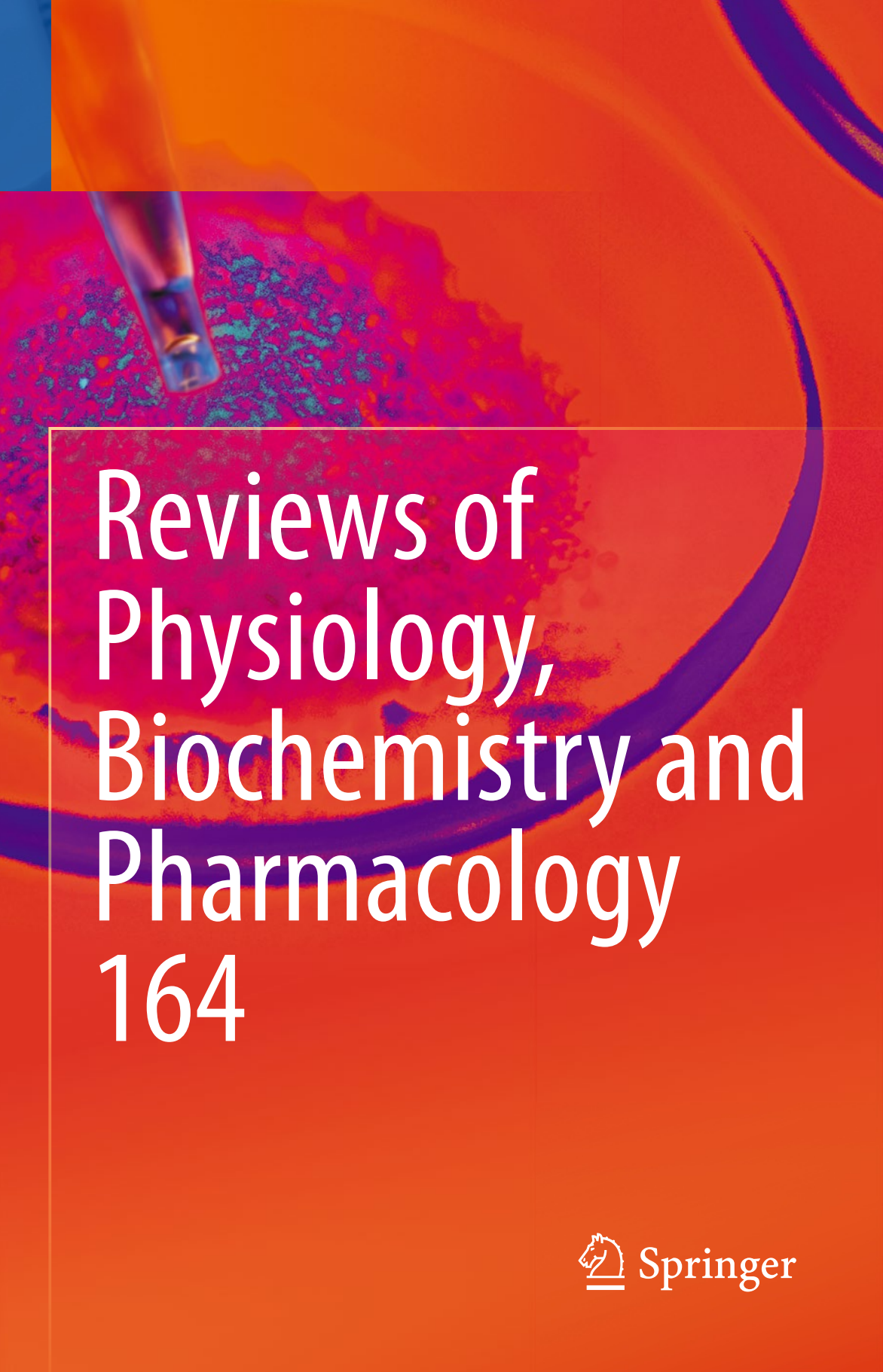
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Contents

Spices: The Savory and Beneficial Science of Pungency

Bernd Nilius and Giovanni Appendino

"In the Beginning Was the Spice." S. Zweig, "Magellan", 1938

Abstract Spicy food does not only provide an important hedonic input in daily life, but has also been anedoctically associated to beneficial effects on our health. In this context, the discovery of chemesthetic trigeminal receptors and their spicy ligands has provided the mechanistic basis and the pharmacological means to investigate this enticing possibility. This review discusses in molecular terms the connection between the neurophysiology of pungent spices and the "systemic" effects associated to their trigeminality. It commences with a cultural and historical overview on the Western fascination for spices, and, after analysing in detail the mechanisms underlying the trigeminality of food, the main dietary players from the transient receptor potential (TRP) family of cation channels are introduced, also discussing the "alien" distribution of taste receptors outside the oro-pharingeal cavity. The modulation of TRPV1 and TRPA1 by spices is next described, discussing how spicy sensations can be turned into hedonic pungency, and analyzing the mechanistic bases for the health benefits that have been associated to the consumption of spices. These include, in addition to a beneficial modulation of gastro-intestinal and cardio-vascular function, slimming, the optimization of skeletal muscle performance, the reduction of chronic inflammation, and the prevention of metabolic syndrome and diabetes. We conclude by reviewing the role of electrophilic spice constituents on cancer prevention in the light of their action on

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pro-inflammatory and pro-cancerogenic nuclear factors like NFκB, and on their interaction with the electrophile sensor protein Keap1 and the ensuing Nrf2-mediated transcriptional activity. Spicy compounds have a complex polypharmacology, and just like any other bioactive agent, show a balance of beneficial and bad actions. However, at least for moderate consumption, the balance seems definitely in favour of the positive side, suggesting that a spicy diet, a caveman-era technology, could be seriously considered in addition to caloric control and exercise as a measurement to prevent and control many chronic diseases associate to malnutrition from a Western diet.

Contents

1 Introduction

Chemesthesis is the sensation induced by the chemical activation of gustatory receptors others than those for taste and odor. These receptors mediate pain, touch, texture (mechanical), and thermal perception, substantially modifying what is perceived as "food taste". The notion that the same food tastes differently when warm or cold, when shredded or coarse, when plain or seasoned with tiny (catalytic, in the lingo of chemistry) amounts of spices may seem a truism, but its molecular bases and its implications are not. In general, the acceptance of food depends not only on taste, but also on olfactory, tactile and visual cues, as well as on memories of

previous, similar experiences and social expectations. Food palatability and its hedonic value play therefore a central role in nutrition, and one of the most fascinating aspects of these relationships is how food taste is modified by receptors that mainly provide a spicy flavor. Humans are the only animals which deliberately and systematically consume spices with a pungent, "hot", or even slightly painful note, raising the issue of the biological significance of this behavior, and what its possible evolutionary impact might have been. If, during evolution, taste has determined the discrimination between beneficial and harmful nutrients, chemesthesis has probably added another quality, namely, a hedonic experience associated to some health benefits. While there is no shortage of review articles and even books on the beneficial effects of spices, the molecular bases of their perception as such has received little attention outside the realm of neurophysiology, where spice constituents have provided the tools to identify a series of sensory receptors of wide biomedical relevance. This review tries to fill this gap, summarizing the relationships between the basic mechanisms of taste and those of chemesthesis. The mechanisms by which chemesthetic TRP channels control the intake of a host of spicy, often electrophilic, food compounds will be analyzed, discussing how spices might provide a sensory clue to potentially beneficial health effects.

2 A Historical Cultural Sojourn: The Role of Spices in History

The detection of eatable food was dramatically changed when our ancestors stood up on 2 ft. Not anymore close with their nose to earth, they complemented the decrease of anterograde olfaction with sight, taste and retrograde olfaction (Shepherd 2012), developing anticipative taste experiences to control food intake, often a decision of life or death (Wrangham 2009). In consideration of the brain neuronal network associated to food intake, *neuronutrition* might be a justified neologism for the "neurological" integration of the inputs from taste, olfaction, and chemesthesis into a decision on the palatability of a specific food source. Spices have the potential to upregulate our response to food, and this explains, in part, the role they have played in human history. The amazing, pantagruelic appetite of Europe for spices was not only a matter of culinary taste, but also of social and emotional reasons. We are amazed when we read that the Roman Emperor Heliogabalus, the quintessence of depravation, was seasoning his roasts with gold powder, but his contemporaries would have been even more startled by seeing the profligacy by which spices like cinnamon, pepper, and cloves are nowadays used in cuisine and even in soft drinks. Thus, ginger ale contains ginger, Coca Cola is rumored to contain a huge variety of spices like a smörgåsbord that includes cinnamon and nutmeg, and spices are used in profligacy to fortify energy drinks. Incidentally, we have managed to outperform Heliogabalus, since not only gold, but also edible silver and even platinum are now commercially available for culinary use, and are claimed to improve brain function (see the site eat gold and also the moonhill.jp website).

There is convincing evidence for the trade of cloves from the remote and minuscule Spice Islands in Indonesia, where *Syzygium aromaticum* (L.) Merril & Perry is endemic, to the Middle East as early as in 1700 BC (Turner 2004), and most spices were well known in the Ancient World. Thus, we know that cinnamon was more valuable than gold in the ancient Egypt (2000 BC), and a plethora of spices are mentioned in the Egyptian *Ebers Papyrus* (1550 BC), a description of 700 natural agents used for medical purposes and the oldest example of a pharmacopoeia. Over 1,000 years later, *Hippocrates of Cos* (460–377 BC) described the use of spices (out of 400 natural agents) as remedies for digestion disturbances (in Corpus Hippocratium) (Ji et al. 2009), also suggesting that broccoli, which contain activators of the ion channel TRPA1, can be useful to treat, inter alia, headache. The ancient literature is full of "anticipations" of modern discoveries, generally vaguely expressed and better recognized *a posteriori*. For instance, wormwood (*Artemisia absinthium* L.) was already recommended as an anti-malaria remedy, probably because of its apocalyptic bitterness (Touwaide 2012), and even clues on the molecular mechanism of action of spices can be identified in the ancient literature. Thus, in his *De Anima* (translated as "The soul" in English, DA II.7–11), *Aristotle* (384 BC–322 BC), while discussing senses (in the following order: sight, sound, smell, taste, and touch – one chapter for each, and, incidentally, giving more relevance to touch than to olfaction) (Hamlyn 1968; Sachs 2011), merged heat, cold and touch together, anticipating the critical involvement of TRPs in all these sensations.

After the Romans discovered the burning and irritating taste of the Oriental ingredients during their expeditions and wars, the Western World could not miss anymore the "especerias" from India and Arabia, that became a pleasure and not a necessity to survive. The Roman cuisine made abundant use of many herbs and spices, to the point that the Greek historian *Plutarch* (c. 45–120 AD), bemoaning the need to use so many spices to treat meat, commented that: "we mix oil, wine, honey, fish paste, vinegar, with Syrian and Arabian spices, as though we were really embalming a corpse for burial". On the other hand, the frugal Roman statesman *Cato the Elder* (234–149 BC) recommended his Roman citizens to cultivate broccoli, and to use them as a remedy against gastro-intestinal diseases (Touwaide 2012). Although modern Europeans associate spices with India and the Far East, the most celebrated and expensive spice of the ancient world was silphion, a product coming from the Mediterranean area. Silphion is a gum-resin, obtained from a *Ferula* species that grew exclusively around Cyrene, in today's Libya. Silphion was more expensive than silver and gold, and acquired a sort of status symbol all over the Greek-Roman world. After centuries of over-exploitation, gastronomic merits and alleged aphrodisiac properties eventually condemned Silphion to extinction in the first century AD. Silphion is considered the first documented case of the extinction of a plant by humans (McGee 2001). The replacement of *Silphium cyrenaicum* with the cheaper *Silphium particum* (asafetida, a.k.a. *Stercum diabuli*) suggests that, just like the infamous garum based on fermented fish. Also silphium had a rather strong flavor. Interestingly, some Mediterranean *Ferula* species contain high concentration of ferutinin, one of the most potent phytoestrogens known,

Fig. 1 Alaric sacking Rome in 410. A ransom including, inter alia, 3,000 pounds of pepper was necessary to liberate Rome from the Goths devastation during his first approach in 408 (a miniature from the Fifteenth Century, Wikipaintings, Public domain. Courtesy of Wikipedia)

suggesting that the ancients, even in their vague ideas on the physiology of reproduction, might have been well aware of the "hormonal" properties of silphion (Appendino et al. 2002).

Pepper was a currency. When Alaric (or Alarich), the King of the Visigoths invaded Italy and laid siege to Rome in late 408, starvation and disease rapidly spread throughout the city. The Roman Senate negotiated with Alaric, giving him precious metals but especially the demanded 3,000 pounds of pepper (Scheiper 1993). This ransom ended Alaric's first siege of Rome. However, Alaric instituted a second siege and blockade of Rome in 409, a ransom was paid again. In 410 Alaric came back and ravaged Rome (the sack of Rome, Fig. 1).

Pepper was for a long time the universal currency of the world, a sort of fragrant dollar, and still in 1937, the King of England was getting 1 pound of pepper as a symbolic rent from the major of Launceston in Cornwall. Its appreciation continued unabated during the whole Middle Age, and, as an example, William I. (1143–1214), King of Scotland, the Lion, honored his host, the king and later crusader Richard (Lionheart) I. from England (1157–1199), with a daily gift of 2 pounds of pepper. Roman and Medieval Europe was dependent on spices just like it depends from oil today. Scouting for spices was, indeed, the main driving force behind the "*Age of Explorations*", and it is a pity that this historical period has been so systematically stripped of its spicy flavor in school curricula. The root of Western imperialisms can, indeed, be traced to the control of the spice trade (Haedrick 2010), and all great explorers were essentially spice-hunters, just like Marco Polo, who discovered the Chinese food and described a host of exotic spices and new tasty dishes, if he indeed existed. Columbus sailed West trying to find a shortcut to India and its "spicy sky" of Shakespearian flavor ("*Midsummer Night's Dream*"), and was looking for an Old World, not a new one! From the Eastern front, Vasco da Gama (1460 or 1469–1524), who first circumnavigated Africa and reached Malabar in India sailing from Europe, wrote in his log book: "We are searching for Christians and Spices! His ships came back to Portugal loaded up with

cinnamon, black pepper, black cardamom, saffron, and nutmeg, much to the disappointment of Columbus who was coming back from the Caribbean full of everything except the spices he had been searching. Columbus did indeed brought chili from America, but the chili plant could be easily cultivated also in Europe, and lacked the glamour of pepper, whose plant source was still unknown to the Europeans, and could still therefore feed their dreams for the exotic. In a world devoid of the xanthinic pleasure of coffee, tea, chocolate as well as of the nicotinic stimulation of tobacco, spices played, undoubtedly, the role that science-fiction plays today in a World that can be "scanned" by Google Map on a computer screen. Remarkably, the expedition of Fernão de Magalhães (1480–1521) around the globe was aimed at establishing the location of the Spice Islands, and ascertaining if, on the basis of the treaty of Tordesillas, they belonged to the Spanish or to the Portuguese zone of influence (for a terrific description see the novel of Stefan Zweig 1938) (Zweig 1938, p. 326)". They belonged to Portugal, but both contenders were soon displaced by the Dutch. Religions and philosophies were changed by the spice trades, and the fall or rise of empires was determined by taste products (Schivelbusch 1990; Turner 2004). Of note, in 1667 New Amsterdam, currently New York was exchanged by Holland to England for the small island of Run in the remote Spice (Banda) Islands of Indonesia. Run, nowadays even difficult to locate on maps, was then a sort of Honk Kong of the spice trade, while New Amsterdam was simply a trading post for the much less glamorous fur trade, and a poor compensation for annexing of Run to the Dutch spice empire in the Far East.

Spices were long considered the quintessence of health, and were used to retard the spoiling of food, as well as corpses. In this context, the evangelic description of Jesus passion provides a remarkable example of the various uses of spices. A wine preparation of myrrh, a spice containing furanosesquiterpenoids endowed with opioid activity, is the only mercy offered to Jesus (who refuses it) to ease the pains of the cross, and is body is then anointed with the mixture of myrrh and aloes brought by Nicodemus. In general, the exorbitant prices of spices mean that they could only be afforded by wealthy people who had no problem in purchasing fresh foodstuff to avoid intoxication from rotten food. This oxymoronic situation is somewhat similar to what happens today with some expensive dietary supplements (omega-3, bilberry), that are purchased by wealthy people who would not have any problems in getting them from their costly food sources (wild salmon for long-chain omega-3, imported bilberries from South America during European and US Winter time for the bilberry anthocyanosides). The European fascination for spices was more hedonic and social than medicinal. Nevertheless, it was plague that fuelled the nutmeg frenzy of the seventeenth century, since this spice was rumored to fight this disease. The craving from nutmeg was ultimately responsible for the Run-for-New Amsterdam exchange between the English and the Dutch, the most economically insane deal in history. It is, nevertheless, remarkable that the antibacterial activity of spices was discovered by the same scientist (Antony van Leeuwenhoek, 1632–1723) who first saw bacteria. Thus, in a letter dated 9 October 1676, the father of microbiology described the decline in the number and activity of

"animalcules" (bacteria from tooth) in a sample of well water following addition of pepper (see also Miranda 2009).

An unsuspected testimonial of the medicinal relevance of spicy and bitter plants nowadays confined to the realm of liqueurs was *Jean Jacques Rousseau*, who, in one of the critical passages of his autobiographic *Confessions* describes how Claude Anet, the gardener-lover of M.me de Warens, at the request of a physician once made an excursion to the higher Alps to collect genepi (*Artemisia genipi* Weber, a plant containing the potent TRPA1 and bitter activator costunolide, and the source of the homonymous celebrated Alpine liqueur), only to "heat himself" so much, that he was seized with a pleurisy, which genepi could not relieve, though said to be specific in that disorder (*ce pauvre garçon s'échauffa tellement qu'il gagna une pleurésie don't the Génipi ne put le sauver, quoiqu'il y soit, dit-on, spécifique*). Anet was next replaced by the young Rousseau in the favors of the wealthy lady, who, apart from making him abjure Calvinism for Catholicism, took also care of his sentimental education. Given the role that Rousseau, the father of environmentalism, played in shaping our current thinking, the humble genepy was the modern equivalent of the chaste tree under which Socrates was teaching in Athens.

The father of a molecular approach of eating and even "molecular" gastronomy was Sir *Benjamin Thompson*, Count Rumford (1753–1814), one of the early pioneers in the science of food and cooking as well the founder of thermodynamics. Interested in the economization of energy and ingredients in cooking, he invented the cast-iron Rumford stove to economize fuel consumption, and was one of the early proponents of the replacement of salt (expensive in some places also at his times) with herbs. Just like salt, herbs can increase our sensitivity to taste, a concept that has led to the current success of herbs-salt mixture to economize the intake of sodium, a dietary pariah in current nutritional sciences. The aim of his gastronomical investigations was the clarification of the chemical and physical mechanism(s) involved in the culinary transformations and processing of food, paying attention to its social, artistic, and technical aspects (see for more details and references Benjamin Thompson).

