFA are beneficial is also unclear. Is the reduction in cardiovascular risk due to EPA, DHA, or the combination of the two, and what is the dosage of the effective combinations? This would build on the results of the JELIS Study. Lastly, clinical studies are needed to determine whether ALA from vegetable oil can effectively substitute for fish oil-derived EPA plus DHA [44].

31.4.2 Dietary Fiber and Other Supplements

A number of observational studies have identified dietary fiber as being potentially protective against coronary heart disease. Moreover, over the years a number of plausible and potential mechanisms have been postulated that could explain the link between dietary fiber and decreased coronary heart disease. For example, dietary fiber may slow the absorption of fats and sugars from the intestines, thereby attenuating postprandial blood glucose peaks. Dietary fiber may also improve satiety, leading to eating less and less weight gain. Also, foods high in dietary fiber tend also to be high in other potentially beneficial nutrients, such as antioxidants and phytosterols [45].

A number of research studies have been done in this area, and these have been recently reviewed in a systematic review. This review identified 22 cohort studies reporting total dietary fiber intake, fiber subtypes, or fiber from food sources, and their relation to cardiovascular events [46]. They concluded that greater dietary fiber intake was associated with a lower risk of cardiovascular events. Specifically, they found that increasing total dietary fiber intake by 7 g/day was associated with a 9% reduction in cardiovascular events. They did note that there was some heterogeneity in the results. More recent studies have also not conclusively demonstrated the link [45].

While there is some evidence that increasing dietary fiber is protective against cardiovascular disease, the evidence is not yet overwhelming, and we can identify no trials at this current time that provide convincing evidence that dietary fiber can prevent future cardiovascular events in patients that have suffered a heart attack.

As noted in a number of the studies above, varying vitamin supplements have also been examined with regard to reducing cardiovascular events. The studies we identified are all primary prevention studies. Some of these studies were described above as interventions evaluated in factorial designs with PUFA and their negative results noted. More recently, a large randomized controlled trial, also part of a complex factorial design, examined the effects of multivitamins in the Physicians Health Study II. This study of 14,641 male physicians from the United States aged 50 years or older (mean 64.3 years, SD 9.2) found no reduction in the primary outcome (a composite of major cardiovascular events, including non-fatal MI, stroke, and CVD mortality) in intervention compared to control groups (11.0 and 10.8 events per 1000 person-years for multivitamin vs placebo, respectively; hazard ratio [HR]: 1.01; 95% CI: 0.91–1.10; $P = 0.91$) [47]. As such, currently there is no convincing evidence that multivitamins reduce cardiovascular events.

31.5 BARRIERS TO CHANGE

A number of studies have now shown that improving diet after an acute coronary event will improve clinical outcomes. The pathways to this seem mainly through diet effects on modification of biological risk factors such as blood pressure, glycemia control, and lipid management. The literature and guidelines appear to be in general agreement that improving diet quality, and increasing the proportions of fruit, vegetables, and probably fish and polyunsaturated versus saturated fats in the diet, helps reduce the risk of repeat events after an acute coronary event. It is less clear, however, whether specific dietary supplements are effective in reducing risk. Vitamin supplements do not appear to have effect, and there is still mixed evidence for the benefits of supplements containing ω-3 fatty acids.

Separately from this, however, it is clear that adherence to dietary guidance is poor even after patients have suffered an acute coronary event [15]. The reasons may differ for regions; for example, in low- and middle-income countries the low intake of fruit and vegetables may be due to lack of access, and in high-income countries this may be due to an environment overwhelmed with a supply of processed and poor quality foods [48]. Demographics also seem to play a role, with younger people less likely to make changes, and women, older adults, and more educated individuals more likely to make changes. It has also been observed that those who adhere to one behavior recommendation are more likely to change others. For example, adults with a lower BMI and non-smokers were also found more likely to meet diet recommendations [49].

Following diet recommendations can be difficult if given complicated instructions, and decreased dietary compliance has been observed when additional and separated food needed to be prepared to meet new diet recommendations. Occurrence of medical complications, poor self-discipline, lack of information, and tendency to eat out have all been identified as factors that decrease compliance [50,51]. Also patients reported that their barriers to healthy eating included experts who kept changing their advice, limited choices for healthy food when eating out, and having to give up eating the foods that they liked [52]. Psychosocial factors have been shown to influence dietary adherence. Support from family members, empowerment through provision of practical advice, recent diagnosis of disease, and the authority of the person providing the advice all increase adherence [50]. Following on from this, interventions including telephone follow-up, provision of feedback, and the use of videos and nutritional tools have been shown to increase dietary adherence; employing multiple different techniques is even more effective [53].

31.6 FINAL THOUGHTS

Much still needs to be done to better understand the components of a heart healthy diet, but taking simple steps to improve diet quality in patients with acute coronary syndromes is important and should be enforced to improve their chances of longer and higher-quality lives. Additionally, in better understanding the important components of diet that are needed to improve outcomes consideration needs to be given to how such interventions can be implemented, and in particular what additional facilitators are required to enable people to modify their dietary behaviors, which are often well ingrained by the time they suffer a heart attack.

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CHAPTER

32

The Effects of Vitamin B12 and Folic Acid Deficiencies on Stroke, and Vitamin B12 and Folic Acid Supplements

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32.1 INTRODUCTION

In recent years substantial breakthroughs have been recorded in medicine, and the average life expectancy has extended accordingly. However, incidence of diseases related to advanced ages has increased. Vitamin B12 deficiency caused by poor or inadequate diet leads to both aging and the emergence of some diseases directly related to aging [1].

Low folic acid and high homocysteine levels directly damage the neurons in the brain. High levels of homocysteine are toxic to neurons. High level plasma total homocysteine is a risk factor for major cardiovascular events, including stroke. Vitamin B12 and folic acid, added to the metabolism of homocysteine as a co-factor, play an important role in keeping plasma homocysteine levels in balance [2].

In this chapter, the relation between vitamin B12 and folic acid deficiency and stroke in elderly patients, and B12 and folic acid supplement for prevention of stroke, will be discussed.

32.2 VITAMIN B12 AND FOLIC ACID METABOLISM

Vitamin B12 is a substance from the group of cobalt-containing cobalamins. It acts as a co-factor for two enzymes, methionine synthase and methylmalonyl coenzyme A mutase, in the human body. Two forms of metabolically active vitamin B12 are methylcobalamin and adenosylcobalamin. Adenosylcobalamin serves as a co-factor for methylmalonyl CoA mutase and converts methylmalonyl CoA into succinyl CoA. This reaction is essential for the synthesis of branched-chain amino acids and single-chain fatty acids. Methylcobalamin is a co-factor for methionine synthase, and catalyzes remethylation of homocysteine (Hcy) to methionine. This reaction is essential for methylation of phospholipids, amines, neurotransmitters, DNA, RNA, and myelin basic proteins. Lack of vitamin B12 leads to deficiency in methylation reactions and increased Hcy levels in the circulation [1,3].

Folic acid plays a role as a co-factor in many biochemical processes. The folic acid in the human body cannot be synthesized; therefore, all requirements must be taken through nutrients from outside. Dietary folic acids are mainly 5-methyltetrahydrofolate and formyltetrahydrofolate, which are absorbed into the intestinal epithelium the most quickly [2]. This form is the most important one that can be moved in the extracellular fluid, including via the blood–brain barrier. Folate is in three-fold higher concentrations than blood in the brain tissue. The blood–brain barrier limits the input of vitamins, and specific active transport systems are used to transport vitamins into brain tissue. The organism uses tetrahydrofolic acid mostly for the biosynthesis mechanism for the transport of single carbon units. It

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plays an essential role in converting the vitamin B12-dependent methionine synthase extracellular 5-methyltetrahydrofolate into monoglutamyltetrahydrofolate. Monoglutamyltetrahydrofolate can be used directly in the synthesis of nucleotides [4].

Methionine synthase activity is reduced in vitamin B12 deficiency, and tetrahydrofolate production is blocked. Purines, pyrimidines, and 5-methyltetrahydrofolate, which cannot be used in DNA synthesis, are produced by cells. In this case, cells divide more rapidly in the bone marrow and megaloblastic modified red blood cells start to form [1].

32.3 HOMOCYSTEINE AND CEREBROVASCULAR DISEASES

Homocysteine is a sulfhydryl-carrying amino acid produced by demethylation of methionine, which is an essential amino acid. Methionine occurs as a result of methylation of Hcy through methionine synthase. Vitamin B12 and folic acid are the fundamental elements for Hcy remethylation to methionine and cysteine transsulfuration. Homocysteine causes lipid peroxidation, free radical formation, inflammation, and endothelial dysfunction. As a result, atherosclerotic changes are accelerated [5]. Several studies have shown the relationship between hyper-Hcy and aging-related diseases, in particular cerebrovascular diseases. Vitamin B12 and folic acid levels have an inverse relation to Hcy levels. The previous studies on this topic have reported the elderly to be at higher risk than the young in terms of vitamin B12 and folic acid deficiency [6].

32.4 VITAMIN B12 AND FOLIC ACID DEFICIENCY IN THE ELDERLY

In developed countries, vitamin B12 deficiency affects 20% of the elderly. Subclinical vitamin B12 deficiency is more common in the elderly. The lack of folic acid is the most prevalent vitamin deficiency that affects all age groups and both sexes in the general population. This means that vitamin B12 deficiency is accompanied by folic acid deficiency. Vitamin B12 and folic acid deficiency are seen in 63% of healthy elderly people, and in 83% of the elderly who are hospitalized [7].

Vitamin B12 deficiency in the elderly may be due to inadequate oral intake. In addition, *Helicobacter pylori* infection and secondary hypochlorhydria to gastric atrophy can cause vitamin B12 deficiency in the elderly [8,9]. Pernicious anemia is responsible for 15–20% of vitamin B12 deficiency. Parietal cell antibodies are available in 90% of the elderly with pernicious anemia. Vitamin B12 deficiency may occur if B12 vitamin support is not provided after resection of the terminal ileum. The use of metformin is another of the causes of vitamin B12 deficiency. Proton pump inhibitors such as omeprazole and pantaprazol decrease vitamin B12 absorption in older people [10]. The most important reason for the lack of folic acid in the elderly is that it is not taken adequately in the diet. Medical problems associated with increased cell proliferation and malabsorption caused by the use of certain drugs also lead to folic acid deficiency [9]. Severe folic acid deficiency is observed less frequently. Subclinical folic acid deficiency – that is, low-normal levels of folic acid – is more common in the elderly, but it is associated with certain serious diseases [11].

Low vitamin B12 and folic acid levels and high Hcy levels predispose for many diseases, including Alzheimer's disease, dementia, Parkinson's disease, stroke, cardiovascular diseases, and affective disorders [12–14].

32.5 LOW VITAMIN B12 AND FOLIC ACID LEVELS IN STROKE IN THE ELDERLY, AND VITAMIN B12 AND FOLIC ACID SUPPLEMENTATION

32.5.1 The Relation Between Low Vitamin B12 and Folic Acid Levels and Stroke in the Elderly

Stroke ranks fourth among the causes of death in developed countries. Each year, approximately 795,000 people complaining of stroke are admitted to hospitals in the USA. Of the stroke cases, 82–92% are ischemic stroke. The World Health Organization (WHO) reports that 15 million people suffer a stroke each year, of whom 5 million die and 5 million become disabled. Stroke risk is higher in men than in women, and increases with age. The risk is quite high, especially for the elderly; 75% of all stroke patients are over the age of 65 years [15,16].

As stroke ranks fourth among the most common diseases that kill, and causes permanent neurological responses in one-third of patients, researchers have been led to study identification of risk factors, and modification or elimination of the disease, in addition to its treatment. Modifiable risk factors associated with stroke include diet, exercise, smoking, and alcohol. Non-modifiable risk factors are age, gender, race, and genetic factors. Cases of stroke have increased in developing countries along with the changes in diet and lifestyle resulting from industrialization and urbanization, and increased average life expectancy [17]. Epidemiological studies indicate that high homocysteine levels can be risk factors for major cardiovascular events, and it has long been known that folic acid metabolism disorders cause high levels of Hcy [18,19]. High Hcy levels are

associated with premature development of atherosclerosis, venous thrombosis, and arterial and/or venous thrombotic events such as myocardial infarction and stroke. High Hcy levels both lead to vascular endothelial damage and accelerate platelet aggregation. Hyper-Hcy not only is a risk factor for cardiovascular diseases and stroke but also interacts with other risk factors – for instance, a high Hcy level in a patient with a high cholesterol level increases the risk of having a stroke [2].

Folic acid plays a role as methyl donor in DNA synthesis. If folic acid is insufficient, nuclear damage and mitochondrial DNA increases, and the regenerative potential of normal tissue is reduced. Aging is accelerated. Thus, immune dysfunction and cardiovascular and neurodegenerative diseases occur. Folic acid deficiency is associated with increased nuclear and mitochondrial DNA damage, accelerated brain aging, and increased risk of stroke [20].

Weng and colleagues [21] have investigated the effects of inadequate folic acid intake in the diet on the risk of ischemic stroke in a study with 1772 adults over the age of 40. The subjects were followed in terms of the risk of ischemic stroke for 10 years by recording their daily diet properties. In the study, 132 people were diagnosed with ischemic stroke in a 10-year follow-up period. As a result of the study, a significant and independent relation between low folate intake and increased risk for ischemic stroke was identified.

In a similar study, ischemic stroke was determined in 334 patients and hemorrhagic stroke was identified in 62 patients at the end of a 15-year follow-up. As a result, there was no significant relation between inadequate dietary folic acid intake and the risk of ischemic stroke, but an inverse relationship with the risk of hemorrhagic stroke [22]. Weikert *et al*. [23] reported that low levels of vitamin B12 and folic acid increase the risk of stroke and transient ischemic attack (TIA).

Low vitamin B12 levels impact risk not only by increasing Hcy levels, but also by disrupting the blood–brain barrier. Vitamin B12 is involved in maintaining the integrity of the blood–brain barrier, and this integrity is impaired with vitamin B12 deficiency. The disruption of the integrity of the blood–brain barrier is a key factor in initiating small-vessel diseases in the brain. Lacunar strokes are associated with small-vessel diseases: serum vitamin B12 levels were found low in patients admitted to hospital for the first time with lacunar stroke [24]. In a study with 124 patients detected with periventricular white-matter lacunar stroke, and followed for 2 years after stroke, white-matter lesions grew in patients with low vitamin B12 levels [25]. De Lau *et al*. [26] reported in a study carried out on 1919 patients aged between 60 and 90 years that white-matter lesions increased as plasma vitamin B12 levels decreased. It has also been reported in the literature that low vitamin B12 and folic acid levels after stroke increase the risk for development of post-stroke brain atrophy [6].