In those years, the relevance of the complementary antegrade olfaction was experienced as necessary for creativity by the German poet, philosopher, and historician *Friedrich Schiller*, who only could think and create when he smelled rotten apples (i.e. "ethylene, ethene", from ripe, rotten fruits, C_2H_2) (Roth 2005)! The first "*physiology*" of flavor (Brillat-Savarin 1826) was written by *Anthelme Brillant-Severin,* a chemist and physician and deputy to the Estates General at the opening of the French revolution, the greatest gastronome the world has ever known and the inventor of the production of a very tasty low caloric cheese. Olfaction was for him "*the chimney of taste*"! He is still shocking the modern well-educated society by his "*Dis-moi ce que tu manges, je te dirai ce que tu es*." ("The discovery of a new dish does more for human happiness than the discovery of a new star. Tell me what you eat, and I will tell you what you are.") The phrase "*You are what you eat*" was first used by Brillat-Savarin. He wrote in his famous "Physiologie du Gout, ou Meditations de Gastronomie Transcendante" 1826 (see also http://en.wikipedia.org/wiki/Jean_Anthelme_Brillat-Savarin).

The importance of food is also transcendental undermined in Roman Catholic Church: bread and wine of the Eucharist are changed into the body and blood of Jesus (*Thomas Cranmer*, 1549). The German materialist *Ludwig Feuerbach* (friend/opponent of Karl Marx) who wrote in his assay "*Concerning Spiritualism and Materialism*": "*Der Mensch ist, was er ißt.*" (Man is what he eats.) (Cherno 1963) (see for a comment Cizza and Rother 2011)! Only in the 1920s and 1930s, Victor Lindlahr, probably the first dietist, anglicized this phrase in an advert for beef in 1923 "*Ninety per cent of the diseases known to man are caused by cheap foodstuffs. You are what you eat.*" (The *Bridgeport Telegraph* for 'United Meet Markets'). He published 1942, "*You Are What You Eat: how to win and keep health with diet*". The phrase got a new life in the 1960s hippy era (not to speak about the German industrial metal band *Rammstein*). Right now, "*You are what you eat*" had more than 300,000 Google hits in 2011! (you are what you eat!) (for excellent books see also Turner 2004; Freedman 2008)

3 The Taste Machinery

Flavor, the gustatory impression of food, is determined primarily by the chemical senses of taste and smell, while trigeminality is associated to the perception of its temperature and texture. Both flavor and trigeminality are very important to our overall perception of food. From a neurophysiological standpoint, flavor is metabotropic (GPCR-mediated), while trigeminality is ionotropic (TRP-mediated). Another difference is that flavor is purely chemical, while the physiological modulators of trigeminality, at least in a food context, are physical (heat and texture). Just like the flavor of the food can be altered with natural or artificial flavorants, so its trigeminality is affected by a heterogenous group of compounds collectively referred to as "spicy".

Palatability is the hedonic reward provided by food that is agreeable to the "palate" in terms of homeostatic satisfaction of nutritional, hydration, or energy needs. The palatability of food, unlike its flavor or taste, varies with the state of an individual: it is lower after consumption and higher when deprived. Palatability of foods, however, can be learned. It has increasingly been appreciated that this can create a hedonic hunger (food craving) independent of any homeostatic needs. However, we need to remember that food provides us caloric intake which can be even uncoupled from taste, as we know now from studies on the Drosophila fly, which describe a taste-independent metabolic sensing pathway in need to replenish energy stores after starvation (Dus et al. 2011).

Taste physiologically refers to five basic qualities, sweet, umami, sour, salty and bitter. Receptors for these taste mediating substances (tastants) are localized in the tongue's taste buds which are aggregates of 50–100 polarized neuroepithelial cells that detect nutrients and other compounds (Chaudhari and Roper 2010). Three types of taste cells within the buds are identified. Each type can respond to taste stimulation. Type II and III taste cells are electrically excitable (Fig. 2).

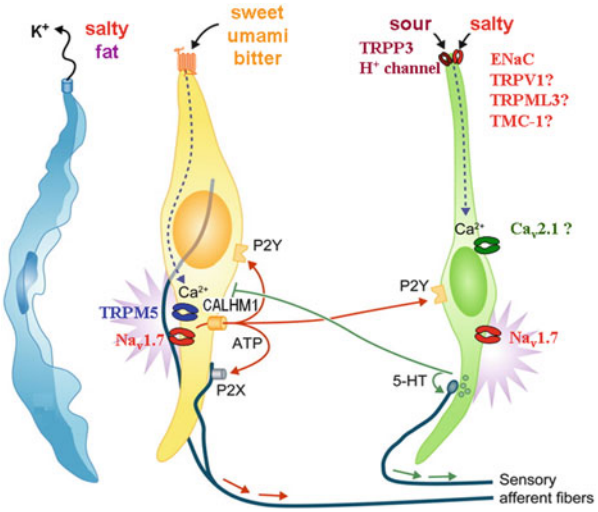

Fig. 2 The taste machinery. Three types of taste cells are located in the taste buds. *Left*, type 1 a support cell with only a putative salt sensor. *Middle*, type II cells or also sensory taste receptor cells (TRC). Taste receptors (for sweet, umami, bitter, G-protein coupled receptors) are located at the apical membrane. Stimulation of these cells causes an intracellular Ca^{2+} release, which activated TRPM5 and generates subsequently a depolarization activating voltage-dependent Na^+ channels ($Na_v1.7$). The large cell depolarization opens the calcium homeostasis modulator 1 (*CALHM1*), a non-selective voltage-gated ion channel (see "Note" at the end of this chapter, Taruno et al. 2013). CALHM1 probably replaces the previous putative member pannexin 1 channels and mediates the release of ATP which binds in an autocrine and paracrine fashion to purinergic receptors on type II and type III TRC. *Right*, type III cells, or presynaptic cells, because these still contain the whole exocitotic machinery. ENaC, likely TRPV1 and TRPML3 are located at the apical surface (salt sensing?) together with TRPP2 (also known as PKD2L1 in association with the surface adhesion protein PKD1L3), and probably a still unidentified H^+ channel. TMC-1 is a putative salt sensor (Adapted from Nilius and Appendino (2011). With permission of *EMBO Reports*)

Type I or glia type cells are dark, have long apical microvilli and an extended nucleus. They express ROMK channels (Kir1.1, eventually for salt taste), epithelial Na channels (ENaC), the ecto-ATPase NTPDase2, as well as the glutamate and norepinephrin re-uptake transporters GLAST and NET (Chaudhari and Roper 2010; Yoshida and Ninomiya 2010; Kinnamon 2011). Type II cells, or receptor cell, appear light, have short apical villi and large, round nuclei. They express the sweet receptors G-protein coupled receptors (GPCRs, T1R2/T1R3 dimers or Tas1r2/Tas1r3), umami receptors (GPCRs, T1R1/T1R3 dimers, or Tas1r1/Tas1R3) and metabotropic glutamate receptors 1 and 4 (mGluR1 and mGluR4), bitter receptors (T2Rs or Tas2r's, e.g. T2R38 or Tas2R38, from 3 to ~66, which do not require heteromerization to function), glutamate receptors mGLuRs, the G protein subunits Gα-gustducin and Gγ13, the phospholipase PLCβ2 and, the non-selective, Ca^{2+} impermeable cation channel TRPM5. Taste cells frequently co-express Fxyd6 and Na,K-ATPase β1 which regulate the transmembrane Na^+ dynamics in type II taste cells (Shindo et al. 2011). It might be of interest that some bitter compounds from

citrus fruit phenolics, like naringin from grapefruit, are potent inhibitors of TRPM3, a role that is not yet understood (Straub et al. 2012). Amazingly, these compounds are antihypertensive, lipid-lowering, insulin-sensitizing, antioxidative, and anti-inflammatory and may reduce stroke risk (Chanet et al. 2012). Type II cells have no Ca^{2+} channels and no proteins of the exocytotic machinery. They express Na^+ channels for the generation of action potential, $Na_v1.7$, $Na_v1.3$ and Pannexin1, which was previously considered as the release channel of non-vesicular ATP release. Quite recently (see "Note" at the end of this chapter), the calcium homeostasis modulator 1 (*CALHM1*), a non-selective voltage-gated ion channel was identified to be required for taste-stimuli-evoked ATP release from sweet-, bitter- and umami sensing taste bud cells and the for sweet, bitter and umami taste perception. Type III cells are presynaptic cells with a single thick apical process and an indented nucleus. They mediate sour taste probably via the TRP-homolog PKD2L1 (TRPP3 or recently renamed TRPP2) which might be associated to the adhesion protein member PKD1L3. Sour transduction might also be mediated via channels sensitive to intracellular pH changes different from PKD2L1, e.g. proton inhibited K^+ channels, or via not yet identified proton channels (Chang et al. 2010). Surprisingly, the putative "sour" channel, PKD1L3/PKD2L1, seems to be inhibited by capsaicin pointing to a spicy-sour (chemesthetic) relation (Ishii et al. 2012).

Salty and sour taste qualities are transduced by changes in the intracellular Ca2+ concentration $[Ca^{2+}]_i$ and can be separated in $[Ca^{2+}]_i$ -dependent and $[Ca^{2+}]_i$-independent mechanisms. Changes in $[Ca^{2+}]_i$ of the taste receptor cells (TRC) in a cytosolic compartment regulate ion channels and co-transporters which are involved in the salty and sour taste transduction mechanisms and in neural adaptation. Changes in taste-cell-$[Ca^{2+}]_i$ in a separate store subcompartment, which is sensitive to inositol trisphosphate, are associated with neurotransmitter release. As outlined, Type III presynaptic cells express PKD1L3/PKD2L1 as putative sour sensing cells. While PKD2L1 and PKD1L3 are reliable markers of sour-sensitive taste cells, PKD2L1 may have some role in sour transduction in fungiform taste cells, but neither PKD2L1 nor PKD1L3 plays a role in sour transduction in circumvallate taste cells. Weak organic acids enter across the apical membranes of TRCs as neutral molecules and decrease pH_i. For strong acids, H^+ entry is dependent upon at least two proton conductive pathways in the apical membranes of sour sensing TRCs that are amiloride- and Ca^{2+}-insensitive. One conductive pathway is activated by cAMP and the second pathway depends upon gp91phox, a component of the NADPH oxidase enzyme. Acidic stimuli depolarize type III cells and increase Ca^{2+} influx through voltage-gated calcium channels (VGCCs) that are involved in the release of the neurotransmitters serotonin and noradrenaline from intracellular vesicles. In fungiform TRCs, Na^+ ions enter across the apical membrane by at least two pathways: the first one involves the apical amiloride-sensitive epithelial Na^+ channels (ENaCs), while the second one involves putative TRPV1 (or a truncated form) non-specific cation channels. Electrophysiological evidence from the chorda tympani nerve (CT) has implicated TRPV1 as a major component of amiloride-insensitive salt taste transduction, but behavioral results have provided only equivocal support (Desimone et al. 2012b; Smith et al. 2012). Type I TRCs may be involved in Na^+ sensing through ENaC or modulation

of potassium channels. The relationship between the increase in $[Ca^{2+}]_i$ and neurotransmitter release is not clear. In addition to its role in the neurotransmitter release, alterations in TRC $[Ca^{2+}]_i$ have been shown to modulate the chorda tympani (CT) taste nerve responses to salty and sour stimuli (Desimone et al. 2012b). Type III cells express in addition to ENaC for salt perception also TRPV1, purinergic receptors (e.g. P2Y), enzymes for neurotransmitter synthesis, e.g. amino acid decarboxylase AADC for amine bioamines and GAD47 for GABA synthesis. They produce the neurotransmitter serotonin, 5-HT. The whole exocytosis machinery is present, chromogranin for vesicle packing, Na+ channels for action potential generation ($Na_v1.2$), voltage gated Ca^{2+} channels (Cav2.1, Cav1.2) and NCAM, SNAP25, a SNARE protein for exocytosis (Chaudhari and Roper 2010; Niki et al. 2010; Yoshida and Ninomiya 2010; Kinnamon 2011). Just recently, a transmembrane channel TMC-1 was identified in Drosophila as a salt sensor. TMC-1 is directly activated by Na^+. Although human *tmc-1* and *tmc-2* genes are probably required for hair-cell mechanotransduction, this new proteins must be added to the potential candidates for salt sensation as a ionotropic sensory receptor (Chatzigeorgiou et al. 2013).

The sensitivity of taste cells and the connection between taste cells and gustatory fibers is critical for taste perception. Broadly tuned taste cells and random connections between taste cells and fibers would produce gustatory fibers that have broad sensitivity to multiple taste qualities. Narrowly tuned taste cells and selective connections would yield gustatory nerve fibers that respond to specific taste quality. Amiloride primarily inhibits NaCl (and LiCl) responses of gustatory fibers that selectively respond to sodium and lithium salts (N type), whereas it hardly affects NaCl responses of fibers that show broad sensitivity to electrolytes (E or H type). The IXth nerve contains primarily E-type fibers but only a very few, if at all, of N-type fibers. About a half population of NaCl-sensitive CT fibers is from the amiloride sensitive type and the rest half is amiloride insensitive. On the other hand, the IXth nerve has almost exclusively the AI type. Norepinephrine is co-released with serotonin, in type III cells (Yoshida and Ninomiya 2010; Yasumatsu et al. 2012). The contribution of TRPV1 to salt perception is not generally accepted. Some reports indicate that TRPV1 does not contribute to amiloride-insensitive salt taste transduction, but may contribute to the oral somatosensory features of sodium chloride sensing as a chemesthetic attribute (Smith et al. 2012). In another context, TRPV1 is also expressed on the insular cortex. In this cortex the primary gustatory area caudally adjoins the primary autonomic area that is involved in visceral sensory-motor integration. This channel influences the electrical activity in this network inducing distinct TRPV1-mediated theta-rhythm firings. The network coordination induced by TRPV1 activation could be responsible for autonomic responses to tasting and ingesting spicy foods (Saito et al. 2012).

In this machinery, gustatory signals initiate in type II cells TRPM5 mediated depolarization, which in turns activates Na^+ channels, triggers action potentials and causes ATP release. Bitter, sweet, and umami tastants are detected by their respective G-protein-coupled receptors that cause, via gustducin and phospholipase Cβ, a brief elevation of intracellular inositol trisphosphate, and induce a Ca^{2+} release-mediated Ca^{2+} transient which is sufficient to gate TRPM5-dependent

currents in intact taste cells. A second type of Ca^{2+}-activated nonselective cation channel that is less sensitive to $[Ca^{2+}]_i$ is involved in this signaling cascade in taste cells. Probably, this channel is TRPM4 (Zhang et al. 2003, 2007b). ATP release causes P2Y activation in type III cells, and autocrine activation of P2X receptors in type II cells, eventually triggering action potentials in sensory fibers from type II cells to the center in the brainstem solitary nucleus. At the same time, ATP excites also type III cells, stimulates them to release 5-HT or NE and also induces a backward inhibition of type II cells.

Importantly, TRPM5 plays a central role in taste, due to its abundant expression in taste receptor cells. Sweet, amino acids, and bitter perception require TRPM5. The distinctive umami taste elicited by L-glutamate and some other amino acids is thought to be initiated by heteromers T1R1(Tas1r1)+T1R3(Tas1r3) (but also metabotropic glutamate receptors, mGluR1 and mGluR4). Single umami-sensitive fibers in wild-type mice fall into two major groups: sucrose-best (S-type), and monopotassium glutamate (MPG)-best (M-type). Each fiber type has two subtypes: one shows synergism between MPG and inosine monophosphate (S1, M1), and the other shows no synergism (S2, M2). In both T1R3 and TRPM5 null mice, S1-type fibers were absent, whereas S2, M1 and M2 types remained. Lingual application of mGluR antagonists selectively suppressed MPG responses of M1 and M2 type fibers. These data suggest the existence of multiple receptors and transduction pathways for umami responses in mouse. Information initiated from T1R3-containing receptors may be mediated by a transduction pathway including TRPM5 and conveyed by sweet-best fibers, whereas umami information from mGluRs may be mediated by TRPM5-independent pathway(s) and conveyed by glutamate-best fibers (Yasumatsu et al. 2012). Perception of flavor is constantly changed during evolution. Carnivorous mammals which are exclusive meat eaters loose for instance sweet taste, as well as mammals which swallow food without chewing loose the receptors for sweet and umami by pseudogenization (Jiang et al. 2012b). Of interest for the "spicy aspect" of this review is also the recent finding that a "truncated" from of TRPV1 might also be involved, in addition to salty taste and via a Ca^{2+} dependent mechanism, in the perception of umami (Desimone et al. 2012a; Dewis et al. 2012)

Dietary fat was for a long time considered to be tasteless, and its primary sensory attribute was believed to be its texture. However, free fatty acids activate taste cells and elicit behavioral responses consistent with a taste of fat. Fat taste requires activation of TRPM5 (Sclafani et al. 2007). The long-chain omega-6 unsaturated free fatty acid linoleic acid (LA) depolarizes mouse taste cells and elicits a robust intracellular calcium rise via TRPM5. The required increase in the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, to activate TRPM5 comes exclusively from endoplasmic reticulum calcium stores. Several fatty acids are also able to activate trigeminal sensory neurons via a similar signaling cascade (Yu et al. 2012). The LA-induced responses depend on G-protein-phospholipase C pathway, in accordance with the involvement of G-protein-coupled receptors (GPCRs) in the transduction of fatty acids. TRPM5 plays therefore an essential role in fatty acid transduction in mouse taste cells, suggesting that fatty acids are capable of activating taste cells in a manner consistent with other GPCR-mediated tastes. Mice lacking TRPM5

channels exhibit, in fact, no preference for fat and even show reduced sensitivity to it (Liu et al. 2011). The exact mechanism of fat signaling is still under discussion. To date, several candidate genes are implicated in fat perception: a delayed-rectifying potassium (DRK) channel sensitive to *cis*-polyunsaturated fatty acids (PUFAs) (Gilbertson et al. 1997), the fatty acid (FA) transporter (translocase) CD36/FAT (Laugerette et al. 2005), and G protein–coupled receptors, GPR40, GPR41,GPR43, GPR84, and GPR120 (see for reviews Gilbertson et al. 2010; Mattes 2011). In human, long-chain fatty acids (LCFAs) as the main taste-activating component of lipids, have the specific receptors for fat taste GPR40 and GPR120. The GPR40 gene is not expressed in gustatory tissue while GPR120 is detected in taste buds, in the surrounding epithelial cells and in nongustatory epithelia and plays a major role in human gustatory fatty acid perception (Galindo et al. 2012; Martin et al. 2012). GPR120 has a critical role in various physiological homeostasis mechanisms such as adipogenesis, regulation of appetite and food preference. GPR120-deficient mice fed a high-fat diet develop obesity, glucose intolerance and acquire fatty liver with decreased adipocyte differentiation and enhanced hepatic lipogenesis, and eventually develop insulin resistance. In human, GPR120 is expressed in adipose tissue, and its expression is significantly higher in obese patients, who very often carry a mutation R270H that inhibits GPR120 signalling activity. GPR120 has a key role in sensing dietary fat and, therefore, in the control of energy balance in both humans and rodents (Ichimura et al. 2012). The chemoreception of dietary fat in the oral cavity can be attributed trigeminal nerve fibres which also recognize textural properties of fat. In addition, free fatty acids are capable of activating trigeminal neurons via intracellular calcium rise from the endoplasmic reticulum in this subset of trigeminal neurons (for an overview see Table 1) (Yu et al. 2012).

Tas2R38, a taste receptor for bitter thiourea compounds and identified to be responsible for phenylthiocarbamide (PTC) bitter sensitivity (supertaster), is also involved in the mediation of fat taste. Genetic variations of the Tas2R38 gene is likely associated with a the nutrient intake pattern and might be linked with healthy eating (Feeney et al. 2011). Interestingly, TasR38 haplotypes influence food preferences (like cruciferous vegetables and fat foods). Therefore, fat taste is genetically modified. Humans with the single nucleotide polymorphism (SNP) of Tas2R38 (P49A) have aversions to green tea, mayonnaise and whipped cream, but not sweet/fat foods (Ooi et al. 2010). A genetic variant of CD36, which plays a critical role in fat preferences, has been discovered in African-American adults. They carry a variant in the CD36 gene, rs1761667. Individuals with this genotype find mayonnaise salad dressings creamier than those who have other genotypes and report higher preferences for added fats, oils, and spreads (for example margarine) (Keller 2012).

Another important finding adds the stromal interaction molecule 1 (STIM1) to the players in fat taste (Abdoul-Azize et al. 2012; Dramane et al. 2012). STIM1, a sensor of Ca^{2+} depletion in the endoplasmic reticulum, mediates fatty acid-induced Ca^{2+} signalling in the mouse tongue and fat preference. Linoleic acid (LA) generates arachidonic acid (AA) and lysophosphatidylcholine (Lyso-PC) by activating multiple phospholipase A2 isoforms via CD36 thereby triggering Ca^{2+}

Table 1 Taste transduction mechanisms for fatty acids (Adapted from Gilbertson et al. 2010; Mattes 2011)

Mechanism	Site	Stimuli
DRK (delayed rectifier K^+ channel) inhibition	Fungiform papillae, foliate and circumvallate papillae	Fungiform: long-chain, *cis*-polyunsaturated fatty acids (PUFA)
(KCNA5 or Kv1.5)		Foliate and circumvallate: long-chain PUFA and monounsaturated fatty acids
CD36	Foliate, circumvallate, epithelial cells surrounding papillae, non-gustatory epithelial cells, trigeminal neurons	Long-chain saturated fatty acids (LCFA) and PUFA
GPR40 (G_s,cAMP,PKA)	Circumvallate papillae, pancreas β-cells, trigeminal neurons	Fatty acids C10–C16
GPR41 (G_i/G_o)	Foliate and circumvallate papillae, adipocytes, trigeminal neurons	Short-chain fatty acids
GPR43 (G_i/G_o; G_q)	Foliate, circumvallate, trigeminal neurons	Short-chain fatty acids
GPR84 (G_i/G_o)	Foliate and circum vallate papillae, trigeminal neurons	LCFA
GPR120 (G_q, PLCβ)	Fungiform, foliate, circumvallate papillae, trigeminal neurons, enteroendocrine cells	Short-chain fatty acids and unsaturated fatty acids C14–C20
Fatty acid transporters (FATP1, FATP2, FATP3, FATP4, FATP5)	Lingual and palatal epithelium	Fatty acids C10–C26

influx in CD36-positive taste bud cells. STIM1 regulates LA-induced opening of multiple store-operated Ca^{2+} channels. This effects is absent in *Stim1*$^{-/-}$ mice which also fail to release serotonin upon fat sensing (Dramane et al. 2012).

What about other TRPs and the taste machinery? From genetic studies of adults twins with stable and heritable differences in taste, (e.g. the sensitivity to cinnamon, androstenone, galaxolide, cilantro, and basil), it seems likely that also TRPA1 is involved in taste perception (Knaapila et al. 2012).

Taste information is modulated by hormones and other endogenous factors. The fat cell specific anorectic hormone leptin, which regulates the appetite and stops food intake, inhibits, via binding to LEP-R in type II cells, the sweet responses. Conversely, endocannabinoids bound to CB1 receptors on type II cells enhance sweet responses. These peripheral modulations of taste information influence preferences of food intake, and play therefore important roles in regulating energy homeostasis (for excellent reviews see Chaudhari and Roper 2010; Yoshida and Ninomiya 2010). It is also remarkable that mice with knock-out of the sweet/umami receptor Tas1r3 (T2R3) are attracted to the taste of Polycose® (a highly digestible glucose polymer obtained by controlled hydrolytic degradation of corn starch), but not sucrose. In contrast, *Trpm5* KO mice are not attracted to the taste of sucrose or Polycose®. Tas1r3 KO mice overindulged in the Polycose® diet and eventually

became obese. The *Trpm5* KO mice, in contrast, showed little or no overeating on the sucrose and Polycose® diets and gained slightly or significantly less weight than WT mice on these diets. Food must be highly palatable to cause carbohydrate-induced obesity in mice and induce a binge-eating pattern (Glendinning et al. 2012a).

Obviously, there are genetical differences in our taste sensation. The best known is the case of "supertasters". Propylthiouracil (PROP) gives supertaster a bitter sensation at the fungiform papillae at the tip of the tongue. They dislike vegetable, alcoholic beverages, coffee, grapefruit, but like more spicy food, olives and are thin. Food preference studies showed that supertasters dislike bitter vegetables and generally strong-tasting foods, while expressing lower preference for sweet foods, sweet drinks, and salad dressings. PROP tasters are also more sensitive to food texture. Supertasters and medium tasters consume fewer vegetables and added fats than do nontasters. Non-PROP taster like fat, sweet, alcoholic beverages, are heavy, have a higher risk of alcohol overconsumption (Bartoshuk et al. 1994; Hayes et al. 2008; Negri et al. 2012)! Supertasters have a polymorphism in the bitter taste receptor Tas2R38. Three substitution A49P, A262V, and V296I determine bitter taste. PAV (P47/A262/V296) is the major determinant of taster status (medium tasters, ~61 %) and AVI (A49/V262/I296) is the major nontaster haplotype (~23 %). Individuals with two copies of the AVI allele are basically nontasters, whereas individuals with one or two copies of the PAV allele are medium tasters or supertasters (~16 %). In children, more supertasters were identified (~30 %) (Negri et al. 2012).

Another genetic difference in taste that has been thoroughly investigated is that to the soapy flavor of *Cordiandrum sativum* L. (cilantro in American English and coriander in British English). The dislike of cilantro has a genetic trait, being more widespread in Caucasians (17 %) and east Asians (17 %) than in Latin Americans, south Asians and Arabians (3–7 %) (Mauer and El-Sohemy 2012) The web site IhateCilantro.com has collected hundred of short verses in the form of haikus dedicated to the dislike of cilantro, with "*O soapy flavour/Why pollutest thou my food?/Thou me makest retch*" being one of the most popular ones. Although not so genetically clear-cut as PROP-based supertasting, the aversion to cilantro has, nevertheless, sparkled genetic studies that have eventually related the perception of the soapy taste of cilantro to variations in genes encoding taste or taste-related receptors (TasR2R50, TRPA1, the guanine nucleotide-binding protein G(t) subunit α-3, or gustducin α-3 chain GNAT3) as well as the olfactory receptor 7D4 OR7D4 (Knaapila et al. 2012) Interestingly, both TRPA1 and OR7D4 are sensitive to aldehydes, and, indeed, cilantro contains a relatively high concentrations of electrophilic 2-alkenals. In a remarkable practical application of biochemical "foodology", it has been suggested (see the following website (cilatro)) that crushing cilantro in a pesto-style moderates its soapy taste by triggering aliphatic aldehyde reductase activity that degrades the aldehydes responsible for the soapy taste (To Quynh et al. 2010). Cilantrophoby seems to be old, since the name *Coriander* is related to the Greek word for bedbug, and, indeed, the flavor of the plant has been compared by some cilantrophobists to that of bug-infested bedclothes.

4 The Alien Taste Receptors

Surprisingly, the TRPM5-cascade is expressed in not only in the oral cavity, but also in many other organs. We sense tastants with more than with our tongue. TRPM5 has been also found on the basolateral surface of taste receptor cells, in other chemosensory organs such as the olfactory epithelium and the vomeronasal organ, and also in epithelial cells of the respiratory and gastrointestinal tract. These epithelial cells are co-immunostained with different epithelial markers and have brushes (brush cells or tuft, fibrillovesicular, multivesicular or caveolated cells). It is suggested that these brush cells are chemosensors. However, a distinct biological function is still missing (Kaske et al. 2007).

The vomeronasal organ (VNO) detects pheromones and other semiochemicals to regulate innate social and sexual behaviors. This semiochemical detection generally requires the VNO to draw in chemical fluids, such as bodily secretions, which are complex in composition and can be contaminated. Solitary chemosensory cells (SCCs) reside densely at the entrance duct of the VNO. In this region, most of the intraepithelial trigeminal fibers innervate the SCCs, indicating that SCCs relay sensory information onto the trigeminal fibers. SCCs express TRPM5 and the PLCβ2 signaling pathway. SCCs express choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT). Inhibition of TRPM5 resulted in larger amounts of bitter compounds entering the VNOs, i.e. they may limit the access of non-specific irritating and harmful substances (Ogura et al. 2008).

Bitter sensing has a special function beyond taste in the respiratory system, contributing to mechanical and chemical defense against pathogens. All eukaryotic cells have cilia, e.g. primary cilia which serve as sensory organelles, whereas motile cilia exert mechanical force. The motile cilia emerging from human airway epithelial cells propel harmful inhaled material out of the lung. These cells express sensory bitter taste receptors, which localized on motile cilia. Bitter compounds stimulated cilia beat frequency. Airway epithelia contain a cell-autonomous system in which motile cilia both sense noxious substances entering airways and initiate a defensive mechanical mechanism to eliminate the offending compound (Finger et al. 2003; Kinnamon and Reynolds 2009; Shah et al. 2009). Pathogens cause different diseases in the respiratory tract, e.g. the prevalence of chronic sinusitis in the absence of systemic immune defects indicates that there may be local defects in innate immunity associated with mucosal infections. Bitter taste receptors (Tas2R46, Tas2R38) in airway epithelial cells also have a direct anti-bacterial action, sensing bacterial factors involved in microbial aggression. Thus, bitter acyl-homoserine lactones serve as quorum-sensing molecules for Gram-negative pathogenic bacteria, and detection of these substances by airway chemoreceptors offers a means by which the airway epithelium may trigger an epithelial inflammatory response before the bacteria reach population densities capable of forming destructive biofilms (Tizzano et al. 2011). Chemosensing receptors (chemosensors and also addressed in the airway as *chemodefensors!*) are located throughout the respiratory system involving diverse components of the canonical taste transduction cascade, but always express TRPM5.

These diverse cells types in the airways utilize taste-receptor signaling to trigger protective epithelial and neural responses to potentially dangerous toxins and bacterial infection (Tizzano and Finger 2013).

A direct anti-infective role of bitter taste receptor was recently proposed. Thus, the taste receptor Tas2R38, highly expressed in the upper respiratory epithelium, was identified as a key regulator of the mucosal innate defense mechanism, triggering the calcium-dependent production of NO and the stimulation of mucociliary clearance (Lee et al. 2012). Since NO has anti-bacterial activity and is gaseous, it rapidly diffuses into the airways, spreading the anti-infective message. Remarkably, polymorphisms of the Tas2R38 gene correlates with the ability of kill and clear bacterial cells and the susceptibility to respiratory infections. Tas2R38 is exquisitely sensitive to limonin (Meyerhof et al. 2012), the bitter nor-triterpenoid constituent of Citrus seeds, making us wonder whether the "flu-fighting" properties of oranges might have a basis outside the highly debated anti-infective role of ascorbic acid.

A specific relationship between bitter receptors and a human disease has been discovered in asthma. Thus, a remarkable correlation exists between the expression of Tas2Rs and several clinical markers of asthma severity, qualifying bitter receptors as a novel target for asthma (Pietras et al. 2012). The activation of respiratory bitter taste receptors in airway smooth muscles has a bronchodilatatory action, whose potency is, however, debated. The initial report that activation of bronchial bitter receptors outperformed the dilatation induced by adrenaline (Deshpande et al. 2012) could not be reproduced (Morice et al. 2011), and the clinical significance of the high expression of bitter receptors in the airways is still debated. Nevertheless, the increased expression of bitter receptors associated to asthma might be a compensatory mechanism for the growing obstruction of the aerial pathways that characterizes this disease.

Nasal trigeminal chemosensitivity, mediated at least in part by epithelial solitary chemoreceptor (chemosensory) cells (SCCs), also affects breathing. Bitter substances applied to the nasal epithelium activate the trigeminal nerve and evoke changes in respiratory rate. The chemosensory cells at the surface of the nasal epithelium serve as a sensor for bitter compounds that can activate trigeminal protective reflexes. The trigeminal chemoreceptor cells are likely to be remnants of the phylogenetically ancient population of solitary chemoreceptor cells found in the epithelium of all anamniote aquatic vertebrates (Finger et al. 2003). SCCs express elements of the bitter taste transduction pathway including Tas2R (bitter taste) receptors, GPR89-gustducin, PLCβ2, and TRPM5. SCCs respond to the bitter receptor ligands (Gulbransen et al. 2008). These substances evoke changes in respiration indicative of trigeminal activation.

The TRPM5 cascade is also expressed in the gastric mucosa and mediates response to glutamate. Parietal cell fraction exclusively expressed umami receptors T1R1 and mGluR1. Representative taste cell specific markers such as PLCb2 and TRPM5 were specifically expressed in the smaller gastric endocrine cell fraction. Multiple glutamate sensors, probably different mechanisms from taste buds, contribute to the glutamate sensing in the gastric mucosa (Nakamura et al. 2010).

The whole "sweet" sensing machinery is also expressed in some enteroendocrine cells in our intestine supporting digestive and absorptive processing of carbonehydrate-rich food, which is digested to simple sugars (glucose, fructose, galactose). The activation of sweet receptors stimulates TRPM5 which in turn enhances the secretion of the incretins GLP-1 (glucagon-like peptide 1) and GIP (gastric inhibitory peptide or also: glucose-dependent insulinotropic polypeptide or GIP). These incretins stimulate expression of a glucose transporter (SGLT1) in the gut which promotes absorption of glucose and also stimulates the insulin release from β-pancreatic cells. Thus, in addition to our tongue, our gut tastes "sweet" (Margolskee et al. 2007; Sclafani 2007; Young et al. 2009)

TRPM5 is expressed in taste enterocrine cells, and sense changes of sugar concentration in the lumen. Regulation of glucose transporters into enterocytes is induced by the sensing of sugar of the enteroendocrine cells through activation of sweet taste receptors (T1R2 and T1R3) and their associated elements of G-protein-linked signaling pathways (e.g. α-gustducin, phospholipase Cβ2 and TRPM5). GLUT2, GLUT5 and SGLT1 are expressed in TRCs (Merigo et al. 2011). Fatty acid-induced stimulation of enteroendocrine cells leads to release of satiety hormones like cholecystokinin (CCK). Fatty acid activated G-protein-coupled receptor, GPR120, has been shown to mediate long chain unsaturated free fatty acid (FFA)-induced CCK release from the enteroendocrine I cells. Linoleic acid (LA) activates TRPM5 which is involved in LA-induced CCK secretion in I cells (Shah et al. 2011). Ghrelin is a hunger hormone with gastroprokinetic properties and is released from P, D1, A-like enterocrine cells in the stomach. Bitter taste receptors (Tas2R) and the gustatory G proteins, α-gustducin and transducin are expressed on these cells. The mouse stomach contains two ghrelin cell populations: cells containing octanoyl- and desoctanoyl ghrelin, which were colocalized with α-gustducin and transducin, and cells staining for desoctanoyl ghrelin only. Increase in food intake is followed by inhibition of gastric emptying, partially counteracted by ghrelin. T2R-agonists have a direct inhibitory effect on gastric contractility. Activation of bitter taste receptors stimulates ghrelin secretion (Janssen et al. 2011). Modulation of endogenous ghrelin levels by bitter tastants provides novel therapeutic applications for the treatment of weight- and gastrointestinal motility disorders. Bitter herbs and liqueurs prepared from them were a mainstay of the European pharmacopoeias. In L-cell of the gut, Glucagon-like peptide-1 (GLP-1), an incretin hormone, is released. It regulates appetite and gut motility and is released from L cells in response to glucose. GLP-1-expressing duodenal L cells also express T1r taste receptors, α-gustducin and PLCβ2, and TRPM5. Gut-expressed taste-signaling elements underlie multiple chemosensory functions of the gut including the incretin effect. Modulating hormone secretion from gut "taste cells" may provide novel treatments for obesity, diabetes, and malabsorption (Kokrashvili et al. 2009).

Chemosensory cells residing in the mucosa of the GI tract express gustducin and TRPM5. Two critical stages have been considered: the suckling period when the neonates are nourished exclusively on milk and the weaning period when the diet gradually changes to solid food. At early postnatal stages, only a few α-gustducin-

or TRPM5-expressing cells have been found. At the time of weaning, numerous gustducin- or TRPM5-positive cells are present in the gastric mucosa and are isomorphic to adult chemosensory cells. The typical accumulation of gustducin and TRPM5 cells at the border between the forestomach and corpus region and the characteristic tissue fold or "limiting ridge" have not been observed at early postnatal stages but are complete at the time of weaning, strategic positions (Sothilingam et al. 2011)!

The sweet tasting machinery is also present in β-cells of the pancreas. Fructose activates sweet taste receptors on β cells and synergizes with glucose to amplify insulin release in human and mouse islets. TR signaling in β cells seems to be is triggered, at least in part, in parallel with the glucose metabolic pathway, and leads to increases in $[Ca^{2+}]_i$ due to activation of phospholipase Cβ2 andTRPM5. Thus, the regulation of insulin release by postprandial nutrients involves β-cell sweet TR signaling (Kyriazis et al. 2012).

The intestinal expression of functional taste receptors can have far-reaching nutritional implications, being involved, inter alia, in the growing debate on the role of artificial sweeteners in the global epidemic of obesity. Sweeteners dissect the taste and the caloric properties of sugars. By activating intestinal sweet receptors, they set in motion the metabolic machine associated to the absorption of sugars, and signal to the brain the illusory presence of a carbohydrate-rich food. The long-term consequence of this metabolic "illusion" and the disregulation of an otherwise perfectly tuned glucose homeostasis are unknown. A correlation between the consumption of artificially sweetened soda drinks and the development of metabolic syndrome has, indeed, been suggested (Lutsey et al. 2008), raising the possibility of a link between the current gargantuan consumption of artificially sweetened soft drinks and the development of cardiovascular disease and diabetes. Remarkably, anti-sweet compounds are used for the management of diabetes and obesity in folk medicine as well as mainstream drugs. The Indian anti-diabetic plant *Gymnema sylvestris* contain anti-sweet triterpenoids (Kanetkar et al. 2007), and the activity of the lipid-lowering and anti-diabetic fibrates might be related to their inhibitory properties on sweet taste receptors, in addition, or even preferentially, to their action on the Peroxisome proliferator-activated receptor α (PPAR-α), a nuclear receptor protein (Maillet et al. 2009). Remarkably, fibrates only inhibit human T1R3, and do not show any affinity for the murine version of this type I taste receptor. This behavior is also shown by anti-auxin phenoxy herbicides, one of the most popular class of agents both in agriculture and in landscape turf management (Maillet et al. 2009). These compounds, exemplified by 2,4-D, have high leachability and are prone to enter the human food chain, with the potential to exert biological effects in humans that could not have been detected in rodents.