Folic acid deficiency has been shown to be associated with larger areas of infarction in several previous experimental studies. It has been proven by experimental studies that infarct volume is two-fold larger than that of the control group in a middle cerebral artery (MCA) ischemia–reperfusion model of rats fed with a folic acid-deficient diet for a period of 3 weeks. Folic acid deficiency is not just a risk factor for ischemic stroke; it is also a risk factor that increases the width of the ischemic lesion [27].

Low vitamin B12 and folic acid levels in patients with ischemic and hemorrhagic stroke are indicators of a more severe clinical picture and poorer prognosis. The 3-month prognosis has been reported to be poorer in patients who have ischemic stroke along with low vitamin B12 and high Hcy levels. A relationship was found between low vitamin B12 levels at admission and lower Glasgow Coma Score (GCS) and high hospital mortality rate in patients with ischemic stroke in a study conducted on patients diagnosed with stroke in the emergency department [28]. In the same study, it was reported that there was a relationship between low folic acid levels and low GCS and higher hospital mortality rate in patients with hemorrhagic stroke [28].

Hyper-Hcy was found at the rate of 60.6% in a study conducted on patients with ischemic stroke. The 3-month prognosis was worse in the patients with high homocysteine levels and low vitamin B12 levels [29].

Researchers followed 500 patients with a mean age of 81 years, who had previously suffered ischemic stroke, for 31 months and established that high Hcy levels are independent risk factors for a new stroke. According to this study, every 1 μmol/l increase in plasma Hcy level increases the risk of having a stroke at a rate of 1.079% [30].

As can be seen, low vitamin B12 and folic acid levels and elevated Hcy levels increase not only the risk of stroke in the elderly. Low vitamin B12 and folate levels and elevated Hcy levels also increase the likelihood of patients with stroke having a more severe clinical course, poorer prognosis, and higher mortality rate, and suffering another, more significant, stroke.

Therefore, researchers emphasize that the risk of stroke can be reduced in the risk groups by decreasing homocysteine levels via vitamin B12 and folic acid supplementation. The results of the studies on this subject, however, are conflicting.

32.5.2 Vitamin B12 and Folic Acid Supplementation in Stroke in the Elderly

In a study in which a total of 5522 patients aged 55 years and over with cardiovascular risk (diabetes, vascular disease history) were followed, the study group was

given 2.5 mg folic acid, 1 mg vitamin B12, and 50 mg vitamin B6 daily [31]. In this study, patients were compared with the control group patients receiving placebo, and it was concluded after 5-year follow-up that folic acid, vitamin B12, and vitamin B6 supplementation did not reduce the risk of major cardiovascular events (stroke or ischemic cardiac-related death, stroke, acute myocardial infarction) [31].

Flicker and colleagues [32] followed patients supplemented with 2 mg folic acid, 25 mg vitamin B6, and 400 µg vitamin B12 daily for 2 years in a randomized, placebo-controlled study on 299 male patients aged 75 years and above. This therapy was observed to have maximal lowering effect on total Hcy levels, particularly in patients with low vitamin B12 levels and hyper-Hcy.

In another clinical trial in which 43,732 people were followed up for 4 years, it was concluded that ischemic stroke incidence decreased in the 40–75 years age group administrated high folic acid in their diet [33]. In another study of 2125 patients with a mean age of 66 years, a group administered high-dose vitamin supplements (25 mg vitamin B6 + 0.4 mg vitamin B12 + 2.5 mg folic acid) was compared with a group administered lower-dose vitamin supplements (200 µg vitamin B6 + 6 µg vitamin B12 + 20 µg folic acid). In this study, the primary outcome was stated as ischemic stroke and coronary heart disease, and death was stated as a secondary outcome. The stroke, coronary ischemic event, and death rate of the high-dose group was 21% lower than that of the low-dose group [34].

Total Hcy levels decreased by 3.8 µmol/l after a year in patients treated with 2.5 mg folic acid, 0.5 mg vitamin B12, and 25 mg vitamin B6 daily [35]. It was observed in the studies with folic acid and vitamin B12 supplementation in the diet that Hcy levels fell at the rate of 25–30%. A 25% decrease in Hcy levels reduced coronary ischemic events by 10% and the stroke rate by 20% [36].

According to a meta-analysis in which 15 placebo-controlled randomized studies investigated the effects of folic acid supplement on the prevention of stroke, it was found that folic acid supplementation reduces the risk of stroke in the population. It was determined in 10 of the studies analyzed that stroke risk was 11% lower in the folic acid supplement group than in the placebo-treated patients. When a 0.4- to 0.8-mg daily dose of folic acid was compared with higher doses in a meta-analysis, it was reported to have the same effect on reducing risk [37].

In another meta-analysis where 26 randomized controlled studies were examined, endothelial damage decreased in the group administrated a daily low dose (0.4 mg) of folic acid, and additional benefits were not observed in the group administered a high-dose (5 mg) of folic acid supplement. It was concluded in this meta-analysis that folic acid supplement can reduce the risk of stroke that may develop later [38].

Folic acid supplementation not only reduces the risk of stroke. Experimental studies have shown that folic acid supplementation helps the improvement of cognitive functions after ischemic stroke [39]. When patients who had suffered a stroke or transient ischemic attack (TIA) (mean age 63.6) were given 2 mg folic acid, 25 mg vitamin B6, and 500 µg vitamin B12 daily, no changes were observed in cognitive functions after the average 2.8-year follow-up period [40]. On the other hand, Almeida *et al*. [41], in a randomized, double-blind, placebo-controlled study, supplemented patients who had had a stroke (mean age 63) with 2 mg folic acid, 25 mg vitamin B6, and 500 mg vitamin B12 daily, and followed them for an average of 10.5 years. It was reported at the end of the follow-up period that the risk of developing major depression in patients decreased by 50% more than in the patients given placebo. Folic acid and other B group vitamin supplements are recommended to prevent chronic diseases associated with advanced age, including stroke in the elderly [42].

Oxidative damage was found to be markedly reduced in the patients who start B group vitamin supplementation within the first 12 hours after the initiation of stroke symptoms [43]. In patients (mean age 77 years) who were given oral 5 mg folic acid, 5 mg vitamin B2, 50 mg vitamin B6, and 0.4 mg vitamin B12 daily during the acute phase after stroke, the plasma total antioxidant capacity, malondialdehyde (MDA) levels, total Hcy level, and C-reactive protein (CRP) levels after 14 days were significantly lower than those in the group (mean age 79 years) given no supplement [43]. Although studies have been conducted with only folic acid supplement to prevent recurrence of stroke attack, or to affect the prognosis positively, researchers have usually argued that vitamin B12 supplement should be given along with folic acid. In a study dealing with this issue, Sato *et al*. [44] reported that vitamin B12 supplementation accompanied by folic acid supplementation during the acute phase of stroke created a synergistic effect in patients. This study included 191 patients diagnosed with acute stroke at the hospital. Patients were divided into three groups. Group A ($n = 63$, mean age 65.6) was given 1500 µg/day vitamin B12 orally; Group B ($n = 64$, mean age 64.1) was given 5 mg/day folic acid orally; and Group C ($n = 64$, mean age 65.8) was given 1500 µg/day vitamin B12 and 5 mg/day folic acid orally. After the total 8-week follow-up period, plasma Hcy had decreased to 10.9% in Group A, 22.4% in Group B, and 38.5% in Group C. As a result of the study, the researchers stated that secondary prevention can be achieved in stroke patients by giving vitamin B12 along with folic acid. According to a meta-analysis investigating 13 randomized controlled studies including a total of 39,005 patients, the risk of stroke was found not to be affected in the group given folic acid supplement only. It was

reported that the risk of primary stroke decreased when folic acid supplementation was accompanied by vitamin B6 and vitamin B12 [45].

In contrast, in a clinical study where patients were followed up for 6 weeks, a significant difference was found between the rate of decrease in plasma Hcy levels of patients administered folic acid only, and the rate of decrease in plasma Hcy levels of patients administered a combination of folic acid + vitamin B12 + vitamin B6 [46].

While folic acid is the major determinant of plasma Hcy levels, vitamin B12 and vitamin B6 have a significant effect on plasma Hcy levels albeit at a lower rate. Therefore, the US Food and Drug Administration (FDA) suggested that all cereal grain products should be enriched with folic acid, and since 1998 it has been mandatory that whole grain cereals be fortified with folic acid. The daily folic acid supplement recommended by FDA can be up to 100 µg. The safe uppermost limit of folic acid that can be taken and is specified by the FDA is over 1000 µg daily, and side effects on these doses are minimal [47]. The American Heart Association (AHA) has proposed a folic acid-, vitamin B12-, and vitamin B6-rich diet, and accompanying vitamin preparations (folic acid 400 µg–1 mg/day, vitamin B12 400–600 mg/day, and vitamin B6 2–10 mg/day), for patients who have previously suffered a stroke and have higher Hcy levels, in order to prevent primary and secondary stroke attacks [48].

In conclusion, a high Hcy level is one of the risk factors of stroke, which is the most significant cause of mortality and morbidity in those aged over 65 years. Endothelial damage caused by stroke can be reduced with folic acid and vitamin B12 supplementation in the diet. Although numerous studies have been made in this regard, the primary and secondary risks of stroke have been reported not to be affected in only very few of the patients administered folic acid and vitamin B12 supplementation. The results of studies mostly with elderly patients and in high-risk groups suggest that a folic acid- and vitamin B12-rich diet program should be implemented in the elderly population who have high level Hcy and low folic acid and vitamin B12 levels, or who have suffered stroke before. Either folic acid and vitamin B12 can be added to the foods in the cereal grain group, or special supplement preparations in accordance with the recommendations of AHA and FDA can be used for this purpose.

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CHAPTER

33

Nutritional Data in the Prevention and Therapy of Peripheral Arterial Disease

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33.1 PERIPHERAL ARTERIAL DISEASE

Peripheral arterial disease (PAD) is one of the clinical syndromes of atherosclerotic disease, which has become the first cause of death and morbidity in Western countries. PAD can affect any artery outside the heart, but most commonly describes ischemia of the lower limbs. It can present clinically as intermittent claudication, when peripheral arterial supply fails to meet the metabolic demands of the active leg muscles and thus the patient experiences leg pain on exercise that is relieved by a short rest; or as critical limb ischemia (CLI) in its most severe stages, when the arterial blood supply is so diminished that the patient experiences pain at rest and/or tissue loss, and limb viability is compromised (Table 33.1).

PAD affects 4–20% of the population older than 50 years, and its prevalence increases with age [1,2]. It is directly linked to smoking, and is often associated with metabolic comorbidity such as diabetes mellitus, hypertension, dyslipidemia, obesity, and metabolic syndrome (Box 33.1). The atherosclerotic burden of PAD is seldom limited to the legs; coronary artery disease (CAD) is present in up to 50% of patients, and cerebrovascular disease in 5–15% of patients [1]. PAD conveys a high risk of non-fatal and fatal cardiovascular events (myocardial infarction and stroke), and premature death. Each decrease in the ankle/brachial index (ABI) of 0.10 is associated with a 10% increase in relative risk for a major vascular event. The 5-, 10-, and 15-year morbidity and mortality rates are 30%, 50%, and 70% respectively [1] (Figure 33.1).

Even though PAD is more common than CAD in the general population, it is not as well known and has not been as thoroughly researched. PAD patients are often underdiagnosed and undertreated compared with CAD patients, regarding both pharmacologic therapies and management of risk factors, lifestyle, and metabolic disorders. Treatment of PAD focuses on improving functional capacity, limb salvage in the most severe presentations, and reducing cardiovascular risk. Lifestyle adjustment is crucial, with smoking cessation and daily exercise the cornerstones of treatment; routine medication includes antiplatelets and statins. Revascularization procedures, both open and endovascular, are also common, and, unfortunately, repeat interventions are often necessary. Thus, the costs of PAD for any given healthcare system are great, and increasing.

33.2 NUTRITIONAL ASSESSMENT

A good nutritional status is essential for the maintenance of good health and for recovery from illness. Illness and surgical recovery imply increased metabolic demands and require physiological adjustment to limit catabolism, preserve organ function, and promote wound healing and defense against infection [3,4]. Nutritional deficiencies can impair this adjustment and response. Malnutrition occurs when the net nutrient intake is less than requirements. It leads to metabolic disorders, organ and tissue dysfunction, and loss of body mass, all of which can be accelerated by concurrent inflammation, infection, stress,

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TABLE 33.1 Classification of Peripheral Arterial Disease

Fontaine		Rutherford		
Stage	**Clinical presentation**	**Grade**	**Category**	**Clinical presentation**
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	1	Mild claudication
IIb	Moderate to severe claudication		2	Moderate claudication
			3	Severe claudication
III	Ischemic rest pain	II	4	Ischemic rest pain
IV	Ulceration or gangrene	III	5	Minor tissue loss
			6	Major tissue loss

BOX 33.1

RISK FACTORS FOR PERIPHERAL ARTERIAL DISEASE

- Black race
- Male gender
- Increasing age
- Smoking
- Diabetes mellitus
- Hypertension
- Dyslipidemia
- Systemic inflammation (increased serum levels of C-reactive protein)
- Hyperviscosity
- Hypercoagulability (increased plasma levels of fibrinogen)
- Chronic renal failure
- Diet rich in saturated fat, cholesterol, and sodium; low in fiber, folate, magnesium, and vitamins D and E
- Abdominal obesity
- Reduced physical activity

or trauma. Malnutrition can impair respiratory muscle function, cardiac contractility, renal and immune function; increase thrombogenicity; and affect emotional and behavioral responses, leading to apathy, depression, loss of will to recover, and further anorexia, thus exacerbating malnutrition [4–7]. Disease and nutrition interact so that disease can cause secondary malnutrition, and malnutrition can worsen the underlying disease [5,6]. Thus, the final outcome can be multifactorial. Malnutrition has been associated with higher postoperative complication and death rates, longer hospital and intensive care unit stays, and an increase in healthcare costs [3,6]. Approximately 30–55% of hospitalized adult patients suffer some degree of malnutrition, which makes nutritional assessment part of the integral evaluation and care of the critically ill and surgical patient [4,8]. Malnutrition can be previous to hospital admission or develop during the hospital procedure, as a result of the catabolic or hypermetabolic state and insufficient nutrient intake [6,8]. It is potentially reversible with targeted interventions, but, if unaddressed, the nutritional deficits will persist after discharge, affecting complete patient recovery and functional status. These patients have increased early hospital readmission rates and 1-year mortality rates [6].