The studies on the anti-sweet properties of fibrates and phenoxyherbices was triggered by their structural analogy with lactisol, a coffee bean constituent that causes a wash-out after-taste sweet sensation in humans (Schiffman et al. 1999). In the presence of lactisol, the basal activity of sweet receptors is lowered, and when the compound is washed out, removal of this inhibition is interpreted by brain as a sweet sensation. Interestingly, artichoke has long been known to make water sweet

with a similar mechanism, triggering a never-ending debate within food savants on which wine should accompany artichokes (http://www.oceanmist.com/products/artichokes/artichokepair.aspx), considered by many as a wine-killer because of the sweet taste it impart to it. Sensitivity to the after-taste sweet sensation of artichoke might have a genetic basis, since a *Science* article of 1972 (Bartoshuk et al. 1972) failed to detect the effect in all participants to the study. While the activity of cynarine, the caffeoylquinic constituent of artichoke responsible for the sweet after-taste of artichoke, on T1R3 has never been investigated, artichoke is known for beneficial effects on blood lipids in humans (Bundy et al. 2008).

Surprisingly, also brainstem neurons contain signaling molecules similar to those in taste buds which may sense bitter, i.e. the bitter-responsive type 2 taste receptors (T2Rs), their associated G-protein α-gustducin, the downstream signaling molecules phospholipase C isoform β2 (PLC-β2) and TRPM5. α-gustducin and PLC-β2 were also identified at multiple cardiorespiratory and CO_2/H^+ chemosensory neurons in the rostral ventral medulla, solitary tract, hypoglossal and raphe nuclei. In the medullary raphe, α-gustducin and PLC-β2 were colocalized with a subpopulation of serotonergic neurons, a subset of which has respiratory CO_2/H^+ chemosensitivity. Presence of these taste transduction pathway proteins in the brainstem implies additional functions for taste receptors in the central nervous system (Dehkordi et al. 2012). In addition to the brain stem, Tas2R4, Tas2R107 and Tas2R38 were also detected in the cerebellum, cortex and nucleus accumbens. At least, Tas2R4 expressed in these cells is functional and is involved in G-protein mediated calcium signaling after the application of exogenous ligands for Tas2R4, denatonium benzoate and quinine to these cultured cells, suggesting that endogenous Tas2R4 expressed in these cells is functional (Singh et al. 2011). Noteworthy, the "pungent" channel TRPA1, as discussed later in detail, seems to be involved in the fine-tuning of inhibitory GABA-nergic synapses in the brain, e.g. in the hippocampus (Scimemi 2013).

Bitter receptors are expressed in the testis. Ablation of bitter receptors led to a smaller testis and removed the spermatid phase from most of the seminiferous tubules. The entire taste transduction cascade (α-gustducin, Gg13, phospholipase Cβ2) was detected in spermatogenesis; TRPM5 appears in the spermatid phase. Taste transduction cascade may be involved in spermatogenesis (Li and Zhou 2012).

The bitter taste cascade is also expressed in the auditory tube. The luminal composition of the auditory tube influences its function. Solitary cholinergic brush cells are expressed in the mouse auditory tube epithelium. They express the vesicular acetylcholine (ACh) transporter and proteins of the taste transduction pathway such as α-gustducin, phospholipase Cβ2 and TRPM5. Brush cells in the auditory tube are equipped with all proteins essential for sensing the composition of the luminal microenvironment and for communication of the changes to the CNS via attached sensory nerve fibers (Krasteva et al. 2012).

Representation of taste information in the brain requires an input via N. VII (facial nerve), N.IX (glossopharyngeal nerve) and sensory vagal afferents (N.X). Taste fibers connect in the brain stem to the Nucleus of solitary tract (NST). From NST projections areas are in the brain the ventral posteromedial thalamic nucleus

(VPM), the operculum, insular and the somatosensory cortex (Shepherd 2006). However, information like spiciness, pungency, but also mechanical information such as texture is mainly transmitted via the *N. trigeminus* (N.V) and the trigeminal ganglion.

As for the classical taste sensing machinery localized in ectopic but strategically important position, also the fat sensor, GPR119, is expressed in pancreatic β cells and in enteroendocrine cells. GPR119 can be activated by oleoylethanolamide and several other endogenous lipids containing oleic acid, generated in several tissues and in the gut lumen. Stimulation of GLP-1 release by dietary fat is probably mediated in large part through the luminal formation of 2-monoacylglycerol acting on the 'fat sensor' GPR119. In the pancreas GPR119 is may activated by 2-monoacylglycerol generated from pancreatic triacylglycerol. GPR119 will be crucial for the fat sensing bypassing the taste sensory system (Hansen et al. 2012).

There is, undoubtedly, a growing interest for the "off-target" activity of sweet- and bitter compounds (Clark et al. 2012). In this context, it should be remarked that, while sugars can be considered "neutral" from a pharmacological standpoint, most bitter compounds have specific pharmacological actions (alkaloids) or can interact with biological membranes in a non specific way (bitter ammonium soapy salts like denatonium chloride). In evolutionary terms, bitterness might have originally been invented by plants to signals the occurrence of poisonous compounds to predators, and, indeed, many poisonous compounds like alkaloids are bitter. Presumably later, this strategy was adopted by "impostor" plants, in a closer analogy to the mimicry of poisonous butterfly by harmless relatives. Curiously, the bitterest natural products known, the sesquiterpene lactone absinthin from wormwood and the iridoid glycoside amarogentin from gentian are not toxic, and their plant sources are popular ingredients of liqueurs. It seems that, by focusing on harmless compounds of this type and not on pharmacologically acting bitter agents like quinine or strychnine, a clarification on the "off target" role of bitter compounds could be achieved.

5 The Chemesthetic System

We always evaluate our food, and express our appreciation or dislike of it in terms of flavor. Flavor is the complex interplay of chemesthesis, taste and palatability, and requires all of our five senses. *Shakespeare* (1564–1616) might have already perceived this complexity when he wrote the lamentation of Jacques on old age in "As you like it" (Shepherd 2012):

Last scene of all,
That ends this strange eventful history
Is second childiness and mere olivion,
Sans teeth, sans eyes, sans taste, sans everything
Act 2, scene 7.

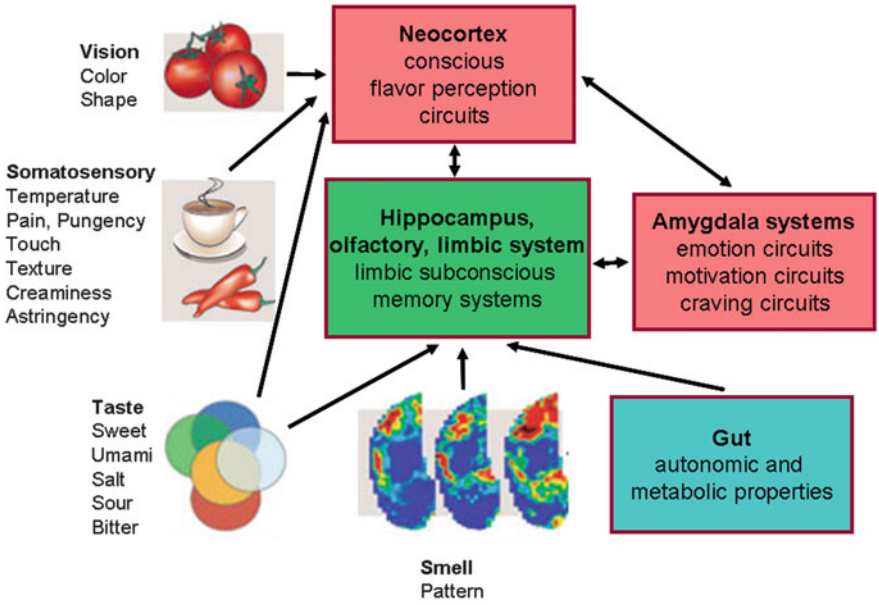

Fig. 3 The evaluation system for food. Taste sensing, odor perception are included but especially chemesthetic evaluation via somatosensors (e.g. temperature, mechano-sensong, pain, vision and a chemosensory input from the gut) (Adapted from Shepherd (2006), Shepherd (2012). With permission of *Nature*)

Chemesthesis or chemesthetic sensations arise when chemical compounds activate gustatory responses associated to senses that mediate pain, touch, and thermal perception (Fig. 3). These chemical-induced reactions do not fit into the traditional sense categories of taste and smell. Examples of chemesthetic sensations include the burn-like irritation from chili pepper, the coolness of menthol in mouthwashes and topical analgesic creams, the stinging or tingling of carbonation in the nose and mouth, and the tear-induction of onions. Often, they refer to spicy qualities or piquance. Also chemosensory signals from our gut modulate the taste sensation. All signals are evaluated in the central nervous system (hippocampus, olfactory, limbic system, nucleus amygdalus) to form finally a picture (neocortex) about the flavor of our food (Fig. 3) (Shepherd 2006, 2012).

Chemesthetic sensations arise by direct chemical activation of ion channels on sensory nerve fibers, e.g. of TRP channel including TRPV1, TRPV3, TRPV4, TRPA1 or TRPM3, TRPM4, TRPM5, TRPM8. Alternatively, irritant chemicals may activate cells of the epithelium to release substances that may active the nerve fibers indirectly.

Chemesthetic properties are the evolutionary gift of TRP channels to our food! Importantly, this quality of chemesthesis is connected to behavior, such as like bait shyness, avoidance of sick-making and rotten food and built up an efficient form of memory. They determine what we describe as mouth-sense, mouth-feel, and food-

texture: sweet feels more viscous, sour feels more fluid, hot food taste stronger. Other flavor qualities include TRPV1 for the metallic taste of sweeteners and the avoidance of too salty food. Excitingly, just beyond TRP channels, food can coordinates courtship, a drosophila fairy tale! A new ionotropic glutamate receptor (IR84a) is described in the neuronal circuitry of the fruit fly drosophila which is required for courtship. This receptor is activated by phenylacetic acid and phenylacetaldehyde, which have aromatic odors and origin from food sources such as fruits and other plant tissues. These food-derived compounds activate neurons in neuronal circuits in male drosophila which trigger and coordinate courtship behavior. This is a very interesting finding: evolution couples feeding with reproductive behavior! I will love you if you eat the correct food (Grosjean et al. 2011)!

Importantly, TRPV1 is expressed on the tongue epithelium together with calcitonin gene-related peptide (CGRP). Strong expression is restricted to the apex of the tongue and CGRP-expressing nerve terminals were in close apposition to the strongly TRPV1-expressing epithelium of fungiform papilla (Kawashima et al. 2012). A chemesthetic connection to pain seems now unraveled. Tachykinins, i.e. substance P (SP) and neurokinin A (NKA), are present in nociceptive sensory fibers expressing TRPV1 which are found extensively in and around the taste buds. Tachykinins are released from nociceptive fibers by irritants such as capsaicin, the active compound found in chili peppers commonly associated with the sensation of spiciness. Such compounds induce an increase in $[Ca^{2+}]_i$ in taste cells, which is inhibited by blocking the SP receptor NK-1R. Tachykinin signaling in taste cells requires Ca^{2+}-release from endoplasmic reticulum stores. Mouse taste buds express NK-1R and NK-2R. Tachykinin-responsive taste cells were Type I (Glial-like) and umami-responsive Type II TRCs. Stimulating NK-1R had an additive effect on Ca^{2+} responses evoked by umami stimuli in Type II (Receptor) cells. This data indicates that tachykinin release from nociceptive sensory fibers in and around taste buds may enhance umami and other taste modalities, providing a possible mechanism for the increased palatability of spicy foods (Grant 2012).

Taste and chemosthesis comprise probably all five senses to determine the quality of food. An intriguing anectode can be find in Gordon M. Shepherd's book on "*Neurogastronomy*" in which a French Chef defines the ideal wine by: "*it satisfies by vision (color), smell (bouquet), touch (freshness), flavor (taste) and hearing (it's glou-glou (sic)).*"*(hearing the wine in Moliére, The doctor in Spite of himself (Le médecin malgré lui), act 1, scene 5)* (Shepherd 2006, 2012).

6 Spicy Plants

The leading ethnobotanist James Duke, creator of databases on phytochemicals at the *US Department of Agriculture*, claims that human, having co-evolved with plants, have developed the ability to maintain homeostasis by selectively using the compounds they need from food plants. In this context, spices are a concentrate of

bioactive compounds. Turmeric (*Curcuma longa* L.) is estimated to contain 5,000 biologically active chemicals (but Duke puts this number to 50,000), and most spices might have as many. Spice constituents often show a triad of anti-inflammatory, antioxidant, and anti-infective activity, and sometimes outperforms mainstream pharmaceutical drugs in pre-clinical assays. According to Duke, synthetic drugs, even when efficacious, "disturb homeostasis", and he suggests to accompany pharmaceuticals with selected spices in order to "homeostatically balances the patient". Despite this vague rationale, Duke claims that challenging the use of herbs, and especially turmeric, during cancer chemotherapy, "*borders. . .on criminal*", and predicts that 1 day in the near future, computers will be able to select from among 250,000 herbs those best suited to an individual with a particular ailment. Duke's spice database lists about 200 plants, most of which are not only used for culinary purposes, but also for medical indications. The database shows evidence for activity in many diseases, and Dukes gives good example by using capsaicin from peppers (*Capsicum*) for back pain, scoliosis and spondylosis, drinking "BackBracer" tea with spearmint (*Mentha spicata*) or peppermint (*Mentha piperita*), ginger (*Zingiber officinale*), and capsaicin or pepper sauce. Mints have analgesic menthol. Adding wintergreen (*Gaultheria procumbens*) to the tea adds methyl salicylate for pain-relieving effects. He alternates this with a mustard (*Brassica juncea*) plaster and takes hot baths with lemon balm (*Melissa officinalis*), wintergreen, and peppermint. Other mints, yarrow (*Achillea millefolium*), thyme (*Thymus* spp.), bayberry (*Morella cerifera*), ground ivy (*Glechoma hederacea*), West Indian lemongrass (*Cymbopogon citratus*), mallow (*Malva neglecta*), flax (*Linum usitatissimum*) seed, and walnut (*Juglans nigra*) also have compounds that can provide back pain relief (Freedman 2008; Duke 2010). Each spice has a unique aroma and flavor and are known as "phyto-chemical" or "secondary compounds" because they are only "secondary" in the basic metabolism of a plant, but provide recipes for survival in the coevolutinary struggle against biotic enemies (Sherman and Billing 1999).

Spices have been used over millennia without knowing how they work and what they are really doing to us. Cultural and climate differences determine substantially the use of spices. Obviously, Japanese dishes are often "delicate," Indonesian, Indian and Szechwan dishes are "hot," and middle European and Scandinavian dishes "bland". A statistics on recipes from the Indian cuisine included 25 different spices, 9.3 were used per recipe. In Norway only 10 spices were used which accounted for 1.6 spices per recipe (Sherman and Billing 1999).

Highly educated gourmets like *Brillant-Savarin* where aware that, in addition to taste, spices also improve digestion, salivary-, gastric- and intestinal secretion, stimulate the production of bile, and activate gut motility. High caloric diets increase the risk of chronic diseases, and reducing energy can protect various tissues against disease, possibly also increasing lifespan. Pungent spices now have a target: TRP channels and especially TRPV1 and TRPA1, both expressed on the whole sensory system. Spices often contain ingredients that activate TRPV1 or TRPA1 in the sensory nerves of the mouth cavity and the palatine, where activation of these channels modulates the taste. Furthermore, they are widely

expressed on the gastro-intestinal, the nervous, the respiratory, and the cardiovascular system. Therefore, culinary use of the pungent spices has not only taste attributes but also potential "systemic" health effects.

A TRP-related prickly note has made hot pepper, ginger, fresh garlic, and horseradish popular ingredients in cuisine around the world. Within mammals, only humans deliberately consume hot food. They obviously enjoy its aversive effects! Hot cuisine may be considered as the culinary equivalent of a benign masochistic activity like a parachute drop, a hot bath, an icy shower, or a horror movie and experience a pleasant thrill when confronted with a "constrained risk", a "rollercoaster" drive (Nilius and Appendino 2011). Hot food mimics the danger of fire. Our oral cavity "senses fire" in the absence of any tissue damage, in a complete dissection of the sensory and pathology of burning. In turn, the sensation of danger and pain might cause our brain to release pain-relieving or mood-lifting endogenous compounds, hence explaining the additive culinary properties of spices.

Another beneficial gustatory effect of spicy food is the increased responses to salt, an effect mediated by TRPV1. Salt laced with spices is commercially available to reduce dietary sodium intake. Hydroxy alfa-sanshool is an active component of Szechuang pepper (*Xanthoxylum bungeanum* Maxim and related species) which also contains citral, a TRPA1 agonist. Hydroxy alfa-sanshool is a pungent TRPV1 activator, and is both the archetypal inducer of tingling, a sort of paralytic pungency and an excellent replacement of salt in French Fries. Because of its binding to the endocannabinoid CB2 receptor, it makes Szechuan pepper a favoured target in neuroculinary research! (Chef Shirley Cheng personal communication and http://menuscience.ciachef.edu/node/529). Interestingly, Szechuang pepper, although pungent, can reduce the pungency of hot pepper, and is used to moderate the hotness of chilli sauces, acting as a veritable chemical antidote to hot pepper pungency (McGee 2001). Finally, when applied topically, the alkamide fraction from Szechuan pepper temporarily reduces superficial wrinkles, acting as a sort of short-acting Cinderella-style Botox (Artaria et al. 2011). Due to the multitude of targets, the sensory profile of hydroxy alfa-sanshool is combinatorial, and cannot be reduced to interaction with a single end-point. In this context, hydroxy alfa-sanshool is the "curcumin" of neurosciences.

TRPs are also involved in the sensory and pharmacological effects of alcohol. Ethanol potentiates the responses of TRPV1, mediating both beneficial effects and some drawbacks of alcohol consumption. Potentiation of TRPV1 responses on perivascular sensory nerve terminals leads to an increased secretion of CGRP and SP, causing vasodilation and beneficial effects at coronary level.

Another example, the practice of consuming hot dishes implies that our tongue gets easily insensitive to e.g. capsaicin, with an associated insensitivity to other irritant spices as well (mustard, pepper, and ginger). Trained people can assume large amounts of chili pepper without any adverse effect (gastronomic mytridatism). In the light of the various beneficial effects of dietary food, evolution seems to have installed in humans an unconscious quest to optimize accepted flavors (see for review Appendino et al. 2008).

6.1 The Case of TRPV1

TRPV1 is activated by pungent *Capsicum* spices that produce as active components capsaicinoids. The genus *Capsicum* encompasses 22 wild species, 5 of which cultivated [*C. annuum, C. chinense* (Habanero), *C. fructescens* (Tabasco), *C. baccatum, C. pubescens*], and well over 3,000 varieties, The genus *Capsicum* is endemic in the highlands of Peru and Bolivia. Capsaicinoids are the amides of a phenolic amine (vanillamine) with medium-sized, mostly branched, fatty acids. Over 12 major capsaicinoids have been characterized from chili, the most abundant ones being capsaicin and dihydrocapsaicin. Homocapsaicin, homodihydrocapsaicin and nordihydrocapsaicin are only half as pungent as capsaicin and dihydrocapsaicin. Capsaicin is produced only in the placenta of the fruits, where most of it (ca. 86 %) is located, with minor amounts leaking into the pericarp (ca. 6 %) and the seeds (ca. 8 %). The leaves contain only minor amounts of capsaicin. In contrast to capsaicin, its ester analogue (capsiate) is not pungent (see below).

In vivo activation of TRPV1 receptors by natural agonists like capsaicin is associated with a sharp and burning pain, perceived as pungency. Remarkably, the sensory properties of TRPV1 agonists and their activating potency are substantially unrelated, with pungency decreasing in the order capsaicin > piperine > RTX > arvani > olvanil, and potency in the order RTX >> olvanil and arvanil > capsaicin > piperine. Pungency of TRPV1 agonists is critically dependent on lipophilicity: highly lipophilic agonists are less pungent because they cause slow TRPV1 activation, delaying, or even suppressing, its ability to trigger action potentials in sensory neurons (Ursu et al. 2010). TRPV1 agonists mediate thermogenic effects, i.e. systemic administration causes a drop in core temperature and subsequent activation of thermogenesis and energy dissipation. Therefore, non-pungent activators represent attractive dietary ingredients to support weight loss. Nevertheless, all other TRPV1 related effects will be maintained (Chu et al. 2009), including those on thermogenesis. These considerations underlie the development of capsinoids, a group of non-pungent ester isosters of capsaicinoids, as slimming agents. These compounds were discovered in the late nineties in a sweet pepper cultivar (*CH-19 Sweet*) from Thailand (Kobata et al. 1998; Kobata et al. 1999), and later found in high concentration also in the Japanese cultivar *Himo*. The production of these compounds is associated to specific mutations in the putative aminotransferase gene (*p-AMT*) responsible for the reductive amination of vanillin, the key step in the biosynthesis of capsaicin in pepper fruits. In the absence of a functioning aminotransferase, vanillic alcohol, and not vanillamine, is produced, and this alcohol is eventually acylated to capsinoids in a striking example of biosynthetic tinkering (Lang et al. 2009; Tanaka et al. 2010). In CH-19 Sweet, a nonsense mutation (insertion of a T nucleotide at base pair 1291, with formation of the stop codon TGA) prevents translation of the gene (Lang et al. 2009), while in Himo a single-nucleotide substitution (T → C) at base pair 755 results in a cysteine-to-arginine change in the pyridoxal 5-phosphate binding domain of the enzyme, with loss of function (Tanaka et al. 2010). Capsinoids are exemplified

by capsiate, the ester isoster of capsaicin, and share the thermogenic and metabolic properties of capsaicinoids, but not their pungency (Iida et al. 2003) (Josse et al. 2010). Interstingly, olive oil aromatized with hot pepper is a common condiment in the Mediterranean area. Transamidation of capsaicin to *N*-oleylvanillamine (olvanil) can occur. Olvanil, a trace capsaicinoid in hot pepper, is non pungent, but much more potent than capsaicin as an anti-inflammatory agent, and might contribute the health effects traditionally attributed to olive oil flavored with chili (see for a review also Nilius and Appendino 2011).

Also other plants produce pungent chemical components. Ginger is the rhizome (underground stem) of *Zingiber officinalis* Roscoe, family Zingiberaceae. The plant is of Indian origin, but is now cultivated in the tropics and, in greenhouse, also in Europe. Plants from the family Zingiberaceae are important spices and major ingredients of curry. The most important ones are turmeric (*Curcuma longa* L.), zedoary (*C. zedoaria* Roscoe), galangal (*Alpinia galanga* L.), cardamomon (*Elettaria cardamomum* L.), and grains of paradies (*Aframomum melegueta* K. Schum.). Ginger has a remarkable culinary range of uses, from sausages to fish dishes, sweets and soft drinks. It combines the refreshing note of lemon to a pepper-like pungency and a floral note, complementing other flavors rather than dominating them. Fresh ginger contains gingerols that are partially dehydrated to shogaols during drying. Shogaols are twice more pungent than gingerols, and dry ginger is more pungent than fresh ginger. Gingerols are classified with a number in bracket that refers to the number of carbon atoms from the oxymethine to the terminal methyl. [6]-Gingerol is more pungent than its longer homologues ([8]-and [10]-gingerols). Cooking triggers the retro-aldolization (crotonization) of gingerols and shogaols to zingerone, a much less pungent compound with a sweet-spicy aroma. The typical constituents of the essential oil are bisabolane sesquiterpene. The major one is α-zingiberene that on storage is dehydrogenated to *ar*-curcumene. The essential oil of Australian ginger has a high content of citral, and, indeed, Australian ginger smells like lemon.

Another important component of TRPV1 mediated pungency comes from pepper, the berry of the Indian vine *Piper nigrum* L. (family Piperaceae). The plant is cultivated in India, Indonesia, Malaysia and South-America, with a world production approaching 230,000 t, and second only to hot pepper. There is a remarkable rainbow of peppers. Green pepper comes from whole immature berries frozen, pasteurized or conserved in acidic solution; black pepper from dried mature (not yet fully red) berries let browning in the air; white pepper from ripened (red) berries from which the pericarp has been removed; pink pepper from ripened berries preserved in brine and vinegar to avoid browning. The alkamide piperine is the pungent principle of pepper and activates TRPV1. Piperine is ~100-fold less pungent than capsaicin, but is more potent than capsaicin as a TRPV1 desensitizer, inducing its presence in the inactive dephosphorylated state. Piperine is localized in the fruit layer and in the surface layer of the seeds. The major aroma compounds of pepper are localized in the outer fruit layer, and therefore white pepper lacks most of the aroma of black pepper. Piperine is light-sensitive, and pepper loses its pungency if exposed to light, because pungent piperine is converted to almost

tasteless isochavicine. This photochemical transformation exemplifies the relevance of *cis*/*trans* isomerism in chemesthesis. Pepper must be protected from light! Other TRPV1 activators are obtained from water pepper (*Polygonum hydropiper* L.), used as a cheap pepper replacement in the Roman and Medieval peasant cuisine. Water pepper was once cultivated, but is now rarely employed in the Western cuisine. Water pepper contains polygodial, a sesquiterpene dialdehyde that activates TRPV1. Other source for TRPV1 activators are the grain of paradise (*Aframomum melegueta* K. Schum.). The plant grows in Africa's Equatorial West Coast, and its name testifies how praised it was in medieval times. Also *Xylopia aethiopica* (Dunal) A. Rich (grains of Selim), the source of negro pepper, grows in tropical Africa, and was trade to Europe especially in Medieval times. The plant contains unique diterpenoids, but no information on their activity on pungent TRP receptors has been reported so far.

Rutaecarpine, a spicy alkaloid, is found in certain herbs including *Evodia rutaecarpa* (Peng and Li 2010). Essential oils from rose, thyme geraniol, palmarosa, and tolu balsam contain constituents which activate TRPV1, like citronellol (main constituent of rose oil) and geraniol (main constituent of thyme geraniol and palmarosa oils) (Ohkawara et al. 2010).

There is another aspect of a role of TRPV1 channels. Salt taste is legend! It triggers two divergent behavioural responses, since high concentrations of saline solutions elicit aversion, whereas low concentrations are considered hedonic and attractive. The attractive salt pathway is probably mediated via the epithelial sodium channel (ENaC) in type III cell. However, the aversive functions is due to a non-selective detector for a wide range of salts which is amiloride-insensitive and might be coupled with TRPV1 or a truncated variant TRPV1t expressed also in type III cells (Chandrashekar et al. 2010). Here, TRPs give us the unpleasant feeling of the badly oversalted meal (Lyall et al. 2005, 2010). However, there is still a ENaC independent salt detection in the TRPV1 ko mouse, indicating that additionally, there may be other amiloride-insensitive salt transduction mechanisms in taste receptor fields that maintain normal salt detection performance in the KO mice (Treesukosol et al. 2007). TRPV1 also contributes to the bitter taste by inducing high concentrations of Ca^{2+} and Mg^{2+} and sensations like salty, metallic, astringent and sour. TRPV1 mediates the bitter aftertaste sensation of artificial sweeteners (AS) like saccharin and acesulfame-K, which all activate the channel and sensitize it to acid and temperature. TRPV1 can be also activated by $CuSO_4$, $ZnSO_4$ and $FeSO_4$, which all produce a metallic taste sensation. Thus, activation of TRPV1 provide a molecular mechanism that account for off tastes of sweeteners and metallic tasting salts (Riera et al. 2007, 2008, 2009). TRPV1 ko mice show NaCl presence. As a conclusion, TRPV1 is rather involved in avoidance of high salt concentrations rather than sensing salt (Ruiz et al. 2006). However, single nucleotide polymorphisms (SNPs) in TRPV1 has been shown to modify supra-threshold taste sensitivity and causes a higher sensitivity to salt solutions than in normal genotype populations (Dias et al. 2012). TRPV1 has become also interesting because it mediates the taste of Maillard-reacted peptide (MRP), an arginine-free peptide which was considered to be a key substance which gives the characteristic

flavour (mouthfulness and continuity) of Miso soup (味噌 miso shiru), a traditional Japanese soup consisting of a stock called "dashi" into which softened miso paste is mixed. MRPs are salt taste enhancers which vary in effect depending on the conjugated sugar moiety. Cooked meat gets its flavor (and its brown color as well) from the Maillard reaction, a chemical reaction between amino acids and reducing sugars that is triggered by heat. The reactive carbonyl group of the sugar reacts with the nucleophilic amino group of the amino acid, in a replica of the protein glycation that plays havoc in diabetic patients. This pleasant salt taste modulation requires TRPV1t and is additive with TRPV1 activation by the common hot spices (Katsumata et al. 2008).

In many cases, more or less complex combinations of spices and herbs, usually (but not invariably) including fresh or dried hot capsicum peppers, are used for culinary purposes. The well known *curry* (from Tamil word *kari* meaning 'sauce') includes coriander, turmeric, cumin, fenugreek, and red pepper in their blends. Curry is alien to authentic Indian cuisine, that see it as a globalized and standardized version of the marvelous diversity of its masalas, that use a variety of additional ingredients (ginger, garlic, asafoetida, fennel seed, caraway, cinnamon, clove, mustard seed, green cardamom, black cardamom, nutmeg, long pepper, black pepper) to selectively modulate the flavor of dishes.

6.2 "Irritant" Pungency: TRPA1 A New Player

TRPA1 is a major target of electrophilic molecules. The first signal of the intake of possibly beneficial electrophiles comes from the taste sensation they induce by activating TRPA1 on sensory nerves in our mouth, palatine, and tongue. Electrophiles in our food are present in cruciferous vegetables like broccoli, cauliflower, watercress, Brussels sprouts, Japanese radish, black mustard, papaya, wasabi. They all cause special taste sensations, exploited by good chiefs, via TRPs, especially TRPA1. Probably everybody has experienced the pungent and irritating taste of mustard! Its active component is allyl isothiocyanate (AITC), one of the most efficient activators of TRPA1. This channel is present in the sensory nerves of the mouth cavity and the palatine where activation of these channels modulates the taste (for a review see Nilius et al. 2012).

Many culinary *Brassica* plants have pungent and even obnoxious properties. Cruciferous plants are used for a multitude of purposes in cuisine, the most famous product being the *Moutarde de Dijon* and horseradish sauces. Cruciferous (wasabi, mustard, Brussels sprouts, and horse-radish) and related plants (capers, nasturtium) contain offensive lachrymatory principles known as isothiocyanates. Isothiocyanates are not genuine plant constituents. They are accumulated as glucosinolates and compartmentalized (physically separated in distinct biological structures) from those that contain their hydrolytic enzyme (myrosinase). Tissue damage triggers the glucosinolate bomb, resulting in the myrosinase-catalyzed hydrolysis of glucosinolates into isothiocyanates. Besides isothiocyanates, nitrilesare are often formed upon

glucosinolate hydrolysis due to the presence of proteins that modulate the outcome of glucosinolate hydrolysis without having hydrolytic activity on glucosinolates. The concentration of glucosinolatesin plants can reach 4 g/kg in Brussels sprouts. Different cruciferous plants contain different glucosinolates, derived from distinct amino acids.

A garlic/onion bomb also exists. Garlic contains the odorless amino acid alliin, a cysteinyl derivative (ca. 0.25 %). Alliin (from *Allium* plants) is the substrate of alliinase, an enzyme stored in different types of cells or compartment. Crushing fresh garlic puts alliin and allinase in contact, generating a sulfenic acid that dimerizes spontaneously to allicin, a pungent compound. In many cultures, e.g. in East European countries, the addition of garlic to many meals is highly favored. Allicin is an irritating compound that activates TRPA1 (Bautista et al. 2005).

Allyl isothiocyanate from mustard and allicin from garlic are the archetypal dietary TRPA1 activators. In striking diversity to chili and black pepper, TRPA1-mediated pungency is not present in the plant, but is unleashed by a cascade reaction triggered by an enzymatic process, with only its final products being capable of activating TRPA1. This enzymatic activity is heat-sensitive, and is significantly lost by heating, that therefore moderates or nullify the pungency of mustard, garlic and onion, while the TRPV1-mediated pungency of peppers is heat-stable.

Tingling is relatively alien to Western cuisine, but is the main sensory element of several spices used in the Eastern cuisine. Tingling is due to the occurrence of polyunsaturated alkylamides. Extracts of Sichuan and Melegueta peppers evoke pungent sensations mediated by different alkylamides [mainly hydroxyl α−sanshool (a-SOH)] and hydroxyarylalkanones (6-shogaol and 6-paradol). This pungent sensation is accompanied by pleasant tingling, cooling and numbing sensations. Tingling involves a complex mechanism of sensory neuron activation via several ion channels, under which inhibition by sanshool of K^+ channels, such as the two-pore K^+ channels KCNK3, KCNK9, and KCNK18. Inhibition of these leak channels may cause depolarization and may support the action of depolarizing TRP channels (Koo et al. 2007; Bautista et al. 2008; Menozzi-Smarrito et al. 2009; Riera et al. 2009; Klein et al. 2011).

Another plant widely used in the Asian cuisine is perilla [*Perilla frutescens* (*L*) Britt.] This plant has interesting taste and somatosensory properties. In Korea, leaves from perilla are used as food, and its seeds are used to make edible oil. Sometimes, the seeds are added to soup for seasoning. It is also used in traditional Chinese medicine for inducing diaphoresis, dispelling heat, moving, and strengthening the stomach and digestion. Perillaldehyde and perillaketone are among the components of the aromatic extracts from perilla, and they all activate TRPA1, providing a molecular mechanism for the chemesthetic properties of this plant (Bassoli et al. 2009).

The first leaves of *Kalopanax pictus Nakai* (Araliaceae) are used for a delicious piquant tea, which is used in Korea to treat several diseases under which neuralgias such as lumbago. The major active constituent is methyl syringate, which is a selective TRPA1 activator (Son et al. 2012a, b).

TRPA1 agonists are also derived from terpenoids with an α,β-unsaturated 1,4-dialdehyde moiety in the active compounds, like miogadial, miogatrial, and polygodial. These compounds have a broad distribution in Nature, having been isolated from plants, mushrooms, insects, and marine organisms. Dialdehyde-containing "spices" (a term that here even embraces marine molluscs) are used in cuisine because of their pleasant pungent taste. They also have antimicrobial and anti-fungal activity, and show anti-cancer properties in many pre-clinical assays. Dialdehydes are contained in the flower buds of the myoga plant (*Zingiber mioga* Roscoe). Flower buds are shredded and used in Japanese cuisine as a garnish for miso soup, sunomono, and dishes like roasted eggplant.

In Korean cuisine, the flower buds are skewered alternately with pieces of meat, and then pan-fried, providing a combination of TRPA1 activation, taste modulation, and possible beneficial health effects, due to the anti-bacterial, antifungal and anticancer properties of myogadial (Iwasaki et al. 2009).

Electrophiles in our food are present in cruciferous vegetables like broccoli, cauliflower, watercress, Brussels sprouts, Japanese radish, black mustard, papaya, wasabi. They all cause special taste sensations, exploited by good chiefs, via TRPA1, and combine hedonic effects with chemoprevention by reducing oxidative stress (see for an excellent review Nakamura and Miyoshi). Phytochemicals like curcumin, the main curcuminoid of the popular Indian spice turmeric, also act on TRPA1, and, just like species from the family Alliaceae, it causes expression of genes encoding cytoprotective proteins, including antioxidant enzymes, protein chaperones, growth factors and mitochondrial proteins (Mattson 2008). Curcumin causes complete desensitization of TRPA1, and exhibits a marked tachyphylaxis upon subsequent application (Leamy et al. 2011). Curcumin also inhibits TRPV1 (Yeon et al. 2012), but an important new mechanisms has been recently discovered which might be critical to understand many "sensory" and systemic properties of curcumin. Curcumin and the caffeic acid phenethyl ester (CAPE), a constituent of European propolis, inhibit the store-operated Ca^{2+} entry, CRAC currents, in Orai1/STIM1-co-expressing cells. Both compounds contain electrophilic α,β-unsaturated carbonyl groups that potentially form Michael addition with cysteine residues. Cysteines (especially Cys195) in Orai1 are sensitive to curcumin and CAPE. Covalent modification causes an inhibitory effect. Replacing the most sensitive cysteine residue with serine (C195S) reversed the effect of CAPE from inhibition to facilitation and significantly weakened the inhibitory effect of curcumin. Tetrahydrocurcumin, a curcumin metabolite, is a less potent inhibitor of CRAC. This unexpected electrophilic inhibition of CRAC by spices offers probably many explanations for until now not understood effects of electrophilic dietary intake (Shin et al. 2012). CAPE is, per se, inactive in thiol-trapping experiments (Avonto et al. 2011), but is easily oxidized to an electrophilic *ortho*-quinone that might be the actual thiol-trapping species.

Curcumin is credited with a plethora of beneficial effects, and especially anti-cancerogenic properties (Aggarwal 2011; Darvesh et al. 2011). With over 3,000 entries in PubMed, it is, undoubtedly, one of the best investigated natural products. However, the clinical literature on curcumin is very modest, and its clinical study has been hampered by its very low oral bioavailability (Anand et al. 2008). On the

other hand, the local concentrations of curcumin after a curry-laced meal are sufficient to activate the sensory receptors lining the oral and gastro-intestinal surface. For instance, the classic recipe of egg-curry involves the consumption of ca. 250 mg curcumin per serving (calculation made using the recipe reported in http://www.vietworldkitchen.com/blog/2011/08/north-indian-egg-curry-recipe-anda-masala.html, and assuming a 5 % curcuminoids contents for turmeric), leading, after dilution with saliva and gastrointestinal juices, to local millimolar or micromolar concentrations of curcumin, sufficient for interaction with TRPA1, TRPV1 and CRAC.

1′-Acetoxychavicol acetate (ACA), the main pungent component in galangal, does not activate TRPV1, but strongly activates TRPA1, being even more potent than allyl isothiocyanate from MO (Narukawa et al. 2010). Galangal (Galanga, blue ginger) is obtained from the rhizome of *Alpinia galanga* L. Willd., a zingiberaceous species with many culinary and medicinal uses, especially in Indonesia (Sung et al. 2012). The rhizome is also a common ingredient in Thai soups and curries, where is used fresh in chunks or thin slices, mashed and mixed into curry paste, or dried and powdered.