The clinical assessment of nutritional status aims to identify the initial nutritional state of the patient, identify the causes and consequences of malnutrition, assess individual morbidity and mortality risk, provide an insight into the future response to disease and treatment, and identify the patients who would benefit most from nutritional supplementation [3,8]. It is a dynamic process that should be periodically reassessed according to changes in treatment and disease evolution. The nutritional status involves a focused history and physical examination with selected laboratory tests aimed at detecting specific nutrient deficits and patients at high risk of malnutrition [4,5]. The markers for nutritional assessment should be sensitive, accurate, reproducible, easy to perform, generally applicable, and cost-effective [3,6]. Any single parameter can only meet one or two of these criteria, and several parameters should complement each other for accurate global assessment. There is to date no gold standard for the assessment of nutritional status, and the reliability of any given parameter or tool has not been validated [3–5]. Some parameters can be troublesome in acute or critical illnesses, since the interpretation of results can be impeded by changes due to the acute disease or the treatment itself. For example, anthropometrical variables can be altered by changes in water distribution in critical patients, and acute inflammatory processes like tissue necrosis or ulceration may affect plasma proteins [3,5,7,8]. At times a degree of

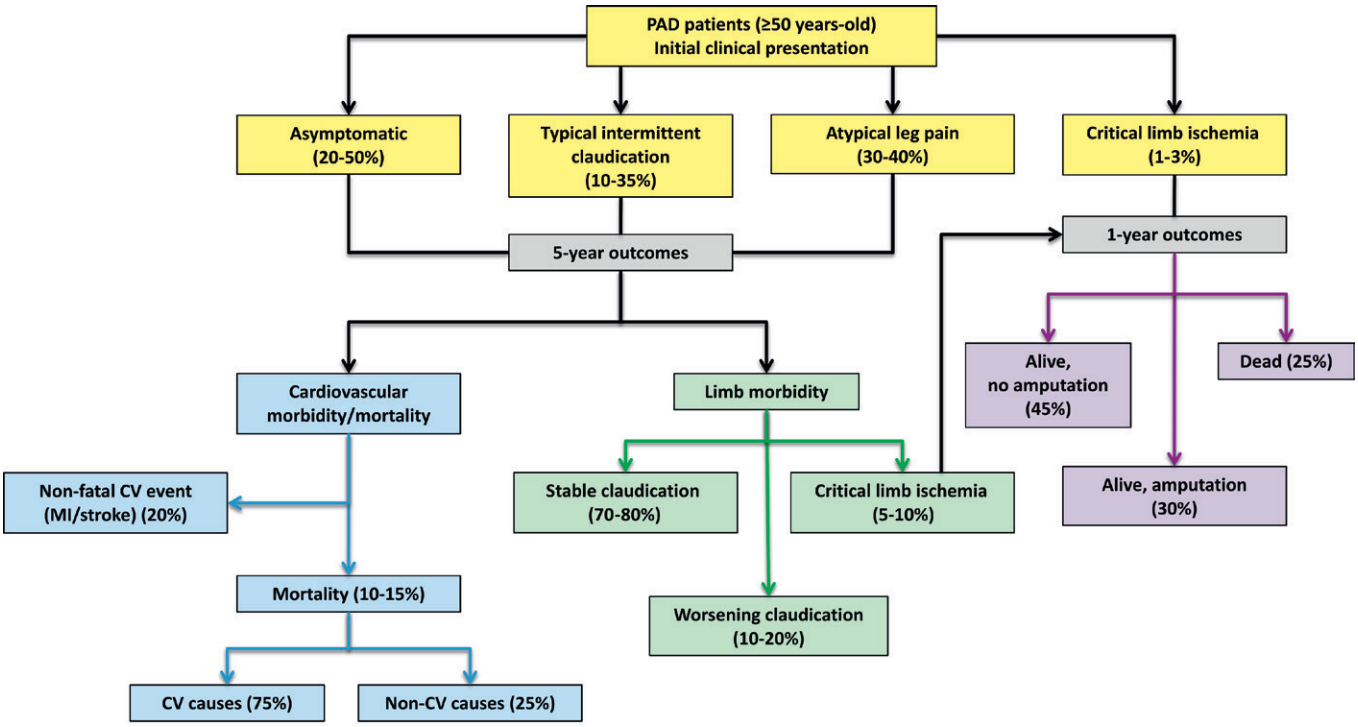

FIGURE 33.1 Natural history of peripheral arterial disease. CV, cardiovascular; MI, myocardial infarction; PAD, peripheral arterial disease. *Figure adapted from Norgren et al. [1].*

subjective clinical assessment will be necessary in some patients to complement physical symptoms and objective parameters [3]. Box 33.2 summarizes currently used parameters for nutritional assessment.

33.3 NUTRITIONAL DATA IN PERIPHERAL ARTERIAL DISEASE

Evidence of the effects of body composition, nutrition, and supplementation on PAD is scarce. Most studies are small and mainly cross-sectional, which allows for the detection of associations but not a temporal relationship or causation, and the clinical trials in which the effects of specific supplementation on the risk or evolution of PAD have been assessed are small, methodologically very diverse, and have proved quite disappointing. Data are basically extrapolated from cardiologic studies and recommendations are based on heart health. Little is known about the peculiarities and specific needs of patients with PAD, but there is increasing evidence of the importance of nutrition in the incidence, severity, and prognosis of PAD.

33.3.1 General Dietary Patterns

Besides common risk factors like age, gender, smoking, diabetes, hypertension, and hypercholesterolemia, the dietary pattern is also considered a risk factor for CAD. The Mediterranean diet, with an abundance of vegetables and poly- and mono-unsaturated fatty acids, has been associated with reduced incidence of cardiovascular events and mortality risk [9,10]. There is increasing evidence that it may also play a role in stroke and PAD [2,9].

The nutritional intake of PAD patients has been found to be below the estimated average requirement for fiber, magnesium, folate, and vitamin E for all ages, genders, and body mass indices (BMI), yet rich in cholesterol, trans-fatty acids, and sodium [11,12]. These dietary patterns counteract the beneficial impact of pharmacologic therapy for dyslipidemia and hypertension, which are often, additionally, insufficiently addressed among PAD patients. High consumption of saturated fatty acids has been associated with higher prevalence of PAD (adjusted odds ratio [OR]: 1.30; 95% confidence interval [CI]: 1.01–1.67; $P = 0.04$) [10]. Higher dietary intakes of linolenic acid and saturated fatty acids have been associated with higher and lower ankle brachial index, respectively, after adjustment for cardiovascular risk factors [10]. Consumption of a high-fat meal has been found to acutely impair endothelial function and exercise performance [12]. In contrast, cereal fiber intake has been associated with reduced inflammation, lower C-reactive protein (CRP) and LDL cholesterol levels, reduced incidence of PAD, and higher ABI values [2,12].

BOX 33.2

NUTRITIONAL DATA IN PAD: KEY POINTS

- Evidence on the impact of nutrition on the incidence, severity, and prognosis of PAD is scarce and low quality
- There are, to date, no established dietary recommendations or evidence-based guidelines for the prevention or treatment of PAD
- Patients with PAD are often underdiagnosed and undertreated compared with CAD patients, regarding both pharmacologic therapies and management of risk factors, lifestyle, and metabolic disorders.
- A diet based on fruits, vegetables, nuts, and whole grains, low on meat and refined grain products, and with no added salt, is recommended for the prevention and treatment of PAD
- Patients with PAD should receive dietary counseling
- Patients with PAD must reduce the consumption of dietary fat, saturated fat, cholesterol, and sodium, and increase the daily intake of fiber, folate, and vitamins D and E
- Abdominal obesity is associated with PAD
- Daily exercise and weight control should be encouraged
- Folate and vitamin D deficiencies are associated with an increased risk of PAD
- Folate and vitamin B/D supplementation cannot be systematically recommended until further evidence demonstrates efficacy on PAD incidence and/or outcome
- Systematic nutritional assessment should be included in the management of patients with CLI
- Anemia and iron, folate, and vitamin B/D deficiency should be actively looked for in patients with CLI, and addressed preoperatively whenever possible
- Clinical assessment of nutritional status should be periodically reassessed according to changes in treatment and disease evolution
- Further research is needed to clarify the impact of nutritional status and supplementation on the incidence, symptoms, progression, prognosis, and healthcare costs of PAD.

CAD, coronary artery disease; CLI, critical limb ischemia; PAD, peripheral arterial disease.

However, even though inverse associations between PAD and intakes of fiber, folate, and vitamins A, B6, C, and E were found in univariate analysis, after adjusting for age, sex, hypertension, diabetes, and smoking, in a very recent cross-sectional study performed in the US, the association lost statistical significance when adjusting additionally for energy intake and physical activity [13]. When total caloric intake is associated with disease, even if not a direct cause, the interpretation of individual nutrient intake is complex and may be distorted by total energy intake. Taking energy intake into consideration is standard in nutritional epidemiology to reduce variability related to general over- and underreporting of food intake and improve precision, yet it can sometimes change the interpretation of the results obtained for one particular food or nutrient. PAD patients, with limited mobility and activity, tend to expend less energy and consume fewer calories and smaller amounts of a wide variety of nutrients. This can help explain why the few published trials on specific vitamin C, B, or E supplementation have failed to prove any significant benefit on cardiovascular events.

33.3.2 Weight

Obesity is a well-established risk factor for cardiovascular disease. Total body weight or body mass index (BMI) are not ideal parameters for the assessment of cardiovascular risk; abdominal obesity, measured by waist and hip circumference, is more accurate, as abdominal adipose tissue is metabolically active and can influence serum lipid concentrations, insulin regulation, inflammatory pathways, and endothelial function. Body density scans analyze percentages and distributions of fat, muscle, and bone mass, and are even better predictors of cardiovascular risk and outcome. PAD is more frequent among heavier individuals, especially those suffering from abdominal obesity and if associated with metabolic disorders (impaired fasting glucose, increased serum triglyceride, and decreased serum HDL cholesterol levels) [2,3]. Obesity is also associated with a higher incidence of adverse PAD-related outcomes, such as progressive decline in walking distance, and non-fatal and fatal cardiovascular events [2].

Both obesity and low BMI ($<18.5\,kg/m^2$), which can be considered different forms of malnutrition, have been associated with significant increase in morbidity, postoperative complications, delays in resumption of oral intake, longer stays in the intensive care unit, higher readmission rates, and higher overall mortality in surgical patients [3,8]. A recent small cross-sectional Portuguese study reported an incidence of malnutrition of 21% among hospitalized patients with critical limb ischemia (CLI), with an additional 40.8% at risk of

malnutrition. The risk of malnutrition increased with age >75 years (OR: 5.12; 95% CI: 3.03–51.47), diabetes (OR: 2.73; 95% CI: 0.88–8.46), and low levels of education (OR: 2.64; 95% CI: 0.78–8.90) [14].

33.3.3 Anemia and Iron

Most patients with CLI will require some surgical procedure for revascularization and/or minor or major amputation. Two studies, published in 2011 and 2010, described 49.5% and 60% prevalence of anemia among patients with CLI, respectively, and this increased with the age of the patients (62.1% CLI patients older than 75 years compared to 22.2% CLI patients younger than 65 years) [15,16]. In contrast, anemia affected only 9.8% of patients with ischemic claudication [15]. Anemia was associated with low albumin levels, elevated CRP levels, and prolonged hospital stay among the CLI patients [16]. Low preoperative hemoglobin has been independently associated with increased risk of major adverse cardiac events (OR for each 1 g/dl drop below the mean = 1.4; 95% CI: 1.13–1.7) and death (OR for each 1 g/dl drop below the mean = 1.5; 95% CI: 1.14–1.86) after peripheral arterial surgery [17]. Thus, the cause for anemia should be assessed and treated, prior to intervention when possible, and thus blood transfusion and postoperative complications minimized.

Anemia in surgical patients can be secondary to iron deficiency or chronic disease. In the 2011 study, 31.9% of the CLI patients had iron deficiency, as assessed by circulating concentrations of iron <58 μg/dl [15]. Serum ferritin and transferrin saturation are good indicators of body iron stores, but they are acute phase proteins and increase similarly to CRP in acute inflammatory situations, so they are of limited use in CLI patients, who may have associated acute or chronic inflammation, and occasionally infection. Several other physiopathological factors can contribute to chronic anemia: disruption in iron homeostasis; impairment of erythroid progenitor cell proliferation and differentiation; decrease in the production and activity of erythropoietin; and decrease in erythrocyte lifespan [16].

Accumulation of iron stores has been hypothesized to contribute to atherosclerotic cardiovascular disease through increased iron-catalyzed free radical-mediated oxidative stress [18,19]. However, a randomized trial published in 2007 did not find any significant reduction in mortality, myocardial infarction, or stroke in PAD patients in whom iron stores were reduced through phlebotomy, although the effects were almost significant in younger patients [18]. Menke found a modest association between ferritin and transferrin saturation and PAD, particularly for men and women with high (≥200 mg/dl) cholesterol levels [19].

Finally, red cell distribution width (RDW) has proved to be an independent predictor of the 10-year estimated risk of CAD events. Higher levels of RDW have also been independently associated with an increased risk of PAD [20].

33.3.4 Albumin

Preoperative malnutrition has shown important prognostic implications for vascular surgery patients. Albumin levels have been reported to be independent predictors of myocardial infarction, stroke, or death after open or endovascular surgical procedures, as well as 1-year death rates [5,8,21]. Albumin levels also emerged as an independent predictor (OR: 0.43; 95% CI: 0.26–0.71) of mid-term all-cause mortality among a cohort of patients with CLI who had undergone lower-limb bypass surgery [22]. The incidence of hypoalbuminemia among CLI patients was 18.1% in the series published in 2011, with significant differences between women (60.9% prevalence) and men (33.8%) [15].

33.3.5 Folate and Vitamin B

Low folate intake has been repeatedly associated with significantly higher prevalence of PAD. Folic acid and vitamin B12 are necessary for adequate homocysteine regulation, and their deficiency leads to toxic accumulation of unmetabolized serum homocysteine, endothelial dysfunction, vasoconstriction, inflammation, apoptosis, and eventually atherosclerotic plaque formation [2,23]. Vitamin B12 and folic acid deficiency were found in 15.7% and 6.4% CLI patients, respectively, in the cross-sectional series published in 2011. In contrast, vitamin B12 and folic acid depletion were rare among patients with intermittent claudication, affecting only 6.7% and 2.9%, respectively [15].