Ginger also encompasses a TRPA1 component. Shogaols are electrophilic compounds that interact not only with TRPV1, but also with TRPA1. In a systematic study on the TRPV1-TRPA1 ligand properties of [6]-gingeroids, some behaved as selective TRPV1 agonists/desensitizers of TRPV1 channels, and others as TRPA1 antagonists (Morera et al. 2012).

Thymol, a major component of thyme and oregano, is used as oral care product, as an astringent and antibiotic. Its distinctive sharp odour and pungent flavour are considered as aversive. Thymol activates TRPA1, and this effect disappears after pretreatment with camphor, a known TRPA1 inhibitor. The related phenols 2-tert-butyl-5-methylphenol, 2,6-diisopropylphenol (propofol, a general anesthetic) and carvacrol also activated TRPA1 (Xu et al. 2006; Lee et al. 2008).

Eugenol is a phenylpropene used in perfumeries, flavorings, and medicine as a local anesthetic. Cloves can be used in cooking either whole or in a ground form, but, due to their strong properties, their use is rather rare. On the other hand, eugenol is a popular anesthetic in dentistry, due to its desensitizing properties on TRPV1 and TRPA1, both highly expressed in dental tissues (Pramod et al. 2006). Ajoene (from garlic *Allium sativum* L.) is an unsaturated disulfide which contains reactive electrophilic chemical groups and has been claimed to show antithrombotic (anti-clotting) properties. It cannot alone activate TRPA1, but subsequent application of ajoene enhanced activation of TRPA1 by electrophiles and also by depolarization. Ajoene is, therefore, classified as a TRPA1 channel enhancer (Yassaka et al. 2010).

Salvia officinalis L. is used as a traditional herbal medicine for gastric disturbances and inflammatory processes. Hydroalcoholic extract (HE) from leaves of sage can reduce both neurogenic and inflammatory phases. Carnosol and ursolic acid/oleanolic acid inhibited the inflammatory phase of formalin and the nociception and mechanical allodynia induced by cinnamaldehyde. HE presents significant anti-inflammatory and also antinociceptive effects. Carnosol and ursolic acid/oleanolic acid contained in *Rosmarinus officinalis* have also antinociceptive properties, possibly through act via a modulatory influence on TRPA1-receptors (Rodrigues et al. 2011).

Ligustilide is the major aroma constituent of celery (*Apium graveolens* L.) and loverage (*Levisticum officinale* L.). It is also present in plants used in traditional Chinese medicine such as *Angelica sinensis* (Oliv) Diels and *Ligusticum chuanxiong* Hort. and North American traditional Medicine osha (*Ligusticum portieri* Coult. & Rose). It is an electrophilic potent TRPA1 activator but is also capable to induce a modest block of activated TRPA1. The action of ligustilide on TRPA1 contributes to the gustatory effects of celery, its major dietary source (Zhong et al. 2011).

This tour around TRPA1-modulating spices shows a more complex scenario compared to TRPV1-modulating spices. Thus, TRPV1 modulators behave as non-covalent ligands, and have a less pleiotropic profile of end-points compared to the electrophilic TRPA1 modulators of spice origin. Furthermore, dietary TRPA1 modulators are much more widespread compared to dietary TRPV1 modulators. In both cases, the overall biological profile of activity is multifaceted and complex, although, in a very rough simplification, we could say that dietary TRPV1 modulators have potential as analgesics, while dietary TRPA1 modulators are more relevant as anti-inflammatory agents.

6.3 A Gustatory and Beneficial TRPM5 Connection

As briefly described, bitter, sweet, and umami perception require activation of TRPM5 (Zhang et al. 2003, 2007b) and the signalling cascade for sweet and bitter depending on TRPM5, is also expressed in some gastric cells. Activation of bitter taste receptors on gastric cells, and thereupon TRPM5, stimulates ghrelin secretion, a hunger hormone with progastrokinetic effects. Modulation of ghrelin by bitter tasting compounds, e.g. in the famous German "*Magenbitter*" (i.e. bitter tasting apertitives or digestives, provide) are tools for treatment of gastrointestinal motility disorders (Janssen et al. 2011). Dietary free glutamate, which triggers umami taste in type 2 cells, is also sensed by specific glutamate sensors in the gastric mucosa and contributes to the regulation of gastrointestinal functions. This signalling is also coupled, just like in taste bud cells, to TRPM5, indicating that the perception of food is supported by glutamate sensing in the gastric mucosa (Nakamura et al. 2010).

Another role of TRPM5 in body weight control has been suggested from studies on rat. Quinine is a natural molecule commonly used as a flavoring agent in tonic water. Diet supplementation with quinine leads to decreased body weight and food intake in rats. Quinine is an in vitro inhibitor of TRPM5. In mice, the same effects were observed which was not present in trpm5 deficient control. Obviously, quinine contributes to weight control in rodents including a contribution of TRPM5 (Cettour-Rose et al. 2013).

As outlined, TRPM5 provides the key signal for the secretion of transmitters, most likely ATP, for transducing sweet, sour, and umami type 2 receptor cells to sensory fibres converging on type 3 cells. TRPM5, importantly, is a thermoTRP, i.e. it is highly activated with a Q_{10} of ~10 by increased temperatures. It is probably this thermoTRP that mediates the temperature sensitivity of our gustatory sensation.

Upon activation of a taste-specific receptor, TRPM5 function as an amplifier of the primary signals evoked by tastant binding. If a bitter tastant (e.g. hop, the flavoring and foam stabilizer of beer) activates its receptor, then the bitter taste is reduced by cooling and enhanced by warm food. This makes cold beer less bitter (and more popular) than warm beer! The same hold for sweet and umami: ice-cold cheese is tasteless, ice-cream becomes pleasantly sweet when it melts on the tongue, not to speak about the right temperature for a red wine (Talavera et al. 2005, 2007)!

7 Spices, TRPs and Health

In general, spices have been used over millennia without knowing how they work and what they are really doing, but a connection with health has always been vaguely implicit in their use. As we have mentioned, Brillant-Savarin was well aware that spices have "systemic" effects beyond their flavour. Incidentally, Brillant-Savarin was also aware that high caloric intake had some risks (he invented a low-caloric cheese!), and that reducing energy intake is beneficial for health and protects various tissues against disease. To begin with, a statistics from the World Health Organization from 2009 shows that India consumes more than 2,500,000 t of spices and has a cancer incidence of less than 100 per 100,000 for males and females. In contrast, the US consumes less than 250,000 t of spices and has a cancer incidence of more than 500 per 100,000 inhabitants (Aggarwal et al. 2009). Pungent spices have now a target, TRP channels and especially TRPV1 and TRPA1, both expressed in the whole sensory system. Spices often contain ingredients that activate TRPV1 or TRPA1 in the sensory nerves of the mouth cavity and the palatine, where activation of these channels modulates taste, but they are also expressed in the gastro-intestinal and the cardiovascular system. Culinary use of the pungent spices has not only taste attributes but, as discussed below, offers health benefits. Spices might be supportive to reduce food intake. Obviously, reduction of food intake has to be taken seriously. Just remember: overweight and obesity are connected to diabetes, hypertension, heart failure, immune deficiency, chronic inflammations but also during midlife to late-life dementia (Kingwell 2011). Capsaicin stimulates gastric acid secretion, but also provides some protective effects on the gastric mucosa. TRPV1 is expressed in gastric mucosa epithelial cells and plays an important role in gastric defense. Capsaicin shows antibacterial activity against *Helicobacter pilori*, the causative agent of gastric ulcer. Antagonists of capsaicin are being developed for pharmaceutical purposes, and gastric toxicity is one of their major side-effects. Food goes often together with alcohol. An interesting link between TRPA1 and alcohol abuse has been considered. TRPV1 and TRPA1 are expressed in oral trigeminal neurons and mediate the aversive orosensory response to many chemical irritants including aversive oral effects of ethanol. In mice, fetal ethanol exposure attenuated the oral aversiveness of ethanol in adult mice. Increased acceptability of ethanol was directly related to this reduced aversiveness (Glendinning et al. 2012b).

7.1 Spices and Obesity

The spicy TRPV1 channel has also a "say" on metabolism and visceral fat, with a connection to obesity becoming more and more evident. TRPV1 agonists have maybe most intensively studied for dietary effects. It has been shown in early studies that addition of red pepper to the breakfast significantly decreased fat and protein intake associated with an increased activity of the sympathetic nervous system (Yoshioka et al. 1999). However, the effects of TRPV1 are complex. In animal models, *Trpv1* knock-out mice are protected from diet-induced obesity (Motter and Ahern 2008). On the other hand, in several tissues such as skeletal muscle, white fat tissue and liver, capsaicin clearly increases the expression of hormone sensitive lipases (HSL), carnitine palmmitoyl transferase 1α (CPT-1α) and uncoupling protein UPC2 which are involved in the lipid catabolic pathway and thermogenesis (Zhu et al. 2010; Lee et al. 2011; Li et al. 2012; Luo et al. 2012; Wang et al. 2012a). In general, it seems that dietary intake of TRPV1 agonists, especially capsaicin, trigger cellular mechanisms against obesity (for a detailed review see Zsombok 2013). However, further investigation is required.

In this context, could non-pungents casaicinoids (capsinoids) from pepper be a magic slimming pill? Capsiate, the archetypal capsinoid, has the same affinity and potency of its pungent amide isoster capsaicin to TRPV1, but is not pungent. A problem with TRPV1 agonist might be their pungency, i.e. in vivo activation of TRPV1 receptors by natural agonists like capsaicin is associated with a sharp and burning pain. However, pungency and activation of TRPV1 activators can be substantially dissected by lipophilicity. Pungency dependents on the lipophilicity of the agonists: highly lipophilic agonists are less pungent because they cause slow TRPV1 activation and delay or even suppress their ability to trigger action potentials in sensory neurons and, ultimately, pungency (Ursu et al. 2010). All other TRPV1 related effects will be maintained. Capsiate is less toxic than capsaicin, and is available as dietary supplement in USA, Japan and Europe for promoting weight loss in association to diet and exercise. A study on the slimming properties of capsiate in humans discovered an interesting association between changes in abdominal adiposity and TRPV1 polymorphism (Snitker et al. 2009). At the recommended low dosage of 1 mg/day, the most pronounced slimming effects induced by capsiate were observed in individual bearing a Val585Ile mutation (Val/Val and Val/Ile variants) in the TRPV1 gene, while hardly any effect was associated to the Ile/Ile variant. Remarkably, variation around residue 585 of TRPV1 has been associated to the insensitivity to capsaicin pungency in birds and rabbits (Jordt and Julius 2002). Genotypic analysis would therefore be important to identify the best responders to capsinoids, and polymorphism in TRPV1 could underlie negative results on thermogenesis reported in some studies on capsiate (Galgani et al. 2010). Clearly, further research is necessary before capsinoids are touted as the modern version of the pill of Epimenides, the magic slimming ingredient inspired by the Greek philosopher who was rumoured not to have eaten for 50 years and next writing a book entitled "*The happiness of fasting*" (Watanabe et al. 2011). Interestingly, sensitivity to the slimming effects of green tea

is also dramatically dependent on polymorphism in COMT (catecholamine *O*-methyl transferase), the thermogenic target of EGCG (epigallocatechin gallate) (Inoue-Choi et al. 2010). These examples suggest that dietary ingredients, because of their long association with humans, might have selected a genetic variation that modulates the sensitivity to their activity. Conversely, the activity of synthetic drugs is less plagued by human genetic variations, because they are "more alien" to our body.

Mice lacking TRPV1 have only subtle alterations of the body temperature or in their responses to thermal challenges, but are hypometabolic (have a lower oxygen consumption) and hypervasoconstricted (have a lower tail skin temperature), i.e. these mice possess a distinct thermoregulatory phenotype, which is coupled with a predisposition to age-associated overweight and includes hypometabolism (Garami et al. 2011) (Wanner et al. 2012). In this study, TRPV1 deficient mice are overweight, suggesting a remarkable influence of TRPV1 on the development of obesity. Obesity-induced inflammation contributes to the development of obesity-related metabolic disorders such as insulin resistance, type 2 diabetes, fatty liver disease, and cardiovascular disease, and dietary capsaicin can reduce obesity-induced inflammation and metabolic disorders such as insulin resistance and hepatic steatosis (Kang et al. 2009).

TRPV1 agonists prevent adipogenesis in preadipocytes, an effect attenuated by knocking down TRPV1. During regular adipogenesis, TRPV1 channels are downregulated. Oral administration of capsaicin for 120 days prevented obesity in male wild type mice but not in TRPV1 knockout mice under high fat diet, thus, dietary spicy TRPV1 activators might be considered as novel players in adipogenesis and obesity (Cioffi 2007; Zhang et al. 2007a). Obesity requires a positive energy balance, i.e. caloric input is higher than the used energy. Food intake is often determined by flavor. Of note, ~30 % of vegetables consumed in the Western diet are French fried potatoes, which also increases the salt load. Szechuan pepper could, at least, be able to decrease this load. Obesity is already initiated by a small but sustained positive energy balance that can be prevented by a corresponding modest alteration of energy expenditure and lowering of appetite.

Mayan inhabitants of Mesoamerica incorporate chili peppers (*Capsicum* species Solanaceae) into a number of medicinal preparations as listed in a collection of 437 Mayan therapeutic remedies. Mexicans eat ~250 mg capsaicin/day. US Americans (only 10 % users) consume 0.6 mg capsaicin/day. Unsurprisingly, obesity incidence is negatively correlated with spice consumption although the adoption of a Western diet is taking its obesity toll also in countries where spice consumption is high (Ludy and Mattes 2012).

TRPV1 can also affect brown adipocytes (brown adipose tissue, BAT), which constitute a metabolically active tissue responsible for non-shivering thermogenesis and the depletion of excess calories. The major molecular mechanisms involved in the control of brown fat activity are the increased β3-adrenergic activity during exposure to cold; the augmentation of thyroid function; the modulation of peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor protein, and of the PPARγ co-activator 1 (PGC1α) which induce expression of the mitochondrial uncoupling protein 1 (UCP1). The stimulation of TRPV1 by capsaicin and monoacylglycerols stimulates BAT and may increase the thermogenic potential

of BAT. This may also be exploited by the development of novel therapies for obese and diabetic individuals (Birerdinc et al. 2012).

Capsinoids enhances fat oxidation, reduce energy intake, increase satiety, and prevent "pungent" adversion, being non-pungent. Hedonically acceptable capsaicin dosages are associated to a ca. 10 kcal negative energy balance (Ludy et al. 2012), and would produce an ultimate weight loss of 0.5 kg over 6.5 years in an average weight, middle-aged man, whereas a 50 kcal negative energy balance, as predicted for encapsulated non-pungent dihydrocapsiate, would yield a total weight reduction of 2.6 kg over 8.5 years. The ingestion of 10 mg of capsinoids increases adrenergic activity and energy expenditure, and results in a shift in substrate utilization toward lipids at rest, with hardly any effect during exercise or recovery. The observed changes confirm previous data on the thermogenic and metabolic effects of capsinoids at rest, and further promote its potential role as an adjunct weight loss aid, in addition to diet and exercise. A recipe to moderate energy intake, preventing obesity and its pre-diabetic complications, might combine cinnamon (6 g/day, uncertain effects on body weight but improved glucose tolerance), ginger (1 g/day to promote gastric emptying and intestine motility), red pepper (100 mg/day) and saffron (175 mg/day). Intriguingly, these spices may also cause positive effects because of potential anxiolytic and anti-depressive effects (Josse et al. 2010; Mattes 2012).

Recent studies on capsinoids showed a clearly potential benefit for weight management: Intake of capsinoids (1) increased energy expenditure; (2) increased lipid oxidation and (3) reduced appetite. It was observed that the consumption of capsinoids increased energy expenditure by approximately 50 kcal/day, producing clinically significant levels of weight loss in 1–2 years. It was also observed that regular consumption of capsaicinoids significantly reduced abdominal adipose tissue levels and reduced appetite and energy intake. The mechanism of action is not presently fully understood, although it is well accepted much of the effects are caused by stimulation of the TRPV1 receptor. Capsaicinoids could play a beneficial role, as part of a weight management program (Whiting et al. 2012).

TRPA1 has also been identified on cranial visceral vagal (nodose) neurons, which transduce chemical signals from the gut to the nucleus tractus solitarii. Afferent nerve fibres from these neurons participate in peripheral satiety signalling. These neurons are also stimulated by cholecystokinin (CCK), a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein. Pungent spices will also activate these neurons mediating satiety, and may contribute to the reduction of food intake associated with spicy diets (Choi et al. 2011). Clinical trials indicate that cinnamon ingestion moderates postprandial glycemia, favourably modifies body composition and decreased food intake. Doses of 1–6 g of cinnamon consumed daily for 40 days resulted in positive effects on serum glucose as well as triglyceride, LDL-cholesterol and total cholesterol concentrations (Mattes 2012). Ginger increases gastric motility, stomach emptying, reduces energy intake and has thermogenic properties which increase energy expenditure.

An important effect of TRPV1 has recently described for prevention of nonalcoholic fatty liver disease (NAFLD), a condition characterized by massive hepatic lipid deposition. Several dietary factors have promising effects on NAFLD. TRPV1

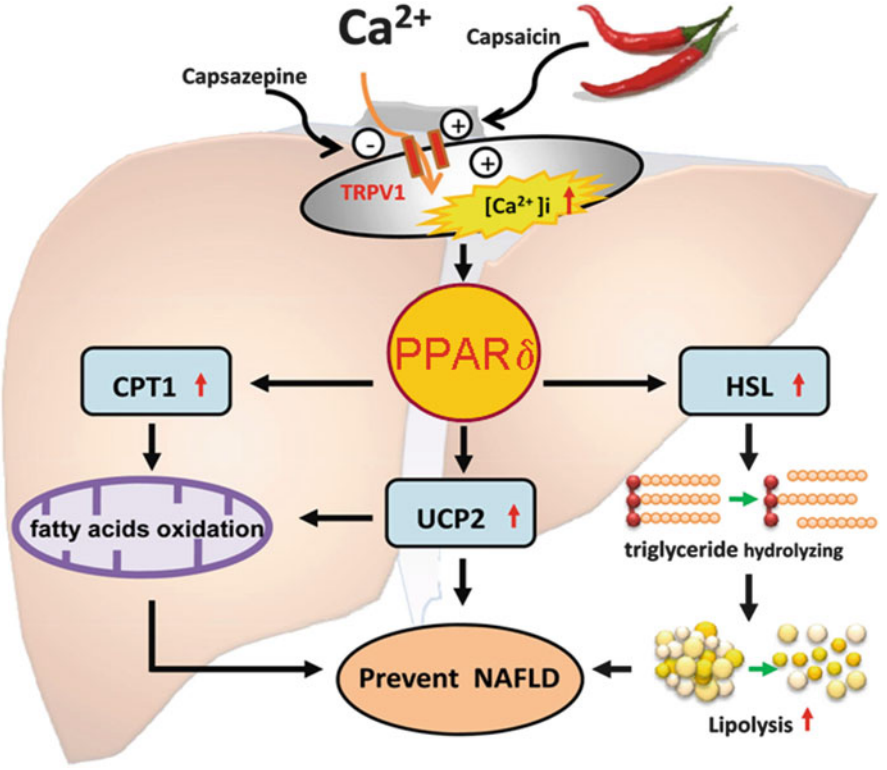

Fig. 4 Capsaicin helps preventing non-alcoholic-fatty-liver disease (NAFDL). Dietary capsaicin stimulate PPARδ through TRPV1 activation via a Ca^{2+} dependent mechanism. This results in the enhancement of expression of the mitochondrial liver uncoupling protein 2 (UCP2) and upregulation of Carnitine palmitoyl transferase I (CPT1), and promotes the free fatty acid oxidation as well as lipolysis (hormone sensitive lipases, HSL), finally prevents the nonalcoholic fatty liver disease (Courtesy of *Prof. Zhiminhg Zhu* summarizing the results presented in Li et al. (2012) and Zsombok (2013))

activation by dietary capsaicin reduces the intracellular lipid droplets and promotes lipolysis by increasing hepatic hormone-sensitive lipase (HSL), carnitine palmitoyltransferase 1 (CPT1), and peroxisome proliferator-activated receptor δ (PPARδ) in wild-type (WT) mice. All these effects are absent in *Trpv1*$^{-/-}$ mice. The TRPV1-mediated PPARδ activation could also significantly increase the expression of autophagy-related proteins (Fig. 4) (Li et al. 2012).