Folate and vitamin B6 supplementation have shown a 24% decrease in the risk of cardiovascular events compared with a placebo group [2]. Folate food fortification or dietary supplementation might reduce the population incidence and morbidity of vascular disease [23].

33.3.6 Vitamin D

The active form of vitamin D, 1,25-dihydroxyvitamin D, regulates the expression of several proteins relevant to the arterial wall, such as vascular endothelial growth factor, matrix metalloproteinase type 9, myosin, elastin, and type I collagen [24]. It also mediates protein synthesis and cellular adenosine triphosphate accumulation, increases troponin C, and increases actin and sarcoplasmic protein expression in striated muscles [25]. Hypovitaminosis D leads to reduced intestinal calcium

absorption, increased renal calcium loss, compensatory hyperparathyroidism, osteomalacia, arterial calcification, and increased atherosclerotic risk. It can also cause myopathy, and reduced strength and muscle function, and further limit mobility.

Vitamin D deficiency has been associated with increased incidence of cardiovascular diseases, namely hypertension, myocardial infarction, heart failure, sudden cardiac death, stroke, and PAD. Cross-sectional studies have reported an association of vitamin D deficiency with prevalence and severity of PAD [2]. For each decrease of 10 ng/ml in the 25-hydroxyvitamin D plasma level, the multivariable-adjusted prevalence ratio of PAD was 1.35 (95% CI: 1.15–1.59) [26]. Hypovitaminosis D can both cause and contribute to the symptoms of PAD. Hypovitaminosis D is especially prevalent among the African American population, due to their reduced ability to convert the sun-derived precursor 7-dihydrocholesterol to previtamin D3, and accounts for approximately one-third of excess PAD risk compared to white or Hispanic populations [2].

So far, no trials have assessed the effects of vitamin D supplementation on PAD patients [2]. However, two randomized trials with supplementation of vitamin D3 in 65- to 85-year-old men and women and 50- to 79-year-old postmenopausal women, respectively, showed no improvement in overall or cardiovascular mortality, and no reduction in cardiovascular events [27,28]. Thus, to date, vitamin D supplementation for either the prevention or the treatment of PAD or other cardiovascular diseases is not endorsed until large-scale randomized, controlled studies demonstrate efficacy.

33.3.7 Niacin, L-arginine, Omega-3 Essential Fatty Acids, Amino Acids, and Antioxidants

Niacin supplementation, which increases serum HDL cholesterol concentrations and inhibits hepatic LDL cholesterol synthesis, has been associated with moderate improvement in walking distance in limited cohorts of patients with intermittent claudication [2]. L-arginine supplementation, which can improve endothelial function, has been reported to improve walking distance in these patients in the short-term, but not at 6-month follow-up [2]. In contrast, supplementation with omega-3 essential fatty acids has repeatedly failed to prove any significant improvement in cardiovascular symptoms or outcome in PAD patients in several small clinical trials. A meta-analysis of 10 trials did show, however, a beneficial impact on arterial stiffness, with a reduction in arterial compliance, cardio–ankle and brachial–ankle pulse wave velocities, in both healthy and obese individuals, in diabetics, and in hypertensive patients [2]. Supplementation with oral amino acids has been proved to increase protein synthesis in calf muscles of patients with intermittent claudication, which could improve calf muscle mass, walking ability, and functional status [29]. Selenium, vitamin E, and β-carotene supplementations have failed to show any beneficial effects on vascular function or PAD prognosis [2].

Spark found reduced total antioxidant capacity in patients with CLI compared to controls [7]. He showed that a reduction in total antioxidant capacity in patients with CLI can increase the risk of perioperative infection, and that this reduction is in part due to their impaired nutritional status. Episodes of ischemia–reperfusion further reduce total antioxidant capacity. In theory, PAD and especially CLI patients could benefit from nutritional and/or antioxidant supplementation. However, three studies have failed to prove a significant effect of antioxidant supplementation (administered as a nutritional supplement or fruit juice) on anti-inflammatory biomarkers in patients with PAD [2]. There is currently an ongoing randomized clinical trial assessing the potentially beneficial effects on cardiovascular outcome of a diet rich in alpha-linolenic acid, antioxidants, and fiber (flaxseed) among patients with PAD; results are expected during 2014 [30].

33.4 CURRENT RECOMMENDATIONS

There are, to date, no established dietary recommendations or evidence-based guidelines for the prevention or treatment of PAD. Still, preliminary evidence suggests that nutritional intervention and weight control might be useful for PAD prevention and treatment (Table 33.2). Using nutritional strategies to lower the incidence and functional impairment of PAD could greatly reduce the burden of the disease, in terms of both quality of life for the patients and healthcare costs – most especially in the context of the economic crises.

The dietary pattern should routinely be evaluated in vascular patients. Improving the everyday diet enhances the patient's overall status and cardiovascular prognosis, and does not increase the number of pills prescribed. Apart from addressing the different well-known cardiovascular risk factors, and the obvious advice to quit smoking and increase daily exercise, as well as specific medication (antiplatelets, statins, etc.), PAD patients should be encouraged to reduce the consumption of dietary fat, saturated fat, cholesterol, and sodium, and to increase the daily intake of fiber, folate, and vitamins D and E. General recommendations would include a diet based on fruits, vegetables, nuts, and whole grains, low on meat and refined grain products, and with no added salt. Counseling and follow-up from a dietician could prove very useful for individualized aggressive diet modification.

TABLE 33.2 Parameters Used for Nutritional Assessment

Anthropometric parameters	Body mass index (BMI) (kg/m^2)
	Weight loss during the previous 6 months
	Mid-arm muscle circumference
	Triceps/subscapular skinfold thickness
	Muscle wasting (deltoids, quadriceps, temporalis muscle)
Dietary patterns	Daily calorie and nutrient intake
	Gastrointestinal symptoms that might impair eating
Biochemical parameters	Albumin
	Prealbumin
	Serum colesterol
	Hemoglobin
	Transferrin
	Creatinine/height index
Immunologic parameters	Delayed cutaneous hypersensitivity
	Total lymphocyte count

Folate and vitamin B supplementation cannot be generally recommended with the currently available evidence. Still, complete evaluation of PAD patients could include homocysteine, folate, and vitamin B plasma levels, and supplementation considered individually according to the specific results. Similarly, to date, no solid recommendations can be offered regarding supplementation with vitamin D, niacin, L-arginine, omega-3 essential fatty acids, oral amino acids, antioxidants, or any other specific compound for either prevention or treatment of PAD until further evidence demonstrates efficacy.

We recommend systematic nutritional assessment in CLI patients as part of their integral management. Anemia, iron, folate, vitamin B12 deficiency, and vitamin D deficiency should be actively looked for and addressed preoperatively, whenever possible. Anthropometric parameters and albumin/prealbumin plasma levels can offer relevant data on the individual risk of malnutrition. The intensive treatment that these fragile patients usually require should include nutritional intervention.

Further research is needed to clarify the impact of nutritional status and supplementation on both the prevention and treatment of PAD. There is a wide area of potential research, such as total dietary patterns, specific nutrient intakes, ethnic and geographical influences, or weight loss interventions, and variables such as incidence, symptoms, progression, prognosis, and healthcare costs of PAD.

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CHAPTER

34

Vitamin D Deficiency and Anemia in Heart Failure

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34.1 INTRODUCTION

In recent decades it has become increasingly clear that vitamin D has a broad range of non-classical actions in the human body besides its well-known effects on mineral and bone metabolism. Low vitamin D status has been associated with various pathologies, including anemia. Anemia is common in heart failure, and is considered to be multifactorial. This chapter summarizes the available evidence for an association between vitamin D deficiency and anemia in heart failure. In addition, possible vitamin D-related mechanisms involved in causing anemia are outlined.

34.2 VITAMIN D

34.2.1 Sources and Metabolism of Vitamin D

Humans can achieve adequate vitamin D supply by skin exposure to solar ultraviolet (UV) B radiation (wavelength 290–315 nm), diet, or supplements [1–3]. Skin synthesis of cholecalciferol (vitamin D3) usually contributes up to 90% of the total human vitamin D status [4]. Dietary intake of vitamin D3 (or vitamin D2) is a second source of vitamin D. However, only a few marine foods, such as eel, herring, and salmon, are vitamin D-rich foods; the vitamin D content of the vast majority of other foods is very small or even zero.

Once in the circulation, vitamin D is converted by a hepatic hydroxylase into 25-hydroxyvitamin D (25[OH]D). This is converted in the kidney, as required, to its active hormonal form 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) in a process which is usually tightly controlled by parathyroid hormone (PTH) and the phosphaturic hormone fibroblast growth factor (FGF)-23 [1]. Briefly, renal $1,25(OH)_2D$ synthesis is activated by PTH and phosphate deprivation. The PTH–vitamin D axis is stimulated by low serum ionized calcium levels, whereas renal 1α-hydroxylation of 25(OH)D is suppressed in case of high serum calcium levels [5]. FGF-23, which is stimulated by hyperphosphatemia, inhibits renal 1α-hydroxylase [6] and stimulates renal 24-hydroxylase-induced inactivation of $1,25(OH)_2D$. [7] Dietary phosphate restriction decreases FGF-23, whereas dietary phosphate loading increases FGF-23 significantly [8].

It is well known that a decline in the glomerular filtration rate (GFR) to less than 30 ml/min/1.73 m^2 is associated with severely depressed circulating $1,25(OH)_2D$ concentrations [9]. This is probably due to the inadequate reserve of the renal 1α-hydroxylase in chronic kidney disease (CKD) stages 4 and 5 [10]. However, upregulated 24-hydroxylase resulting in increased $1,25(OH)_2D$ degradation may also be of importance [11]. Stages 2 and 3 of CKD are associated with a moderate decline in circulating $1,25(OH)_2D$ [12,13], and may explain why mild renal impairment in advanced age is associated with lower circulating $1,25(OH)_2D$ [14,15]. Even in men without CKD, GFR is significantly associated with circulating $1,25(OH)_2D$ [16]. Circulating $1,25(OH)_2D$ also depends on vitamin D status. In case of vitamin D deficiency/insufficiency, renal synthesis of $1,25(OH)_2D$ becomes substrate-dependent – i.e., dependent on the circulating 25(OH)D concentration [17].

A high percentage of the vitamin D metabolites circulating in the blood are bound to vitamin D binding

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TABLE 34.1 Vitamin D Status Classified According to Circulating 25(OH)D Concentrations [19]

Stage	25-Hydroxyvitamin D (nmol/l)	Clinical/biochemical alterations
Deficiency	<25	Rickets, osteomalacia, myopathy, calcium malabsorption, severe hyperparathyroidism, low calcitriol levels, impaired immune and cardiovascular function, death
Insufficiency	25–49.9	Reduced bone mineral density, impaired muscle function, elevated PTH levels, slightly reduced $1,25(OH)_2D$ levels
Hypovitaminosis D/ suboptimal supply	50–74.9	Low bodily stores of vitamin D, slightly elevated PTH levels
Adequacy*	75–372	No disturbances of vitamin D-dependent functions
Intoxication	>372	Intestinal calcium hyperabsorption, hypercalcemia, soft tissue calcification, death

**Does not apply to high-dose oral bolus administration of vitamin D.*

protein (DBP). $1,25(OH)_2D$ acts as the ligand for a membrane-bound and a cytosolic vitamin D receptor (VDR) [2,18–20]. Expression of the VDR has been identified in almost all human tissues [20,21]. Besides the pivotal role in extracellular calcium and phosphate homeostasis and bone metabolism, vitamin D has important pleiotropic effects. Directly or indirectly, 3% of the human genome is regulated by the vitamin D endocrine system and the number of genes known to be regulated by $1,25(OH)_2D$ is still growing [22,23].

34.2.2 Vitamin D Status in Humans

The best indicator for defining human vitamin D status is the circulating 25(OH)D level [21]. That level represents the amount of endogenously produced and orally ingested vitamin D [22]. Circulating 25(OH)D levels are influenced by geographic factors such as latitude and altitude, and individual factors such as skin pigmentation, outdoor activities, sunscreen use, clothing, dietary habits, and body mass index [24–28]. Although there is no definitive consensus regarding the optimal level of serum 25(OH)D, nearly all classifications consider circulating 25(OH)D levels <50 nmol/l and <25 nmol/l as insufficient and deficient, respectively [25,29]. An international expert panel has recommended a target 25(OH)D range of 75–100 nmol/l [29]. These values are within the range of 50–125 nmol/l, which the Institute of Medicine (IOM) has classified to be adequate [30]. However, the IOM considers levels >125 nmol/l as potentially harmful, whereas others deem values up to 372 nmol/l to be safe [1,29]. A classification of the vitamin D status and possible biochemical and clinical alterations is given in Table 34.1. It is, however, also noteworthy to mention that results of 25(OH)D measurements can vary between different vitamin D assays, which may lead to misclassification of human vitamin D status [31–33]. Heijboer *et al*. [32], for example, demonstrated a range of 20% to 52% of intensive care patients to be vitamin D-sufficient, and 24% to 67% of pregnant women to be vitamin D-sufficient, depending on the assay used. To prevent misclassification of human vitamin D status, the adoption of common standards to allow assay calibration is urgently needed [31]. At present, accuracy of vitamin D status classification may be limited if results are summarized from publications that are based on different analytical procedures of 25(OH)D measurement.

Many studies have claimed vitamin D deficiency as a significant worldwide health problem. Hagenau *et al*. reported an average global 25(OH)D level of 54 nmol/l [34], which is close to the insufficiency range. A recently published systematic review found mean values below 50 nmol/l in 37.3% out of 195 studies conducted in 44 countries [35]. The prevalence of insufficient levels (<50 nmol/l) in the US population is 33% [36]. A similar prevalence of vitamin D insufficiency has been reported in Canadians, European adolescents, and Australian adults [37–39]. Despite abundant sunshine and high UVB radiation, 25(OH)D levels below 25 nmol/l are most common in regions such as the Middle East (e.g., Turkey, Lebanon, Jordan, Iran) and South Asia [40–42]. This is most probably due to lifestyle factors such as staying out of the sun, and traditional clothing [4].

Generally, short-term solar UVB exposure or oral supplement use are effective and inexpensive methods for improving vitamin D status. The latter measure is easy to handle in groups such as elderly people and heart failure

TABLE 34.2 Prevalence of Low Vitamin D Concentrations in Heart Failure Patients

Author	Year	Sample size (*n*)	25(OH)D <25 nmol/l	25(OH)D <50 nmol/l	25(OH)D <75 nmol/l
Amin *et al.* [45]	2013	100	n.d.	n.d.	94.0%
Schroten *et al.* [55]	2013	101	n.d.	56.0%	n.d.
Gotsman *et al.* [49]	2012	3,009	28.0%	n.d.	91.2%
Liu *et al.* [54]	2011	548	n.d.	75.0%	n.d.
Zittermann *et al.* [51]	2011	364	n.d.	87.8%	n.d.
Ameri *et al.* [47]	2010	52	66.7%	n.d.	97.8%
Kim *et al.* [53]	2008	289	n.d.	n.d.	83.0%

n.d., no data (available).

patients, whose dietary vitamin D intake and skin synthesis are often limited. Although two compounds (vitamin D2 and vitamin D3) are available for improving circulating 25(OH)D levels, there is evidence that, compared to vitamin D3, vitamin D2 has considerably lower bioavailability due to diminished binding to DPB, non-physiological metabolism, and a shorter shelf-life [43,44]. Consequently, vitamin D3 supplements should be preferred for improving vitamin D status. Usually, daily vitamin D doses of 600–800 international units (IU) are sufficient for achieving a target 25(OH)D level of 50 nmol/l [44].

34.2.3 Vitamin D Status in Heart Failure Patients

Compared with healthy controls, low vitamin D levels are even more common among heart failure patients [45–53]. Table 34.2 summarizes the prevalence of vitamin D deficiency (<25 nmol/l), insufficiency (<50 nmol/l), and hypovitaminosis D (<75 nmol/l) in heart failure patients. While the rates of deficient or at least insufficient 25(OH)D levels vary from 28.0% to 66.7% [47,49] and 56.0% to 87.8%, [51,54,55] respectively, the prevalence of 25(OH)D levels <75 nmol/l rises to 97.8%, thus affecting nearly the whole heart failure community [47]. Low 25(OH)D concentrations are inversely associated with higher New York Heart Association (NYHA) functional classes and impaired left ventricular function [50]. Low 25(OH)D concentrations were found to be associated with an increased risk for all-cause mortality [54]. Thus, vitamin D status seems to be an important prognostic factor in heart failure patients.

Compared with free-living healthy individuals, circulating levels of the vitamin D hormone 1,25$(OH)_2$D are also significantly lower in heart failure patients, especially in younger patients [52]. In endstage heart failure, circulating 1,25$(OH)_2$D levels are inversely related to poor clinical outcome [56].

34.3 HEART FAILURE

34.3.1 Definition and Classification of Heart Failure

The American Heart Association (AHA)/American College of Cardiology (ACC) guidelines define heart failure as "a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood" [57]. The clinical manifestations of heart failure are dyspnea and fatigue. These abnormalities may limit exercise tolerance and fluid retention, which in turn may lead to pulmonary congestion and peripheral edema [57]. Subsequently, this leads to diminished quality of life, and episodes of decompensation leading to hospitalization and premature death, usually due to pump failure or a ventricular arrhythmia [58]. In the majority of patients, the underlying cardiac cause is myocardial disease causing systolic ventricular dysfunction [57,58]. However, disorders of the pericardium, endocardium, great vessels, and heart rhythm, and abnormalities of ventricular diastolic function, can also cause heart failure [57,58]. Based on severity of symptoms and physical activity, heart failure can be classified according to the NYHA functional classification (Table 34.3). This classification is widely used in clinical trials on heart failure [58].

34.3.2 Prevalence of Heart Failure

Heart failure is a major public health problem and enormous medical and societal burden because it is a leading cause of hospitalization [59]. Secular improvements in the treatment of heart failure in combination with the increasing life expectancy are major factors that may increase the caseload of heart failure [60]. In developed countries, 1–2% of the adult population suffers from heart failure [58]. The prevalence rises to ≥10%

TABLE 34.3 NYHA Functional Classification

Class I	No limitation of physical activity
	Ordinary physical activity does not cause undue breathlessness, fatigue or palpitations
Class II	Slight limitation of physical activity
	Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue or palpitations
Class III	Marked limitation of physical activity
	Comfortable at rest, but less than ordinary physical activity results in undue breathlessness, fatigue or palpitations
Class IV	Unable to carry on any physical activity without discomfort
	Symptoms at rest can be present. If any physical activity is undertaken, discomfort is increased

among persons 70 years and older [58,61]. With over 23 million affected individuals worldwide, heart failure is a leading cause of morbidity and mortality [62]. According to the Heart Disease and Stroke statistics report from the AHA, one in nine deaths has heart failure mentioned on the death certificate [61]. The AHA estimates that 5.1 million Americans 20 years and older suffer from heart failure. Projections show that by 2030 the prevalence will increase by an estimated 25% from 2013 [61].

Over the past two decades a broad range of new heart failure therapies has been developed and used, resulting in a fall in mortality rates. By comparing a "historic cohort" (1993–1995) with a "contemporary cohort" (2006–2009) of outpatients with chronic heart failure caused by left ventricular systolic dysfunction, the UK-Heart study [63] indicated a decrease from 12.5% to 7.8% in all-cause mortality at 1 year, which corresponds to an improvement of 37.6%. Another cohort study showed an increase in 5-year age-adjusted survival from 43.0% to 52.0% [64]. Besides new therapies, adherence to evidence-based medicine has been made responsible for these very positive trends.

34.3.3 Vicious Circle of Heart Failure

Untreated heart failure leads to a vicious circle of hemodynamic compensatory mechanisms, which is characterized by progressive worsening of the condition. There are two neurohumoral systems activated in heart failure: the renin–angiotensin–aldosterone system and the sympathetic nervous system [65]. These systems lead to further myocardial injury, and have detrimental effects on the blood vessels, kidneys, muscles, bone marrow, lungs, and liver, leading to a pathophysiological "vicious circle" [65]. This cycle accounts for many of the clinical features, and interruption of the two key processes is the basis of heart failure treatment [66].

CKD is one of the most frequent comorbid conditions in patients with heart failure [59]. Up to 50% of heart failure patients appear to have GFR values <60 ml/min/1.73m^2 [67]. The decreased cardiac output leads to reduced renal blood flow and can thus cause renal dysfunction. Even a slight decrease in GFR is a strong independent predictor of all-cause mortality [67]. In addition, worsening renal function during follow-up is a strong independent predictor of prognosis [67]. However, it is also possible that a primary worsening of renal function (e.g., ischemia, hypoperfusion, glomerulonephritis), resulting in salt and fluid retention and increased filling pressures of the heart [68], can lead to acute cardiac dysfunction (e.g., heart failure, arrhythmia, ischemia) and to cardiovascular events. Consequently, the bidirectional pathophysiological associations between heart failure and CKD have been called cardiorenal syndrome. Often, the primary cause of the syndrome is unclear. A reciprocal negative influence between heart failure and CKD leading to disease progression is obligatory [69].

The kidney is also responsible for the production and secretion of erythropoietin (EPO), a highly glycosylated hormone, which plays a pivotal role in the regulation of red blood cell production in the bone marrow [70,71]. It has been assumed that the reduced kidney perfusion results in a suppressed EPO synthesis. However, clinical studies have demonstrated increased EPO concentrations in patients with more severe heart failure [69], indicating that EPO resistance rather than EPO deficiency is the leading cause of anemia in heart failure patients with CKD [69]. The association of heart failure, renal dysfunction, and anemia has been called the "cardiorenal anemia syndrome," where heart failure may cause progressive renal dysfunction and both may lead to anemia, which in turn can worsen heart failure and renal dysfunction.

34.4 HEART FAILURE AND ANEMIA

34.4.1 Definition of Anemia

According to the World Health Organization (WHO), anemia is a condition in which the number of red blood cells or the oxygen-carrying hemoglobin is insufficient to meet physiological needs. Anemia is a global public health problem. With an estimated prevalence of 24.8%, it affects nearly one in four of the global population, with a more common appearance in preschool-aged children and pregnant and non-pregnant women [72]. The WHO defines anemia as a hemoglobin concentration less than 13 g/dl for men and less than 12 g/dl for women [73], whose appropriateness and clinical applicability has been debated [74]. Some investigators use more conservative definitions (e.g., <12 g/dl for men and <11 g/dl for women). Other definitions do not take gender differences in distribution of hemoglobin values into account. However, the National Kidney Foundation modified its original gender-independent cutoff of ≤12 g/dl to <13.5 g/dl for men and <12 g/dl for women. That way, a higher confidence in capturing the affected patients with CKD can be ensured [71]. Studies in heart failure patients have used these and other study-specific definitions (see Table 34.4).

34.4.2 Prevalence of Anemia in Heart Failure

The estimated prevalence of anemia in heart failure patients varies from 12% to 67% (Table 34.4). This extreme range is partly attributable to the use of inconsistent definitions of anemia [59], although the WHO definition has been used most commonly. In addition, the design of the study has an effect on prevalence. In more selected clinical trial cohorts the prevalence of anemia is lower than estimates reported by observational registries [93]. Furthermore, several clinical characteristics are likely to be responsible for the variability. Besides older age, female gender, race, and locus of enrollment (inpatients or outpatients), the occurrence of comorbid diseases (renal impairment, diabetes mellitus) have a profound influence on prevalence estimates [70,78,93–95] (Table 34.4). In the Val-HeFT database the likelihood of anemia was found to be independently associated with peripheral edema, high brain natriuretic peptide, C-reactive protein, low serum albumen, low diastolic blood pressure, low body weight, renal dysfunction, and diabetes [86]. Therefore, the occurrence of anemia correlates with the severity of the heart failure [69,78,88,96]. While the prevalence of anemia occurs in 17.3% of new-onset heart failure patients [90], it reaches 79.1% in those with NYHA class IV [92]. Thus, the examination of different study cohorts and the use of different anemia definitions lead to the wide variety of prevalence. Groenveld *et al.* [97] performed a comprehensive meta-analysis and reported that 37.2% of a total of 153,160 patients from 34 studies were anemic.

34.4.3 Prognostic Relevance of Anemia in Heart Failure

In heart failure patients, anemia is a common comorbidity and is associated with worse long-term clinical outcomes [70]. The occurrence of anemia in heart failure is an independent predictor for increased risk of hospitalizations and mortality compared to heart failure patients not suffering from anemia [78,86,88,90,94,95,97–99]. In several studies, anemia was associated with a 20% to 50% increased mortality risk [70,78,94,95,97,100]. Anand *et al*. [88] demonstrated that a 1-g/dl increase in hemoglobin is associated with a 14.2% reduction in risk of hospitalization and a 15.8% reduction in mortality risk. However, the correlation of hemoglobin and mortality is not linear [70]. Some studies reported a "J"-shaped relationship between hemoglobin levels and mortality in heart failure patients [98,99]. In these studies, not only levels ≤13 g/dl but also very high hemoglobin levels (≥17 g/dl) independently predicted an increased risk of hospitalization or mortality [69,99].

The cause of the increased morbidity and mortality risk at low hemoglobin levels is unclear at present. In contrast, hemoglobin-induced stimulation of vasoconstriction has been made responsible for the increased morbidity and mortality risk at high hemoglobin levels [69].

In heart failure patients, current anemia treatment strategies include therapy with EPO and other erythropoiesis-stimulating agents (ESAs), intravenous iron therapy, and concurrent treatment with ESA and iron. Iron-deficiency anemia might be due to gastrointestinal malabsorption, uremic gastritis, long-term aspirin use-induced gastrointestinal bleeding [70,101,102], and taking of blood samples [59]. Low iron intake due to low-protein diets and anorexia, especially in coexistence with CKD, could also cause absolute iron deficiency [59]. Iron is essential for erythropoiesis, and therefore it has been postulated that iron supplementation may be beneficial in heart failure patients [102,103].

Early results of treatment of anemia in heart failure have been encouraging. Treatment with subcutaneous EPO and IV iron led to a significant increase in hemoglobin, which was associated with significant improvements in NYHA class and left ventricular ejection fraction [92,104]. In addition, the number of hospitalizations fell significantly [92,104]. Moreover, EPO therapy significantly enhanced exercise capacity in patients with heart failure [105]. However, these small sample-size studies were relatively short in duration and not designed to provide conclusive data on the safety or clinical efficacy of ESA treatment (not blinded, not placebo-controlled).

TABLE 34.4 Prevalence of Anemia in Heart Failure Patients

Study	Year	Sample size (*n*)	Definition of anemia	Prevalence
Conde-Martel *et al.* [75]	2013	1172	Hb <12 g/dl (f, m)	45.1%
Van der Meer *et al.* [76]	2013	1969	Hb <13 g/dl (m)	50.3%
			Hb <12 g/dl (f)	
Klip *et al.* [77]	2013	1506	Hb <13 g/dl (m)	28.0%
			Hb <12 g/dl (f)	
Oster *et al.* [78]	2013	2332	Hb <13 g/dl (m)	55.2%
			Hb <12 g/dl (f)	
Pisaniello *et al.* [62]	2013	1021	Hb <11 g/dl (f, m)	20.3%
Noumi *et al.* [79]	2011	60	Hb <13 g/dl (m)	67.0%
			Hb <12 g/dl (f)	
Okonko *et al.* [80]	2011	157	Hb <13 g/dl (m)	38.9%
			Hb <12 g/dl (f)	
Zittermann *et al.* [51]	2011	364	Hb <13 g/dl (m)	52.6%
			Hb <12 g/dl (f)	
Velavan *et al.* [81]	2010	10,701	Hb <13 g/dl (m)	39.1%
			Hb <12 g/dl (f)	
Adams *et al.* [82]	2009	1082	Hb <13 g/dl (m)	33.9%
			Hb <12 g/dl (f)	
Dunlay *et al.* [83]	2008	1063	Hb <13 g/dl (m)	40.0%
		677	Hb <12 g/dl (f)	53.0%
Tang *et al.* [84]	2008	6159	Hb <12 g/dl (m)	17.2%
			Hb <11 g/dl (f)	
Komajda *et al.* [85]	2006	3029	Hb <13 g/dl (m)	15.9%
			Hb <12 g/dl (f)	
Anand *et al.* [86]	2005	5002	Hb <13 g/dl (m)	22.9%
			Hb <12 g/dl (f)	
Kosiborod *et al.* [87]	2005	50,405	Hct <40% (m)	61.2% (m)
			Hct <37% (f)	52.1% (f)
Anand *et al.* [88]	2004	912	Hb <12 g/dl (f, m)	12.0%
Wexler *et al.* [89]	2004	338	Hb <12 g/dl (f, m)	52.4%
Ezekowitz *et al.* [90]	2003	12,065	ICD-9 codes 280–289	17.3%
Kosiborod *et al.* [91]	2003	2281	Hct ≤37% (f, m)	47.9%
Silverberg *et al.* [92]	2000	142	Hb <12 g/dL (f, m)	55.6%

f, female; Hb, hemoglobin; Hct, hematocrit; ICD, international statistical classification of diseases, injuries and causes of death; m, male.