Cinnamaldehyde (CNA), the pungent principle of cinnamon, is a TRPA1 agonist. CNA fed mice with high fat and high sucrose (HFS) diet had a lower body weight increase than the control animals with the same total food intake. The weight of the mesenteric adipose tissue decreased significantly, and a tendency was measured toward lower perirenal and epididymal adipose tissue. Clearly, CNA diminishes visceral fat deposition in HFS diet-fed mice, in part by stimulating interscapular brown adipose tissue (Tamura et al. 2012).

Although somewhat outside the spice-focus, it is, nevertheless, interesting to highlight that also other TRP channels, and especially TRPV4, play a major role in the modulation of our sensitivity to obesity. It has been shown recently, that treatment of diet-induced obese mice with TRPV4 antagonists increased the expression of thermogenic genes in white fat cells, mediating a "browning" of this tissue, and reduced expression of proinflammatory markers in fat tissue, as well as improved glucose tolerance. Development of TRPV4 antagonists can become an effective strategy in the fight against metabolic disease, reducing obesity, diabetes and chronic inflammation associated to them. The mechanism underlying this action is the inhibition of TRPV4 on the expression of PGC1α (Ye et al. 2012).

Dietary modulation of TRPs seems to have a clear long-term beneficial effect, there is, nevertheless, a host of acquired diseases related to over-activation of these ion channels (Nilius 2007; Nilius et al. 2007, 2012; Rech et al. 2011). So far, most publications refer to the negative, disease-causing view. We should be more inclined to follow the positive trait associated to spices:, "positive biology" as a new paradigm in medical sciences (Farrelly 2012).

7.2 A Skeletal Muscle Connection

TRPV1 is expressed in skeletal muscle, and plays here a preventive role against obesity. This happens via the endocannabinoid anandamide, but the effect might also be associated to dietary TRPV1 agonists. Anandamide improves muscle glucose uptake and activate some key molecules of insulin signalling and mitochondrial biogenesis because of an activating effect on PPARγ and TRPV1, which both trigger positive metabolic effects. Interestingly, anandamide levels are increased after intense exercise. It is still puzzling whether dietary intake of TRPV1 agonists reaches sufficiently high plasma concentration and whether the endocannabinoid system is crucially involved in the positive exercise effects on mitochondrial biogenesis and glucose fatty acid oxidation ins skeletal muscle remains to be confirmed (Heyman et al. 2012).

Another important connection between TRPV1 activation and a beneficial effect of skeletal muscle performance, with switch to a more oxidative phenotype, has recently been discovered by several groups (Ito et al. 2012; Luo et al. 2012). Long-lasting administration of capsaicin enhances exercise endurance in rodents, and prevents obesity induce by high fat diet. Capsaicin increased cytosolic free calcium and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) expression, increasing the expression of genes involved in fatty acid oxidation and mitochondrial respiration, promoting mitochondrial biogenesis. Furthermore, the number of oxidative skeletal muscle fibers is also enhanced. These effects are absent in TRPV1-deficient mice. PGC-1a is a transcriptional coactivator and a master switch for genes involved in energy metabolism. This protein interacts with the nuclear receptor PPAR-γ, making it possible its interaction with multiple transcription factors, such as CREB and nuclear respiratory factors (NRFs), and estrogen related receptors (ERR), PPARα and PPARδ. It establishes a link between

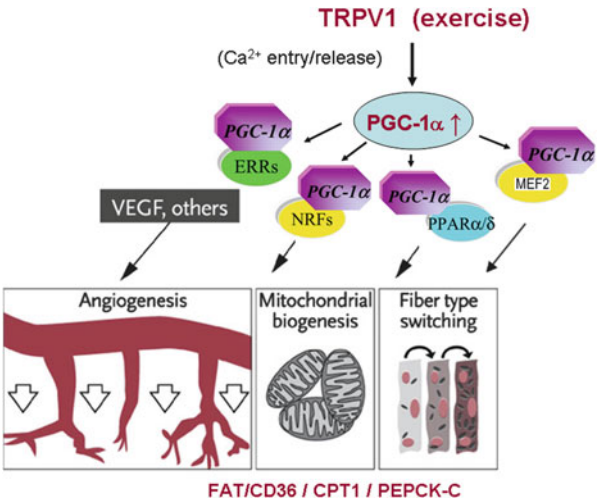

Fig. 5 TRPV1 supports skeletal muscle remodeling (fiber type determination) and mitochondrial biogenesis. Endurance exercise but also dietary intake of capsaicin increase peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) expression. PGC-1α interacts with ERRs (estrogene related receptor) activating angiogenesis via vascular endothelial growth factor (VEGF), activates mitochondrial biogenesis via interaction with nuclear respiratory factors (NRFs) and peroxisome proliferator-activated receptor γ and δ (PPARγ, PPARδ). Mitochondrial activation occurs via FAT/CD36 (fatty acid translocase/Cluster of Differentiation 36), a plasma membrane fatty-acid transport protein, CPT1 (Carnitine palmitoyl transferase I) and PEPCK-C (Phospho-enolpyruvate carboxykinasecoactivator-1α). PGC-1α also triggers the switch to type 1 fibers, which are mitochondria-rich and produce energy primarily through lipid oxidation. This occurs via interaction of PGC-1α with myocyte enhancer factor-2 (Mef2) proteins. All these mechanisms protect also from fatty-diet induced obesity. For details of this mechanistic approach see text (Adapted from the original; courtesy Zoltan Arany, http://hms.harvard.edu/content/metabolic-regulator-has-hand-controlling-vessel-growth, and Arany et al. (2008). With permission of *Nature*)

external physiological stimuli and the regulation of mitochondrial biogenesis, and is a major factor that regulates muscle fiber type determination (Fig. 5).

As with TRPV1 activation, endurance exercise has been shown to activate the PGC-1a gene in human skeletal muscle. This effect is similar to the activation of Sirt3 (silent mating type information regulation 2, homolog 3), a member of the sirtuin family of protein deacetylases with multiple actions on metabolism and gene expression. Expressed in association with brown adipocyte differentiation, Sirt3 is required for responsiveness of cells to noradrenergic, cAMP-mediated, activation of the expression of brown adipose tissue thermogenic genes. The transcriptional coactivator PGC-1a induces *Sirt3* gene expression in white adipocytes as part of its overall induction of a brown adipose tissue-specific pattern of gene expression and full acquisition of a brown adipocyte differentiated phenotype. PGC-1α enhances in fat tissue and skeletal muscle FAT/CD36 (fatty acid translocase/Cluster of Differentiation 36), a plasma membrane fatty-acid transport protein, in mitochondrial membranes, CPT1 (Carnitine palmitoyl transferase I), PEPCK-C (Phospho-enolpyruvate carboxykinase). Capsaicin increased the expression of all these

proteins which favour a more oxidative metabolism. The upregulation of PGC-1α and its target genes, including myocyte enhancer factor-2 (Mef2), also supports the phenotype switch to oxidative fibers in mouse skeletal muscle induced by exercise. These type 1 fibers are mitochondria-rich and produce energy primarily through lipid oxidation. This provides a stable and long-lasting supply of ATP, and these fibers are therefore fatigue-resistant. Conversely, type 2 fibres have fewer mitochondria and rely on glycolysis for energy supply, which encourages lactic acid accumulation in exercise (Luo et al. 2012).

Another beneficial role of TRPV1 activation is connected to the prevention of muscle atrophy. Skeletal muscle atrophy occurs in aging and pathological conditions, including cancer, diabetes and AIDS. Under normal conditions, neuronal nitric oxide synthase (nNOS) regulates load-induced hypertrophy by activating TRPV1. The overload-induced hypertrophy was prevented in nNOS-null mice. nNOSμ is expressed at high levels in skeletal muscle and is found in the sarcolemma bound to α -syntrophin, a member of the dystrophin glycoprotein complex. Loss of dystrophin (and syntrophin), as occurs in Duchenne muscular dystrophy, causes nNOSμ to be lost from the sarcolemma. NADPH oxidase 4 (NOX4) converts NO into peroxynitrite, which functions as an excellent activator of TRPV1. The effect on skeletal muscle protein synthesis is mediated via mammalianTOR. mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. mTOR belongs to the phosphatidylinositol 3-kinase-related kinase protein family (PIP3K). Formation of peroxynitrite, a reaction product of nitric oxide with superoxide, which was derived from NADPH oxidase 4 (Nox4), results in TRPV1 activation, elevation of $[Ca^{2+}]_i$ and eventually activation of the mammalian target of rapamycin (mTOR) (Fig. 6) (Ito et al. 2012).

Following this study, TRPV1 might be is new target for treating muscle atrophy in chronic diseases and cachexia. However, seen the complex signaling mechanism, further investigation is required.

7.3 Spices Against Pain

Chili peppers (*Capsicum* species) were used in folk medicine as topical analgesics. Today, one can readily find capsaicinoids that are designed to ease the pain associated with neuropathy, arthritis, and muscle strain (Hempenstall and Rice 2002; Hempenstall et al. 2005; Setty and Sigal 2005). The approval by FDA of a 8 % capsaicin patch for the treatment of post-herpetic neuralgia (Qtenza®) on November 16, 2009, made capsaicin the first "spiceceutical" to enter the mainstream drug market as a pharmaceutical brand drug. The final approval statement deserves comment to highlight the nomenclature subtelties that trouble capsaicin research. Qtenza® contains capsaicin in its natural form, namely with an *E*-double bond and with a terminal branching, but is, nevertheless, obtained by synthesis owing to the difficulty to getting pure capsaicinoids from their complex mixtures that occur in hot pepper. However, in the FDA statement, Qtenza® is described as

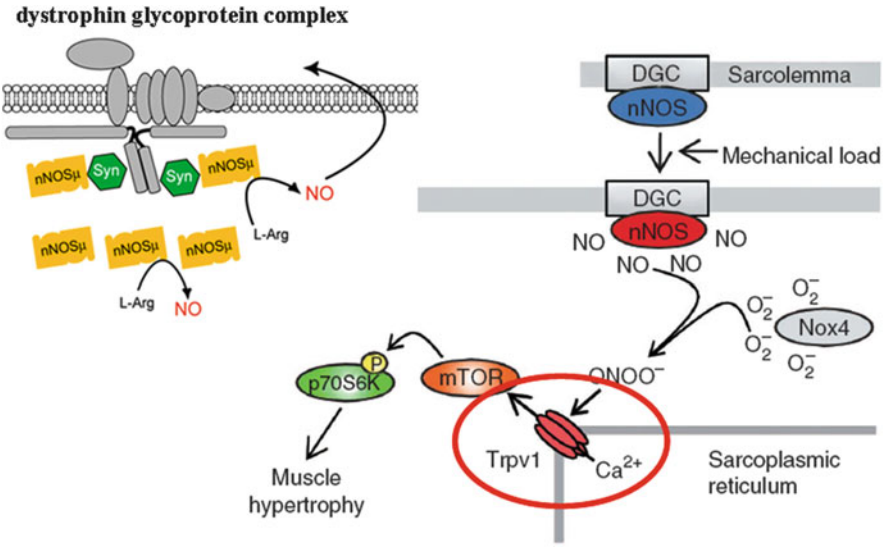

Fig. 6 TRPV1 supposedly mediates skeletal muscle hypertrophy. An isoform of the neuronal nitric oxide synthase nNOS (nNOSμ) is bound to the dystrophin glycoprotein complex via syntrophin (Syn) (*top left*) and releases NO upon physical load. NOX4, a member of the NOX family of NADPH oxidases, converts NO to peroxynitrate ($ONOO^-$) which in turn activates TRPV1 (probably in the sarcoplasmic reticulum). This activation leads to mTOR (target of rapamycin) stimulations which triggers muscle hypertrophy (*below right*) (Adapted from Ito et al. (2012). With permission of *Nature Medicine*)

based on "synthetic capsaicin", a name that, in the food lingo refers to nonivamide, that is, the simplified version of natural capsaicin stripped of its redundant double bond and branching. Another name issue has plagued the development of the *cis*-isomer of capsaicin as a drug. This compound original generic name was zucapsaicin, from the German root of the IUPAC designation of the configurational descriptor of the double bond (*Z* from *Zusammen*, together). However, Winston, the US company that in-licenced the compound from the German company GenDerm, wanted to change the name to civamide, claiming that the prefix *zu* was confusing, since it was used, as an infix, to indicate humanized monoclonal antibodies, as in trastuzumab, alemtuzumab, etc. However, the petition was rejected by USAN (United States Applied Name Council), and the generic name zucapsaicin was maintained. The approval of this change could have had dramatic effects on generic name, triggering a flood of similar request and upsetting the whole pharmaceutical market. Because of this reason, the issue received an enormous media attention (Drahl 2012). Zucapsaicin is currently commercialized in US under the brand name Civanex® (0.075 % cream) and in other countries as Zuacta®.

Clove (*Eugenia caryophyllata*) bud essential oil is extensively used in dentistry, and is as effective as benzocaine for reducing dental pain (Alqareer et al. 2006). [6]-Gingerol, an agonist of TRPV1, is used for the treatment of pain and inflammation. In neuropathic pain models, it significantly decreases secondary mechanical

allodynia and thermal hyperalgesia. [6]-Gingerol could alleviate neuropathic pain by acting centrally at the level of the spinal cord (Gauthier et al. 2012).

All these compounds are TRPV1 agonists, and act by desensitizing TRPV1. In general, few TRPV1 antagonists have been isolated from natural sources, presumably because natural products are mostly produced as part of a biological "chemical war", where offense is favoured over soothing. Nevertheless, the widespread Δ^7-sterol α-spinasterol shows remarkable TRPV1-mediated efficacy in animal models of neuropatic pain (Trevisan et al. 2012). α-Spinasterol, first isolated from the spinach leaves and next as a by-product from the isolation of vitamin K from alfalfa (Fernholz and Moore 1939), shows good oral absorption and high penetration into the brain and spinal cord of mice (Trevisan et al. 2012). Pumpkin seed oil is a major dietary source of α-spinasterol and related Δ^7-sterols. It is a typical seasoning of Styrian cuisine, and is used in phytomedicine for the treatment of benign prostate hyperplasia. Interestingly, over-expression of TRPV1 has also been discovered in prostate cancer tissues (Czifra et al. 2009).

7.4 Spices Against Cancer

More than 500 different gene products have been implicated in cancer development. Since many dietary natural products target multiple genes, they might, in principle, be better suited than targeted products for the prevention, and possibly the treatment, of various chronic diseases, including cancer. This explains the enormous interest in the relationship between the intake of specific dietary ingredients and the development of cancer. Spice-derived compounds can modulate a host of transcription factors, growth factors, protein kinases, and inflammatory mediators that are involved in cancer promotion, initiation and development, and, in general, in chronic inflammation (Sung et al. 2012). An impressive statistics from the World Health Organization GLOBOCAN (Aggarwal et al. 2009) shows that the production and consumption of spices negatively correlates with the incidence of cancer. Obviously, in country with higher spice consumption the cancer risk is reduced.

A direct relation between beneficial anti-cancer effects and the activation of "spicy" channels like TRPV1 or TRPA1 is controversial, and, in general, is complex. The relationship between TRPA1 and melanoma is exemplificative. Activators of TRPA1, such as MO and cinnamon have a clear anti-tumour effect on various melanomas, and functional TRPA1 can be detected in these cells, making it tempting to relate the two observations. However, the anticancer properties of MO and cinnamon turned out to be independent from the activation of TRPA1 (Oehler et al. 2012). TRPV1 has also been added to the potential tumour suppressors in urothelial cancer (Santoni et al. 2012). On the other hand, agonists of TRPV1 reduce proliferation and migration of high-grade astrocytomas (HGAs) and are even suggested by the World Health Organization as potential new HGA therapeutics (Stock et al. 2012).

Chemoprevention, the process of inhibiting, delaying or reversing carcinogenesis, is closely associated to isothiocyanates, a class of compounds that act

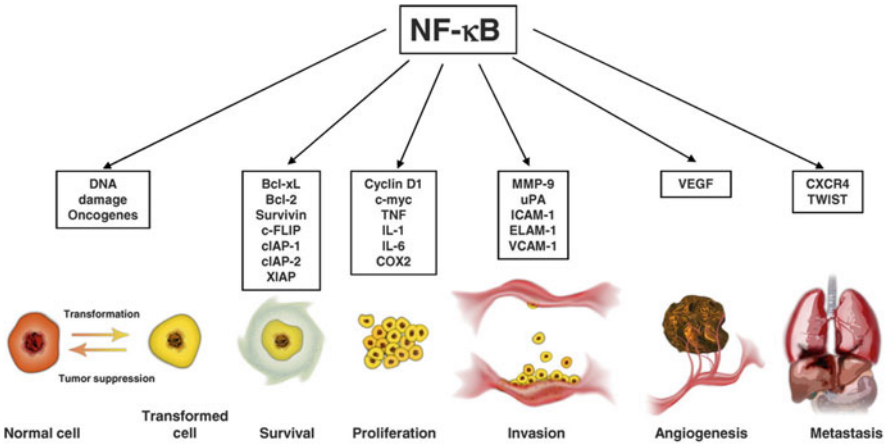

Fig. 7 The proinflammatory, pro-carcinogenic transcription factor NF-κB. NF-κB promotes the expression of proteins and signaling molecules involved in cellular transformation (promoting DNA damage, oncogene expression), cancer survival (expression of apoptosis regulator enzymes Bcl-xL, Bc-2, survivin, ...), cell proliferation (Cyclin D1, c-myc, TNF, IL-1,7), cell invasion (matrix metallopeptidases, urokinase plasminogen activator, adhesion protein ICAM-1, ...), angiogenesis (expression vascular endothelial growth factor, VEGF), and metastasis (expression of the chemokine receptor CXCR4, the basic helix-loop-helix transcription factor TWIST). The effects of NF-κB are counteracted by activation of Nrf2. For details and further explanations see text and Aggarwal et al. (2009) (Adapted from Aggarwal et al. (2009). With permission of the *Experimental Biology and Medicine*)

as blocking agents, inhibiting carcinogen activation and increasing their detoxification. *Broccoli* contain glucorafanin, the glucosinolate corresponding to the isothiocyanate sulforaphane. Sulforafane changes the way our body handles pro-carcinogens. Regular consumption of 300 mg/die of sulforafane cause a rapid increase in glutathione-S-transferase in just 3 weeks, making this compound one of the best known chemo-preventing agents. According to general dietary recommendations, adults should aim for at least five weekly servings of cruciferous vegetables (cabbages, turnips, broccoli, Brussels sprouts, cauliflower). Several epidemiological studies have shown an inverse correlation between the regular consumption of garlic and the risk of stomach cancer. A recent study to evaluate spice compounds used in the traditional Chinese medicine, identified cinnamaldehyde as the most efficient tools to inhibit angiogenesis ($<$100 nM), suggesting a remarkable anticancer potential. Ferulic acid, an ubiquitous plant constituent, shows a similar activity. Ferulic acid is found in high concentrations in various common food plant, like coffee, apple, artichoke, peanut, and orange (Eisenberg et al. 2011).