Therefore, the results have been challenged by proof-of-concept studies like the STAMINA-HeFT study [106]. This Phase II study was randomized, double-blind, placebo-controlled, and designed to evaluate the tolerability and efficacy of treatment with darbepoetin in heart failure patients. Compared to EPO, darbepoetin has two more N-linked carbohydrate sites, which gives it a three-fold longer serum half-life, therefore allowing longer dosing intervals [107]. In this study, treatment effectively raised hemoglobin ≥1.0 g/dl from baseline;

however, the increase was not associated with significant clinical benefits [106]. Therefore, the findings in this multicenter study contrasted with the earlier published data of the small studies. To further explore the potential therapeutic effects of treatment of anemia in heart failure patients, several meta-analyses have been performed [68,108–110]. These analyses suggested a significant reduction in the risk of heart failure hospitalizations [68,109,110]. Analyses found ESA- and IV iron-associated therapeutic effects on parameters of cardiac function, exercise capacity, and quality of life [68,108,109]. No significant protective effect to decrease the risk of all-cause mortality compared to the control group was, however, achieved [108]. Since a meta-analysis is no substitute for an adequately powered randomized trial [110], the recently published randomized, double-blind RED-HF trial [111] evaluated a composite of death from any cause or hospitalization for worsening heart failure in 2278 patients suffering from systolic heart failure and mild-to-moderate anemia. Patients received either darbepoetin or a placebo. Despite increasing hemoglobin values, treatment did not improve clinical outcomes (i.e., heart failure hospitalizations and/or mortality). It has therefore been assumed that anemia may be a marker of disease severity rather than a therapeutic target in patients with heart failure [112].

34.5 VITAMIN D AND ANEMIA

The etiology of anemia in heart failure is not completely understood. In a large cohort of patients with new-onset heart failure, no specific cause of anemia could be identified in 58% of patients. In 21% of patients iron deficiency was diagnosed, while other deficiency states, such as vitamin B12 and folate deficiency, were present in 8% of patients [90]. According to Silverberg, reduced erythropoiesis and inflammatory processes appear to be key factors for anemia in heart failure [113]. Vitamin D deficiency may play a role in several of the suggested causes of anemia in heart failure.

34.5.1 Direct Stimulation of Erythroid Progenitors by Vitamin D

Experimental studies indicate that $1,25(OH)_2D$ can stimulate erythropoiesis in red cell precursor cells via increased erythropoietin sensitivity [114,115]. Alon *et al*. [114] examined the effect of $1,25(OH)_2D$, separately and together with EPO on stem cell proliferation. The authors demonstrated that $1,25(OH)_2D$ synergistically upregulated the proliferative response of hematopoietic cells to EPO. Moreover, data demonstrated that $1,25(OH)_2D$ *per se* has the capacity to upregulate the proliferation of progenitor cells, independent of the presence of EPO. The authors proposed the ability of $1,25(OH)_2D$ to increase EPO receptor expression, at both the mRNA and protein levels, as the underlying mechanisms. Another *in vitro* study confirmed that $1,25(OH)_2D$ improves erythropoiesis by increasing proliferation of erythroid precursors in a synergistic manner with EPO [115]. This was extended by the finding that the effect is dose-related.

Indeed, VDRs have been discovered in several non-renal tissues, including bone marrow [116]. Levels of $1,25(OH)_2D$ in bone marrow are several hundred-fold higher than in plasma [117]. Normalizing 25(OH)D levels may provide an adequate substrate for local tissue production of $1,25(OH)_2D$ in hematopoietic tissues via extrarenal tissue activity of the 1α-hydroxylase enzyme [117] and therefore increased vitamin D-induced erythropoiesis.

34.5.2 Effect on Inflammation

Another important possible mechanism is that vitamin D modulates the level of systemic cytokine production, thus reducing the inflammatory milieu that leads to anemia of chronic disease [117]. Experimental data show that $1,25(OH)_2D$ is able to suppress the release of tumor necrosis factor-alpha (TNF-α) [118,119] and interleukin-6 (IL-6) [118]. While $1,25(OH)_2D$ inhibited the release of these cytokines in a significant dose-dependent manner, 25(OH)D was ineffective. Since 25(OH)D has a multifold less avidity for VDR, the authors suggested that the observed downregulation of cytokine production by $1,25(OH)_2D$ appears to be a VDR-mediated phenomenon [118].

34.5.3 Folate and Iron Absorption

Experimental data indicate that vitamin D also increases intestinal proton-coupled folate transporter, resulting in the therapeutic benefit of enhancing cellular folate uptake [120]. Since only a minority of anemic heart failure patients have low serum folic acid levels [121–123], this approach seems to be of minor importance in heart failure patients. In another experimental study, White Rock chicks were supplemented with 2000 IU vitamin D/100 g of feed and also two oral doses of 5000 IU over a period of 2 days, followed by an oral tracer dose of iron. Data demonstrated that short-term vitamin D treatment increased intestinal iron absorption [124]. The prevalence of iron deficiency has been reported to range up to 21% in heart failure patients [71,125]. Iron participates in the renal activation of 25(OH)D into $1,25(OH)_2D$ [126]. This enzymatic conversion is catalyzed by a cytochrome P450-dependent ferredoxin reductase [9]. Therefore, less available iron can probably compromise the production of $1,25(OH)_2D$, leading to a vicious circle of deficient iron absorption and inadequate synthesis of $1,25(OH)_2D$.

34.5.4 Epidemiological Studies

The assumed association between anemia or EPO resistance and vitamin D deficiency was supported by several epidemiological studies in patients with CKD, heart failure, and in several other cohorts. CKD is a well-known cause of low circulating 25(OH)D [127] and $1,25(OH)_2D$ levels [13].

Studies in CKD patients showed an association between deficient 25(OH)D and/or $1,25(OH)_2D$ levels, low hemoglobin levels, and EPO resistance, which made a higher usage of ESA necessary [117,128–131]. Kiss *et al*. [128] reported a significant correlation between serum 25(OH)D and hemoglobin concentration and the monthly ESA dose in 142 patients on maintenance hemodialysis. While 25(OH)D and hemoglobin concentrations were positively associated, an inverse association was seen between serum 25(OH)D and the monthly ESA dose [128]. The independent association remained significant in both cases, even after statistical adjustments were made for important covariables. In another cross-sectional study in patients with CKD, 25(OH)D and $1,25(OH)_2D$ deficiencies were independently associated with decreased hemoglobin levels [130]. Patients with severe dual deficiency had a 5.4-fold prevalence of anemia compared with those adequately supplied in both. Overall, data suggest that 25(OH)D and $1,25(OH)_2D$ deficiencies are potential risk factors for anemia in CKD patients.

In recent years, the association between anemia and vitamin D deficiency has been found not only in CKD patients but also in individuals not suffering from CKD. A significant association between 25(OH)D deficiency and increased risk of anemia was also found in healthy children [132], in elderly persons aged 70 years and over [133], in HIV-infected Tanzanian women [134], and in pre- and post-menopausal Korean women [135].

In heart failure patients, an independent association of vitamin D deficiency with low hemoglobin values and anemia has been documented as well [51]. Mean hemoglobin concentrations were significantly reduced in the lower tertiles of both 25(OH)D (<18 nmol/l) and $1,25(OH)_2D$ (<40 pmol/l). The odds ratios for anemia of the lowest tertile of 25(OH)D and $1,25(OH)_2D$ were 2.69 and 4.08 compared with their respective highest tertiles (>32 nmol/l; >70 pmol/l). Notably, circulating $1,25(OH)_2D$ was a better predictor of anemia than circulating 25(OH)D. Patients with severe dual deficiency had an OR for anemia of 9.87 compared with those in the highest tertile for both vitamin D metabolites. Another cross-sectional study investigated the association between anemia and circulating 25(OH)D in patients scheduled for cardiac surgery [136]. Anemia risk was highest in the subgroup with severe vitamin D deficiency (<12.5 nmol/l) and lowest in the subgroup with adequate vitamin D levels (50–100 nmol/l).

34.5.5 Intervention Studies

Previous interventional studies in patients with CKD demonstrated an increase in hemoglobin concentration after 12 months of IV $1,25(OH)_2D$ administration and therefore improved control of anemia with reduced need for EPO [137,138]. In a study by Nazem *et al*. in eight hemodialysis patients, IV administration of $1,25(OH)_2D$ decreased the weekly needed EPO dose by 50% [139].

In another study in hemodialysis patients, high-dose oral vitamin D_2 (50,000 IU monthly) has been associated with ESA-dose reductions, while mean hemoglobin levels remained unchanged [129,140]. The benefit was greatest in those patients with 25(OH)D levels <50.0 nmol/l [140]. Since iron storage was unchanged during the study period, the authors assumed that vitamin D2 supplementation may have contributed to improved EPO responsiveness locally in the bone marrow. A recent study evaluated the effect of oral vitamin D2 on the dose of ESA given to children with CKD stage 5 and vitamin D insufficiency [141]. After 12 weeks of vitamin D2 supplementation, the administered ESA dose was significantly lower compared with the initial dose. In a prospective study examining the effect of high-dose alfacalcidol (1α-hydroxyvitamin D) in hemodialysis patients with anemia, hemoglobin levels increased significantly by 2.0 g/dl and 1.8 g/dl at 12 and 18 months, respectively (8.7 ± 1.2 g/dl before treatment versus 10.7 ± 0.9 g/dl; and 10.5 ± 0.6 g/dl) [142].

Nevertheless, the aforementioned intervention studies have some limitations. These studies included only small numbers of subjects and, with one exception [141], no control group. Moreover, they did not assess whether the vitamin D-induced increase in hemoglobin levels was associated with improved clinical outcomes. At present, randomized controlled trials investigating the effect of vitamin D or its metabolites on hemoglobin levels do not exist, either in CKD [143] or in heart failure. Therefore, adequately powered randomized controlled trials with vitamin D metabolites are warranted to assess whether the association between vitamin D deficiency and anemia is causal.

Since the plasma levels of TNF-α, IL-6, and several other pro-inflammatory proteins are increased in heart failure patients and may play a role in the etiology of anemia in heart failure [123], it is of interest that a double-blind, randomized, placebo-controlled trial demonstrated reduced TNF-α levels and increased serum concentrations of the anti-inflammatory cytokine IL-10 by daily vitamin D supplementation of 3300 IU [144].

In a prospective non-randomized study by Gotsman *et al*. [49] in 3009 heart failure patients, vitamin D supplement use (800–1000 IU/day) was associated with reduced mortality risk. The lowest mortality risk was reported in those supplement users who had initial

circulating 25(OH)D levels above the deficiency range, whereas the highest mortality risk was reported in vitamin D non-users with deficient initial 25(OH)D levels.

CONCLUSIONS

Vitamin D deficiency and anemia are prevalent in heart failure, and both are associated with worse clinical outcome. Epidemiological studies suggest a significant association between vitamin D deficiency and anemia in heart failure and other diseases such as CKD. Vitamin D may prevent anemia by direct stimulation of erythroid progenitors, its anti-inflammatory properties, and/or effects on folate and iron absorption. The earlier non-randomized intervention studies reported an increase in hemoglobin concentrations by administration of vitamin D or its metabolites. However, adequately powered, randomized, controlled supplementation trials with vitamin D metabolites are still warranted to assess whether vitamin D and/or calcitriol has beneficial effects on anemia and clinical outcome in heart failure. At present, it must be stated that the role of vitamin D in the prevention and management of anemia in heart failure still remains to be established.

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CHAPTER

35

Immunoprotective Effects of Probiotics in the Elderly

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35.1 INTRODUCTION

It is estimated that more than one-fifth of the world's population will be elderly by the year 2050 [1]. In the United States, those over the age of 85, constitute the fastest growing segment of the population [2]. These dramatic demographic changes bring about new concerns for the health of the aging. Over the past century, medical advancements have significantly increased the number of years that people are living by reducing the prevalence of communicable diseases. However, these advances have also created a shift, making chronic, non-communicable diseases the leading causes of morbidity, mortality, and increased healthcare costs [3]. It is now critical that the medical community looks towards making this extension of time a healthy one. Improved health and well-being in advanced ages will not only help people survive even longer, but also increase quality of life and function.

Aging is considered a process of physiological, economic, social, and psychological factors; however, it is the physiological changes that are most dramatic and scientifically perplexing. Possibly one of the most significant changes in aging, yet one whose intricacies and implications remain a mystery, is the drastic modification in the composition and variability of the large innate community of gastrointestinal (GI) bacteria. This gastrointestinal microflora, or "microbiota," residing in the human GI tract has been recognized to play a profound role in human health and aging. The bacteria that reside in the human gut fulfill a broad assortment of metabolic and functional tasks to benefit the host. The gut microbiota is involved in signaling processes that may have vast implications in inflammation, immunity, overall health, and aging. Modern science has just begun to reveal the significance of these age-related alterations in the gut microbiota, and how they may affect the elderly and their health.

35.2 THE HUMAN GUT MICROBIOTA

The human gastrointestinal tract is the natural habitat for a large and dynamic ecosystem of over 1000 different species of bacteria [4]. Approximately 100 trillion (10^{14}) bacterial cells make up this community – about 10 times the total cells of the human body, and weighing more than 1 kilogram [5–7]. Similarly, the genetic material of these bacterial cells towers compared to our own genetic material. The gastrointestinal tract harbors bacteria throughout its length, although the microbial density significantly increases towards the large intestine, which holds the majority of the gut bacteria. It is reported that the stomach and duodenum contain approximately 10^1–10^4 bacterial cells, the jejunum and ileum between 10^4 and 10^8 cells, and the colon and feces up to 10^{10}–10^{12} cells [8].

It was previously thought that, before birth, the fetus' gastrointestinal tract was sterile. Bacterial colonization of the gut was thought to begin at birth with the exposure of the infant to the mother's bacteria in the vaginal canal, and with exposure of the newborn to the environment. However, new evidence has revealed that there may be maternal–fetus microbiota transfer *in utero*, demonstrated by the presence of bacteria in amniotic fluid, placental membranes and meconium, or the first stool [9]. This vertical transmission of gut bacteria from mother to fetus is hypothesized to dramatically influence the

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development and maturation of the infant's immune system [10]. Interestingly, the bacterial composition of the meconium is altered depending on maternal health, specifically demonstrated in diabetic versus non-diabetic mothers [11]. After birth, an infant's microbiota continually matures through the exposure to different bacteria in the environment as well as maternal bacteria from breast milk.

The gut microbiota varies substantially during the first 1–3 years of life. After the age of 3, it becomes more stable and mature [3]. A less drastic, continuous evolution of the intestinal microbiota occurs throughout the lifespan, and factors that are involved in this evolution include an ever-changing physical environment, diet, disease, physical and mental stress, antibiotics, and pathogenic microorganisms.

The ecosystem of gastrointestinal bacteria, or "microbiota," provides the first line of defense between the body's internal environment and the external environment. Every day the gut is exposed to thousands of external compounds and microorganisms that present a constant challenge to the host, making the barrier integrity of the gut critically important to health [12]. Hence, the bacteria of the microbiota literally lie on, or "hover" over, the epithelial cells of the gastrointestinal lining in a protective fashion. The microbiota also maintains a tight balance between symbiotic and pathogenic organisms within the gut, thus ensuring the protection of the host against harmful compounds and microbes. This balance of organisms influences the pH of the lumen of the gastrointestinal tract to discourage pathogenic bacteria survival. It has also been demonstrated that the bacteria within the microbiota can strengthen the gut barrier integrity by supporting the tight junctions between intestinal epithelial cells, upregulating mucous production, and stimulating immunoglobulins such as IgA and antimicrobial peptides secreted by these cells [13].

The microbiota's interaction and communication with the gastrointestinal lining also involves a more sophisticated protection of the host. Specialized cells within the epithelium, including dendritic cells and specialized epithelial cells called "M cells," constantly "sample" the intestinal lumen content, including the resident bacterial environment. These unique cells, part of specialized immune tissue called gut associated lymphoid tissue (GALT), have the ability to discriminate pathogenic from commensal bacteria. When necessary, these cells present pathogenic antigens to naïve T lymphocytes within the GALT, which activates a further adaptive immune response [14]. GALT is one of the largest lymphoid organs of the body, containing up to 70% of the body's total immune cells, and the largest part of the human immune system [12,15]. GALT is associated with the adaptive immune response, a highly specialized immune reaction that involves antibody and cell-mediated (B and T cell) immune responses. GALT, epithelial cells, and the gastrointestinal microbiota communicate in a complicated and intelligent manner, creating a strong relationship between gut microbiota and overall host immunity.

Besides protecting the host, the gut microflora also participates in metabolic activities that result in the salvage of energy and absorbable nutrients, produces certain vitamins including vitamin K and B vitamins, and has important trophic effects on intestinal epithelia. The gut bacteria hydrolyze complex plant polysaccharides and produce compounds called short chain fatty acids (SCFAs). SCFAs are considered "food" for colonocytes, the intestinal epithelial cells of the gut, and also may play a role in lipid and carbohydrate metabolism.

The numerous effects that the gut microbiota lends to its host beyond these activities are coming to light. It is suggested by current research that the intestinal microbiota may play a more sophisticated role in immune function, inflammatory responses, and metabolic processes such as blood sugar and lipid control [15]. It is also suspected that a healthy microbiota can protect against a vast number of age-related and inflammatory conditions, such as obesity, diabetes, cardiovascular disease, certain cancers, and more. The Father of Medicine, Hippocrates, may have been ahead of his time when he advised centuries ago that "all diseases begin in the gut."

35.3 AGING AND THE GUT MICROBIOTA

Although there is an evolution of the microbiota during the lifespan, the intestinal microbiota is relatively stable from year 3 onwards. Significant changes, however, occur around the seventh decade of life. Scientific studies have revealed that the composition of the intestinal microbiota of the elderly is very different from that of younger adults [16]. These compositional changes are thought to be detrimental to the elderly host's health. The reasons for the dramatic change in the gut microbiota in the elderly years can be attributed to various natural physiological changes in the gastrointestinal tract that occur with aging, as well as common lifestyle changes that may influence the gut.

35.3.1 Physiological and Lifestyle Changes

Various physiological gastrointestinal changes are known to commonly occur in the older years. All of these changes can contribute to an altered habitat for microbiota. Enteric neurodegeneration (loss of nerve functioning in the gut) is widespread in the elderly, and can cause common clinical issues such as chronic constipation and delayed gut transit time, incontinence, and

evacuation disorders [18–20]. Additionally, secretory activity is naturally decreased during aging, therefore, maldigestion and conditions such as hypochlorhydria (low HCl) and atrophic gastritis are also common in the elderly.

A slow yet drastic change in the diet also occurs in the elderly, often due to decreases in taste and smell, decreased dentition, ill-fitting dentures, and swallowing difficulties. Additionally, variation in foods also tends to be less in the elderly population, particularly when the person is in a community home [21].

The diet is a major contributor to the gut microbiota and influences it continually. For example, populations that have a diet high in animal protein and fat, such as that of many Americans, tend to promote greater *Bacteriodes* bacteria in the gut compared to those with diets high in carbohydrates and low in animal protein and fat, such as African children, where *Prevotella* bacteria are predominant [22,23]. Other lifestyle changes, such as a decrease in physical activity, or a decrease in exposure to various bacteria when someone is less active outside the home, can also cause changes in the gut bacteria.

An increase in antibiotic use is also common among the elderly. Antibiotic therapy is known to have a detrimental effect on the intestinal microbiota, and chronic use can have a negative effect on overall bacterial diversity. Antibiotics are known specifically to reduce *Bacteriodes*, *Bifidobacterium*, *Desulfovibrio*, *Clostridium*, and *Faecalibacterium* spp. [21,24]. One study indicated a sevenfold decrease in *Bifidobacterium* species (a bacteria considered to be beneficial to the host) in the elderly after antibiotic therapy. The most dramatic changes in this species were from nucleic acid synthesis inhibitor antibiotics [21]. Antibiotic use is associated with an increased risk of *C. difficile* acquisition – one of the most common causes of acute diarrheal illness in long-term community settings, the symptoms of which can range from mild diarrhea to life-threatening colitis [25]. Although most *Clostridia* bacteria have a commensal relationship with the host, some, such as *C. difficile*, possess pathogenic properties. A retrospective study conducted in Quebec indicated that the incidence of *C. difficile* infections in the elderly increased 10-fold from 1991 to 2003. The factors that were considered to have contributed to this increase were an increase in the mean age of patients, as well as antibiotic use [26]. While antibiotic use alone is considered "not sufficient" to result in the acquisition of *C. difficile*, antibiotic use, particularly broad spectrum antibiotics (and especially cephalosporins), increases the risk by altering the gut microbiota, and the risk of acquiring *C. difficile* without associated antibiotic use is considered "negligible." In fact, one study found that the use of narrow-spectrum antibiotics, in place of broad-spectrum antibiotics, reduced the incidence of *C. difficile* infections [27].

35.3.2 Microbiota Alterations in the Elderly

Two major microbial phyla – *Firmicutes* and *Bacteroidetes* – represent 99% of the bacteria in the gut. Even when taking large individual variation into account, the levels of these two dominant bacterial divisions, are known to shift dramatically with aging. It has been shown that the *Firmicutes* to *Bacteroidetes* ratio decreases from approximately 10.9 in middle-aged adults to 0.6 in the elderly [3]. This dramatic shift is one of the few age-related changes to the gut microbiota that has been consistent in human observational trials, and some have suggested that it could possibly be a parameter in assessment of the health of the gut microbiota [28].

In addition to the changes in bacterial phyla, there are also vast, yet variable, changes within the lower levels of bacterial classifications (order, genus, and species) in the elderly. There are a few common findings, however, that have been reproducible in scientific studies. For example, a significant decrease in *Bifidobacterium* and *Lactobacillus* bacteria has commonly been reported with aging [29–32]. However, newer reports, using updated identification techniques, have indicated that *Bifidobacterium* levels in the elderly are similar to those in younger people [33]. It is commonly found, however, that the diversity of species within these genera is negatively impacted in the elderly. While four or five different *Bifidobacterium* may reside in adults, only one to three species may be found in the elderly [33]. Specifically, *Bifidobacterium angulatum*, *bifidum*, *longum*, and *adolescentis* have been found to be more predominant in the elderly [4]. Accordingly, *Lactobacillus plantarum*, *paracasei*, and *reuteri* species have been shown to be more prevalent in the elderly compared to healthy adults, who have higher levels of *Lactobacillus acidophilus* and *helveticus* [32]. Additionally, studies appear to agree that there are age-related increases in facultative anaerobes, such as *Streptococci*, *Enterococci*, and *Enterobacteria* [28,34].

The majority of the bacteria in the phylum *Firmicutes* fall into two main groups; the *Clostridium coccoides* group (also known as *Clostridium* cluster XIVa) and the *Clostridium leptum* group (also known as *Clostridium* cluster IV). *Clostridium* counts in general have been found to be significantly higher in the elderly; however, *Clostridium* clusters XIVa have been shown to be significantly reduced in the elderly [35]. The bacteria in *Clostridium* cluster XIVa play major roles in the fermentation of carbohydrates within the gut [28]. The major end products of this fermentation in the gut are short-chain fatty acids (SCFAs). Butyrate, one of the best-studied SCFAs, is the main source of nutrition for cells of the gut epithelium. Depletion of butyrate is associated with impairments in the gut barrier integrity. A decrease in *Clostridium* clusters XIVa bacteria can result in a decrease in intestinal fermentation, and, theoretically,

fewer SCFAs, or "food," for the intestinal epithelial cells. SCFAs have also demonstrated anti-inflammatory and immunomodulatory properties [28].

Despite these recognized changes, scientists believe that it is the age-related overall decrease of microbial diversity in gut bacteria that is most detrimental, rather than alterations in one specific species over another. The age-related changes in the composition of the microbiota have been shown to be highly individual, as well as diet and geographically influenced [7,28]. Additionally, variations in bacterial identification techniques and the presence of an estimated >1000 different species of gut microbiota have made it difficult to find consistent age-related changes in specific genera or species in the elderly's microbiota composition [7]. Further understanding of these specific age-related changes of the microbiota, however, could open doors to finding specific, individualized disease treatments, and provide a strategy for the prevention of disease and for healthy aging.

35.4 AGING AND INFLAMMATION

Inflammation is a biological process intended to be a protective response to harmful stimuli. It is a natural yet highly regulated process that is self-limiting and involves minimal damage to surrounding tissues [36]. Chronic inflammation, however, is believed to be pathogenic and unregulated, and can cause unwarranted tissue injury or damage. Inflammation involves a "domino effect" process involving highly regulated cytokines, or messenger molecules, that influence other messenger molecules and pro-inflammatory mediators, and stimulate various cell activities. For example, the activation and control of the polymorphonuclear cell (PMN) is pivotal in regulating inflammation and tissue damage. Signaling molecules, such as prostaglandin E2, control the macrophage's ability to destroy PMNs, thereby controlling the inflammation response [36]. The ability to control this process by releasing counteracting signals, and individual cells' response to these molecules, is crucial and can be the difference between acute versus chronic inflammation. However, the complexity of this messaging process makes it difficult to precisely control through external therapies.

The process of aging is accompanied by the presence of a chronic low-grade inflammatory state, sometimes referred to as "inflammaging" [36,37]. Unfortunately, this chronic inflammation is associated with detrimental effects. The elevation of the inflammation markers C-reactive protein (CRP) and interleukin-6 (IL-6) in the elderly has been associated with an increase in morbidity and mortality [36]. Chronic inflammation has also been associated with many chronic, age-associated diseases, such as cardiovascular disease, arthritis, atherosclerosis, and type 2 diabetes. Cognitive disorders, such as dementia and Alzheimer's and Parkinson's diseases, are also diseases influenced by inflammatory processes [38,39]. Not only do the elderly tend to have an overall general inflammatory state, as indicated by an elevation of systemic CRP levels; they also commonly have low-grade, asymptomatic bowel inflammation [36]. Calprotectin, a marker of gut inflammation, is often elevated in the elderly, and is correlated with increased levels of systemic (serum) CRP levels [3].

The origins of inflammation-mediated aging are not entirely clear. It is also not known whether this inflammatory state reflects a normal deterioration of cells or is a result of disease processes that could be prevented and/or treated [2]. However, it is clear that gut bacteria play a role in inflammation. Several gut bacterial species in the *Faecalibacterium*, *Bifidobacterium*, and *Lactobacillus* genera, which are known to be decreased in the elderly, have also been shown to attenuate inflammation at the level of the gut epithelium [40–43]. Specifically, *B. infantis* and *L. salivarius* were shown to attenuate IL-8 secretion and *S. typhimurium*-induced pro-inflammatory responses *in vitro* [44]. Additionally, *Clostridia* spp. have been found to play a role in inflammation. Some of these bacteria have been shown to induce defined T cell subset responses, specifically T helper 17 (Th17) cells, which are major mediators of "host-destructive pathogenic inflammation" [3]. Therefore, an imbalance in gut microbiota may reduce the regulation of these cells, and increase inflammation. Other studies on the elderly indicate that markers of inflammation, including TNF-α, IL-6, IL-8, and CRP, are intricately associated with changes in gut microbiota [17].

Faecalibacterium prausnitzii, a *Clostridium* cluster IV bacteria, has been shown to have potent anti-inflammatory properties *in vitro* and *in vivo* [41]. In the elderly, *F. prausnitzii* levels are known to be commonly lower than those of healthy adults, and this decrease is associated with frailty, hospitalization, antibiotic therapy, and anti-inflammatory therapy [7,35,45,46].

The associations between gut bacteria and their involvement in regulating the inflammatory response makes it is quite plausible that "inflammaging" could be suppressed via supporting a healthy gut microbiota.

35.5 THE GUT MICROBIOTA AND IMMUNOSENESCENCE

An age-associated decline in immune function, referred to as immunosenescence, appears to affect both the innate and adaptive immune responses. However, while the innate immunity appears to be only slightly affected or sometimes even enhanced, the more sophisticated adaptive immunity, consisting of B cells and T cells, declines. The thymus gland, a critical part of the adaptive

immune system, regresses as part of the aging process in mammals as well as other life forms [47]. Additionally, its ability to produce functional naïve T cells decreases. The elderly often have a decrease of mature T cell numbers (specifically CD4+ cells), a decrease in the functionality changes of NK and dendritic cell proportions, and a loss of diversity of B cells [37,48]. Immunosenescence also contributes to poor vaccine efficacy, increased susceptibility of the elderly to infection, and increased morbidity and mortality from these infections [49]. Gastrointestinal infections, in particular, are several hundred times higher in the elderly compared to young adults [38].

Elderly dysbiosis (an unfavorable shift in the intestinal microbiota) is thought to contribute to immunosenescence [16]. A 2005 clinical study revealed a significant association between fecal microbiota and frailty scores in the elderly [45]. Frailty, a state of a significant decrease in physiological reserves in core functioning, is also associated with a decrease in immune health. Scientists found that in those with a high frailty score (as assessed by the Groningen Frailty Indicator), a significant reduction in the number of *Lactobacilli*, *Bacterioides*, and *Faecalibacterium prausnitzii* was also seen. On the other hand, levels of *Enterobacteriaceae* spp. were significantly higher in the frail elderly. These results indicate that frailty and the associated loss of immune function may be influenced by an alteration of the intestinal microbiota.

Poor vaccine responses, indicating a decline in immune response, are also often observed in the elderly, yet supplementation with beneficial gut bacteria has been shown to enhance the immune response to vaccination [50,51]. In another study, a higher presence of the beneficial bacteria species *Lactobacillus reuteri* in fecal samples of healthy elderly subjects was correlated with a significantly increased white blood cell (WBC) count after adjusting for age, sex, and BMI [52]. Studies like these have started to reveal the microbiota's complex, yet integral, role in immune system functioning in the elderly.

35.6 PROBIOTICS IN THE ELDERLY

Over a century ago, the Nobel Prize-winning Russian microbiologist Elie Metchnikoff theorized that manipulating the intestinal microbiota could prolong life and improve the aging process in his 1908 publication *The Prolongation of Life* [53]. Elie Metchnikoff attributed the long life of Bulgarian peasants to their intake of yogurt containing *Lactobacillus.* With the development and popularization of antibiotics in the 1930s, however, the focus on beneficial gut bacteria and human health declined. Within 20 years, antibiotic resistance was beginning to occur and by the 1950s a focus on beneficial gut bacteria was re-emerging and yogurt with live bacteria was recognized to alleviate gastrointestinal disturbances [54]. Most recently, gut bacteria and its role in the health of humans has re-emerged as a growing field of interest. Current day research has revealed a complicated relationship between aging, the intestinal microbiota, and the decline in immune function that occurs with age.

The strongest evidence of probiotic supplementation's positive influence in the elderly has been human clinical research conducted on its beneficial effects on the immune system. The term "probiotic" literally means "for life," and the term is typically referring to dietary supplements or food containing beneficial live gut bacteria, intended to enrich the gut microflora and offer health benefits.

Probiotic supplementation has been shown to reduce the risk of infection by enhancing certain bacterial populations in the gut, decreasing intestinal pH, and reducing the ability of bacteria to adhere to the mucosal walls. Probiotics have also been shown to reduce or negate some of the age-related effects on the immune system in the elderly. Specifically, probiotics have demonstrated the ability to enhance the activities of various immune cells, reduce inflammation, significantly increase immune responses to vaccines, and reduce the incidence and duration of certain illnesses (see Table 35.1 [33,48–50,55–63]).

Although research has been conducted on a wide variety of bacteria and doses, the majority of research has been conducted on *Lactobacilli* and *Bifidobacterium* species and suggests that various species of bacteria within these genera can beneficially affect the immune system functioning in the elderly.

35.7 ELDERLY GUT CARE

Diet is known to play a central role in influencing the composition of the gut microbiota and unfortunately, due to many common physiological and lifestyle changes, malnutrition is common in the elderly years. Therefore, it is quite plausible that a major contributor to the drastic microbiota changes found in the elderly is alterations in the diet. The elderly should thus be encouraged to consume a diverse diet – one with adequate intakes of macro- and micronutrients, specifically fiber and antioxidant/anti-inflammatory vitamins and minerals, such as vitamins C and E. Diets that include antioxidant and anti-inflammatory foods are also important for the elderly to reduce oxidative damage and overall inflammation. A varied diet based on whole grains, fruits, and vegetables can promote an anti-inflammatory state, a diverse and healthy gut microbiota, and healthy GI functioning.

Community living also enhances the health of the elderly, likely in part due to more bacterial exposure, and therefore a more diverse gut microbiota. It appears that these populations (compared to long-term residential care) have better diets, more SCFAs, less inflammation,

TABLE 35.1 Human Clinical Trials on Elderly Probiotic Supplementation and Immune Function

Study	Number of participants	Bacteria	Dose	Length of study	Primary outcomes	Results
Maneerat *et al.*, 2013 [55]	$n = 36$	*B. lactis* Bi-07 powder, prebiotic, combination, or placebo	10^9	3 weeks	Phagocytosis and oxidative burst by monocytes and granulocytes Various cytokine and chemokine concentrations	Significant improvement of phagocytic activity of monocytes and granulocytes in probiotic group compared to other groups Cytokine and chemokine levels were unaffected by treatment
Akatsu *et al.*, 2013 [56]	$n = 45$; long-stay elderly patients fed by enteral tube feeding, aged >65 years	*B. longum* BB536 powder vs placebo	10×10^{10}	12 weeks	A/H1N1 antibodies VIgM, IgA and IgG levels, and NK cell activity	Significantly higher A/H1N1 antibodies after 6 weeks in those taking the probiotic No significant changes were seen in immunoglobulin levels between groups or NK activity
Akatsu *et al.*, 2013 [49]	$n = 15$; elderly nursing home residents, mean age 75	Jelly containing *L. paracasei* MoLac-1 vs placebo	10^7	12 weeks	A/H1N1, A/H3N2, and B viral antigen response	A/H1N1 and B hemagglutination inhibition titers were significantly higher in those consuming the jelly after immunization
Bosch *et al.*, 2012 [50]	$n = 60$; institutionalized volunteers, aged 65–85	*L. plantarum* CECT 7315/7316 in powdered skim milk (low dose and high dose) or placebo	5×10^8 or 5×10^9	3 months	Influenza-specific IgG, IgA, and IgM	Influenza-specific immunoglobulin (IgG) levels were significantly elevated in the high-dose group compared to the other two groups Influenza-specific IgA levels were significantly elevated in high- and low-dose groups compared to placebo
Shinkai *et al.*, 2013 [57]	$n = 300$; healthy elderly adults aged >65	*L. pentosus* b240 (low and high dose) tablets or placebo	2×10^9 or 2×10^{10}	20 weeks	Incidence of common cold Quality of Life (QOL)	Significant dose-dependent reduction in the incidence of common cold in those taking probiotic (39% and 26% reduction in high- and low-dose groups, respectively) General health perception subscale of QOL scores significantly improved in those taking the probiotic
Mañe *et al.*, 2011 [48]	$n = 50$ institutionalized elderly	Powdered skim milk containing *L. plantarum* CECT 7315/7316 (low and high dose) or placebo	5×10^8, 5×10^9	12 weeks	Leukocyte subpopulations and cytokine levels	High dose resulted in significant increases in the percentages of T-suppressor (CD8+ and CD25+) and NK (CD56+ and CD16+) cells. Low dose resulted in increased T helper lymphocytes (CD4+ and CD25+), B lymphocytes (CD19+), and antigen presenting cells (HLA-DR+) A significant decrease in the immunological cytokine TGF-β1 concentrations was found in both probiotic dose groups compared to placebo
Nagata *et al.*, 2011 [58]	$n = 77$; long-term institutionalized elderly, mean age 84	A probiotic-fermented milk containing *L. casei* Shirota (LcS) or no administration	4×10^{10}	3 months	Prevalence of norovirus gastroenteritis and duration of symptoms	Duration of fever of >37°C was significantly reduced; however, the incidence of norovirus gastroenteritis was not lowered in those taking the probiotic

Makino *et al.*, 2010 [59]	Two studies; $n = 57$ $n = 85$	Yogurt fermented with *L. delbrueckii* spp. *Bulgaricus* OLL1073R-1 and *Streptococcus thermophiles* OLS3059	$2.0–3.5 \times 10^8$ and $6.3–8.8 \times 10^8$, respectively	8 weeks and 12 weeks	Incidence of common cold and influenza NK cell activity and lymphocyte blastoid transformation by concanavalin A (ConA)	Meta-analyses of both trials indicated a lowered risk of catching the common cold or influenza virus; approximately 2.6-fold lower in the yogurt group compared to placebo Lymphocyte blastoid transformation induced by ConA increased in both groups, but significantly greater in yoghurt group
Guillemard *et al.*, 2009 [60]	$n = 1072$; free living elderly (>70 years)	Dairy drink containing *L. casei* DN-114001, *S. thermophiles* and *L. delbrueckii* subsp. *bulgaricus*	10^{10}	3 months; 1 month follow-up	Incidence and duration of upper and lower respiratory or gastrointestinal infections	The yogurt significantly reduced the average duration of common infectious diseases such as upper and lower respiratory and gastrointestinal infections
Ouwehand *et al.*, 2008 [33]	$n = 209$; institutionalized elderly	Oat-based drink containing *B. longum* 2C and 46, *B. lactis* Bb-12, or placebo	10^9 (each species)	6 months	Levels of pro-inflammatory cytokines: TNF-α, TGF-β1 and IL-10	Higher levels of specific *Bifidobacterium* species were associated with lowered TNF-α and IL-10.
Gill *et al.*, 2001 [61]	$n = 27$; healthy adults aged 60–84, crossover trial	Milk powder containing *L. rhamnosus* HN001 or *B. lactis* HN019	5×10^{10} (*L. rhamnosus*) or 5×10^9 (*B. lactis*)	3 weeks	NK activity CD3+/CD56+ ratio *In vitro* tumoricidal activity of PBMCs	CD56+ cells were significantly increased with both probiotic species *Ex vivo* PBMC tumoricidal activity significantly increased with both probiotic species
Gill *et al.*, 2001 [62]	$n = 30$ healthy elderly, aged 63–84	Low-fat milk powder containing *B. lactis* HN019 (low dose or high dose)	5×10^9, or 5×10^{10}	6 weeks	CD4+/CD25+ Natural killer cells PMN and natural killer cell activity	Significant increases in CD3+, CD4+, and activated CD25+ T lymphocytes and CD56+ NK cells after probiotic supplementation Increased activities of cellular immune function were 14.5% to 61.8%, with the greatest effect on NK cell activity
Arunachalam *et al.*, 2000 [63]	$n = 25$ healthy elderly (aged 60–83)	Milk containing *B. lactis* HN019 or placebo milk	3×10^{11}	6 weeks	IFN-α levels Phagocytic capacity and bactericidal activity of PMN cells	Significantly increased production of IFN-α at week 6 Significantly enhanced phagocytic capacity of PMN cells

and less frailty [16]. Physical activity is also often minimal in the elderly. Exercise brings blood flow to the gut and has been shown to lower inflammation in the elderly, so is of utmost importance [64].

Probiotic consumption/supplementation has been shown to improve many aspects of aging, and should also be encouraged in the elderly. Modulation of the intestinal microbiota via probiotic supplementation may be able to prevent the pathogenic bacterial overgrowth that is commonly found in the elderly by reducing luminal pH, enhancing the secretion of antimicrobial compounds, preventing bacterial adhesion, and enhancing the immune response of the host. Most probiotics currently sold provide the *Lactobacillus* and *Bifidobacterium* genera of bacteria; however, further research should identify specific age-related changes in the gut microbiota and interindividual responses to probiotics to provide a more specific treatment to the elderly. For example, because an age-related inflammatory environment is known to exist, it is possible that administration of bacteria such as *F. prausnitizii*, a newly identified anti-inflammatory probiotic bacteria, may be suitable for probiotic products marketed for the elderly.

Probiotic supplementation in the form of a dietary supplement is a simple way for the elderly to consume beneficial bacteria. It is generally recommended that a supplement contains at least a billion living organisms per serving to be effective. In lieu of formulation specifics for the elderly, it is also generally recognized that a broad range of bacterial species most likely offers a broader range of benefits. The species and strains that have been shown in clinical trials to have a positive effect on the immune system of the elderly are good to seek out. Currently, there is also a wide variety of functional foods available in the marketplace, including probiotic-enhanced foods and drinks that are easy to consume and digest, that may benefit the elderly. Probiotic-enhanced milk, juices, coffee, cereals, energy bars, and even chocolate bars have become commonplace in grocery stores. Fermented foods that naturally contain live bacterial cultures are another option: yogurt, certain cheeses, crème fraiche, kefir, sauerkraut, pickles, miso, tempeh, kimchi, or Kombucha can also be incorporated into the elderly diet. A more focused, research and population-based approach to these products in the future is likely to provide the most benefit to the aging community; however, the wide variety of products on the market today offers opportunities for convenient probiotic supplementation that is likely to offer health benefits to the elderly.

35.8 CONCLUSION

The pronounced age-related disruptions to the complicated ecosystem of the human gut microbiota are well recognized. Analysis of the human GI microbiota genome is currently the subject of intense research. Large-scale projects, such as the US Human Microbiome Project (HMP) of the National Institutes of Health (NIH), are working toward the development of a reference set of genome sequences and characterization of the human microbiota, as well as investigating the relationship between disease and the changes in the microbiota (http://commonfund.nih.gov/hmp/overview). Moreover, recent advances in gene sequencing techniques of bacteria are furthering our intelligence of this complicated ecological system.

Although great strides have been made in human microbiota research, there is still a vast amount of uncharted territory in the complex relationship among the gut microbiota, human health, and probiotic supplementation. More information regarding the composition of a possible "core human microbiota," as well as interindividual, geographical, and age- and diet-related variations, is critical to understanding the details of how this internal ecosystem relates to health, immunity, and aging.

The currently available research, however, provides strong evidence of the correlation between the age-related effects on the microbiota and the vulnerability to disease in the elderly, indicating a great need for gastrointestinal health education in this age group. Additionally, the supportive scientific evidence for probiotic consumption/supplementation for the health of elderly people's gastrointestinal and immune system and the high safety profile of probiotics makes them an attractive preventive and therapeutic agent. Modulation of the gut microbiota through consumption of probiotic bacteria or functional foods that contain probiotic bacteria offers a safe, inexpensive, widely available, preventive treatment that may offer an opportunity to preserve health in old age.

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