More and more targets of spice-derived nutraceuticals are identified. First, nutraceuticals may influence the expression of several transcription factors. Nuclear Factor-κB (NF-κB) controls the expression of more than 500 genes which are mostly involved in inflammation, cell survival, proliferation, angiogenesis, invasion and metastasis (Fig. 7).

Translocation of NF-κB from its inactive cytoplasmatic form to its active nuclear form might be critical in all stages of cancer, by, inter alia, enhancing cyclin D1 expression, expression of apoptosis suppressor proteins such as the mitochondrial transmembrane pro-survival proteins Bcl-2, Bcl-xL, proteins for angiogenesis like metalloproteases and the vascular endothelial growth factor (VGEF). NF-κB activation is prevented by a host of spice constituents, the most famous ones being curcumin. However, there is no shortage of other inhibitors of spice origin, as exemplified by capsaicin, gingerols, and zerumbone (Sung et al. 2012). Interestingly, spice-derived NF-κB inhibitors can also act by nasal route, as shown by incensole and incensole acetate from African incense [*Boswellia papyrifera* (Del) Hochst] (Paul and Jauch 2012).

Signal Transducer and Activator of Transcription 3 (STAT3) is associated with cellular transformation required for cancer development. It is constitutive active in cancer but not in normal cells (Aggarwal et al. 2006). STAT3 expression is inhibited by many spice constituents, not only from tropical spices (curcumin and capsaicin), but also by ursolic acid from rosemary (Bhutani et al. 2007; Pathak et al. 2007). Similar examples are reported for the downregulation of STAT5 and AP-1 (activator protein 1).

A main player in spice-connected anti-cancer effects might be the Nuclear Factor-(Erythoid 2-Releated) Factor 2 (Nrf2). It protects cells against oxidative stress. Nuclear translocation activates genes for transcription of antioxidant proteins, such as NQO1, HO-1, GCLC, and many more. Electrophilic spice constituents like curcumin causes Nrf2 activation via its suppressor Keap1, a nucleophilic regulator which is, as TRPA1, activated by covalent modification. Other genes involved in the anti-cancer effects of nutroceuticals comprise β-catenin/Wnt, sonic hedgehog, receptors for growth factors such as EGRF-receptor, HER2, VEGF-receptors, IGF1-receptor, PDGF-receptor, protein kinases (PI3K), AMPK, Raf/Ras, mTOR. For all these targets beneficial effects of spice-mediated compounds have been described and are supported by preliminary clinical trial (for detailed reviews see Aggarwal et al. 2009; Nakamura and Miyoshi 2010; Kim et al. 2012; Sung et al. 2012). Nrf2, central in these signalling cascades, is a polyvalent player to mediate beneficial, anti-cancer, anti-inflammatory and anti-oxidant effects. Transnuclear traffic of Nrf2 is also required for its beneficial action. Keap1 (Kelch-like ECH-associated protein 1, also known as inhibitor of Nrf2, INrf2) has been shown to interact with Nrf2, the master regulator of the antioxidant response, which is important for the reduction of oxidative stress, and inhibits its target protein Nrf2, which has binds Keap1 (Fig. 8a). The Broad complex, Tramtrack and Bric-à-Brac (BTB) domain contains the Cys151 residue, which is important in stress sensing and accepts electrophiles. This domain confers binding in to the proteasome initiating Nerf2 degration (Fig. 8b). The intervening region (IVR) domain contains two critical cysteine residues, Cys272 and Cys288, which are important for the repression of Nrf2 activity. The Kelch domains provide binding of Keap1 to Nrf2. Under quiescent conditions, Nrf2 is anchored in the cytoplasm through binding to Keap1, which, in turn, facilitates the ubiquitination and subsequent proteolysis of Nrf2. Such sequestration and further degradation of

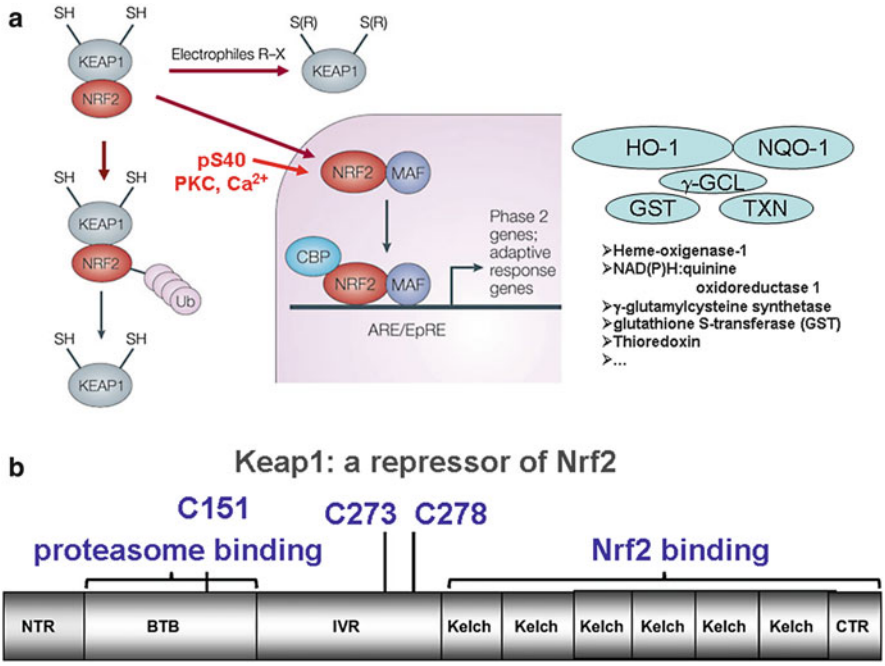

Fig. 8 (**a**) **The signaling cascade of the nuclear transcription factor Nrf2 and its repressor Keap1**. Nrf2 increases the expression of several antioxidant enzymes. Reduced Kelch-like ECH-associated protein 1 (KEAP1) binds NRF2 and retains it in the cytosol, where it has a short half-life and undergoes ubiquitylation. Oxidation or adduction of the crucial thiol residues of KEAP1 releases NRF2 and trigger translocation to the nucleus, where it interacts with small Maf and binds to the antioxidant response element/electrophile response element (ARE/EpRE) to cause enhanced transcription of Phase 2 enzymes (involved in metabolite conjugation) and adaptive response genes. CBP: cAMP-response-element-binding protein. Phosphorylation of the Ser residue S40 in Nerf2 promotes nuclear transition. This step is Ca^{2+} dependent (Shelton and Jaiswal 2013). (**b**) The linear structure indicates the structural domains of Keap1. Marked are the Cys residues which undergo co-valent modification. NTR, N-terminal region; BTB, bric-á-brac domain that binds with Nrf2 ubiquitin factors (proteasome binding for ubiquitylation); IVR, intervening region. The Kelch repeats and CTR regions bind with Nrf2. CTR: C-terminal region. C151 is important for stress sensing, and C273/C278 is required for Nrf2 suppression (Adapted from Liebler and Guengerich (2005). With permission of *Nature Reviews Drug Discovery*)

Nrf2 in the cytoplasm are the mechanisms for the repressive effects of Keap1 on Nrf2. The binding of Nrf2 to Keapl is thought to be more easily disrupted by conformational changes within the interacting Keapl molecules than that of the ETGE motif. The deletion mutants of the Nrf2 protein which disrupt Nrf2-Keap1 binding result in a longer half-life of Nrf2 within the cell, indicating that the binding of Nrf2 to Keapl is associated with proteasome degradation. Direct thiol modifications of Keapl by electrophiles can trigger destabilization and disruption of the binding to the Nrf2 binding motif and profoundly change the conformation of Keap1. Although Cys151 in the BTB region and Cys273 and Cys288 in central linker domain in Keap1 were identified to be essential for regulating Nrf2, this

conformation change was not sufficient to disrupt physical binding between Nrf2 and Keapl mediated by high affinity binding of the ETGE motif (Fig. 8b).

In other words, thiol modification only negated Keap1-mediated Nrf2 ubiquitination and did not disrupt Nrf2/Keap1 binding directly. Recently, alkylation of Keap1 central linker domain cysteines by electrophiles led to a switch in ubiquitination from Nrf2 to Keap1. Direct binding of isolated tumor cells to Keapl was detected in "in vitro" experiments, using recombinant or ectopic expressed Keap1. Preferred adduction sites of human Keapl sites include Cys77 in the BTB domain, Cys226, Cys249, and Cys257 in the central linker domain, Cys489, Cys513, Cys518, and Cys583 in the Kelch repeat domain, and Cys624 in the C-terminal domain, although neither Cys151, Cys273, nor Cys288 was modified except at high concentrations of electrophiles. Recent studies of Keap1 reactions with several electrophiles also indicate that not only targeting selectivity but also the inducibility of ubiquitination switching varies with the electrophile structure. Therefore, activation of Keap1 resembles TRPA1 activation by its electrophilic nature. Previous in vitro experiments advocate the possibility of interaction between ITCs and protein thiols under physiological conditions, because the protein thiols are glutathiolation, nitrosylation, disulfide interchange, and thioether and thioester formation, which are required to execute several intracellular signaling. Therefore, the following candidate proteins have been postulated as targets in tumor cells: Keapl and proteasome components in the Nrf2-dependent phase 2 enzyme induction pathway, thioredoxin and dual specificity phosphatase 8 (M3/6 or JNK phosphatase), gpp1Phox, NADPH oxidase (Nox2) adenine nucleotide translocase, CYP isozyme, histone deacetylase, a transient receptor potential (TRP) family of ion channels (TRPA1) (Nakamura and Miyoshi 2010). Recently, it was shown, although any dietary component was not elucidated, that TRPV1 activation prevents colon cancer via decreasing IL-6, IL-11, STAT3 and NF-kB signaling pathways. TRPV1 exerts a protective role that restricts the initiation and progression of colon cancer (Garcia de Vinuesa et al. 2012). In addition to the Keap1 induced nuclear translocation of Nrf2, PKCδ dependent phosphorylation of Nrf2 seems to be also an important signal for nuclear translocation of Nrf2. It may be possible that this step includes a Ca^{2+} sensitive trigger for which Ca^{2+} entry via electrophilic TRPA1 activation might be supportive (Shelton and Jaiswal 2013).

There is a substantial overlapping of electrophilic ligands between TRPA1 and Nrf2, suggesting that the two systems might be part of the same network, with TRPA1 representing the sensory arm, and Nrf2 its biochemical counterpart. Dietary electrophilic compounds show an hormesic profile of activity, being potentially genotoxic and capable of alkylating DNA (Nakamura and Miyoshi 2010). It is therefore tempting to suggest that sensory receptors like TRPA1 serve as gatekeeper to optimize their intake, avoiding over-exposure and exemplifying the sensory and the metabolic interactions of spicy nutraceuticals. In this scenario, desensitization might be viewed as an attempt to maintain optimal intake of pungent compounds in spite of priming of the metabolizing enzymes and a substantial higher and/or faster inactivation by metabolization. Capsaicin has indeed been shown to promote P450 cytochrome activity (especially of CYP3A4) and speed up the elimination of dietary xenobiotics (Han et al. 2012).

Also other TRP channels might mediate anti-cancer effects. TRPM8, not focused in this review, has emerged as a promising prognostic marker and putative therapeutic target in prostate cancer. Forced overexpression of TRPM8 in PC-3 cells can inhibit the cell proliferation and motility probably through the TRPM8 activation. Dietary menthol input can inhibit the proliferation and motility of androgen-independent prostate cancer (AIPC). It inhibits cell growths, cell cycle progression at the G(0)/G(1) phase. Furthermore, menthol inhibited the migration of cancer cells (Wang et al. 2012b).

Recently, one of the new mechanisms involved in the action of curcumin as potent anti-cancer effects has attracted attention in prostate cancer models. The in vivo inhibition of tumor growth is correlated with enhanced membrane localization of β-catenin. The novel molecular mechanism of curcumin action proceeds via the activation of protein kinase D1, PKD1, in prostate cancer cells (Sundram et al. 2012).

Curcumin is very popular in the cancer population, where its bioavailability troubles are well known, and ingenious ways to promote its oral absorption have been developed, like the incorporation in chocolate (http://margaret.healthblogs.org/life-with-myeloma/discovery-of-curcumin/my-curcumin-protocol/my-curcumin-cocoa-mass-recipe-in-english-and-italian/). Chocolate is a lecithin-rich matrix, and lecithin formulation has been shown to dramatically improve oral absorption of curcuminoids, presumably by preventing their self-aggregation (Cuomo et al. 2011).

7.5 A Cytoprotective and Anti-inflammatory Action of Spices

Many spice constituents directly inhibit NF-κB action, with a general downregulation of the inflammatory status. In general, also TRPA1 activators downregulate the inflammatory status, but indirectly, via activation of Nrf2, that also lead to inhibition of NF-κB. Nrf2, the master regulator of antioxidative responses, causes an increased expression of over 250 antioxidant enzymes mainly via the inhibition of pro-inflammatory transcription factors, like NF-κB and STAT3. The Keap1-Nrf2 system is therefore a major redox-sensitive regulating complex of the inflammatory status. Other anti-inflammatory spice targets are AP-1, HIF-1a, COX2, β-catenin/Wnt (as proinflammatory proteins, see for the signalling pathway Wnt_signaling_pathway) and PPARs (see for excellent reviews Nakamura and Miyoshi 2010; Gupta et al. 2012b). A direct connection to TRPA1 activation has, however, not yet been unveiled for these compounds.

The archetypal anti-inflammatory spice constituent is curcumin, the major curcuminoid of turmeric (*Curcuma longa* L.). Curcumin activate and desensitize TRPA1, and inhibits NF-kB with both a direct mechanism and indirect one mediated by Nrf2. Just like the *Allium* electrophiles, at low dosages, it increases the expression of genes encoding cytoprotective proteins including antioxidant enzymes, protein chaperones, growth factors and mitochondrial proteins (Mattson 2008). To improve bioavailability, curcumin has been formulated in association to peprine, but there is also the possibility of a pharmacodynamic and not only a

pharmacokinetic potentiation of the activity, since piperine is a potent desensitizer of TRPV1 (Aggarwal 2011; Gupta et al. 2012b).

Zerumbone, a TRPA1 activator and the major constituent of shampoo ginger [*Zingiber zerumbet* (L) Roscoe ex Sm], protect the skin from ultraviolet light induced inflammation, photokeratitis, and attenuate corneal photo demaging. These effects have been observed with dietary intakes of the compound, and are probably mediated by downregulation of cytokines (TNFα, NFκB, iNOS), a typical effect of NF-κB inhibitors (Chen et al. 2011a, b). Unsurprisingly, beneficial effects have also been reported in animal models of osteo-arthritis (Al-Saffar et al. 2010).

The anti-inflammatory profile of spices is also echoed by extra-virgin olive oil, that contains oleocanthal, a compound endowed with remarkable anti-inflammatory properties, and sharing the sensory properties of the non-steroid anti-inflammatory drug ibuprofen (Bennett and Hayes 2012). Virgin olive oil causes oral pungency sensed almost exclusively in the throat. This rare irritation pattern is a consequence of the specificity of oleocanthal to activate TRPA1 in sensory trigeminal fibres in the pharynx, and not elsewhere within the oral cavity. The same restricted pharyngeal irritation is shown by ibuprofene (Beauchamp et al. 2005), that also activates TRPA1, providing an molecular explanation for one of the most common forms of dietary and drug-induced sensory irritation This example apparently supports the decade-old rather off-beat idea that the taste properties of a compound can predict its potency in the body (Peyrot des Gachons et al. 2011). In an intriguing anthropological implication, the beneficial consumption of olive oil over the life time, as usual in Mediterranean countries, might be related to the "cultural" development of an appreciation of this TRP-mediated throaty pungency, whereas other cultures, "throaty pungency naïf", do not appreciate it, and prefer more fat, umami oriented notes, like the meat-based American diet shows (Drahl 2011).

Another mechanism that could contribute to the anti-inflammatory activity of TRPV1 agonists is their triggering of myeloid-derived suppressor cells (MDSCs), class of major regulatory cells of the immune system, endowed with potent inflammatory action. It is therefore intriguing to speculate whether dietary activation of TRPV1 could constitute a novel therapeutic modality to treat inflammatory diseases (Hegde et al. 2011; Gupta et al. 2012a).

Another unexpected effect of TRPV1 stimulation by capsaicin is on bone protection during inflammation. Capsaicin suppresses interleukin-1-induced osteoclast differentiation and decreases by this effect inflammatory bone resorption. This effect is mediated due to suppression by TRPV1 via prostaglandin E (PGE) production induced by lipopolysaccharide (LPS). This suppression occurs in osteoblasts, i.e. suppression the expression of cyclooxygenase-2 (COX-2), the membrane-bound PGE synthase-1 (mPGES-1) and PGE production induced by LPS in TRPV1 expressing osteoblasts. Capsaicin may suppress PGE production by inhibiting the expression of COX-2 and mPGES-1 in osteoblasts. PGE, however, is sense by osteoclasts. LPS treatment markedly induced bone loss in the femur in mice, and capsaicin significantly restored the inflammatory bone loss induced by LPS in mice. TRPV1 ligands like capsaicin are therefore potentially useful as clinical drugs targeting bone diseases associated with inflammatory bone resorption (Kobayashi et al. 2012).

TRPA1 is also the target of a very interesting class of natural compound, the Brazilian green propolis phenolics. Propolis or also know as honeybee glue, is a resinous product consisting of sap, bark and bee excreta, accumulates in bee hives and is used by bees to repair their hives, and its composition changes with the origin of the material. *Baccharis dracunculifolia* DC (Asteraceae), a native plant from Brazil, is the most important botanical source of Brazilian green propolis. It is used as a popular health supplement. The ethanol extract of Brazilian green propolis has a herb-like smell and unique pungent taste. The major active component in this extract is Artepillin C, a pungent compounds which potently activates human TRPA1. Artepillin C is a prenylated cinnamic acid derivative is even a more potent TRPA1 activator than allyl isothiocyanate. Remarkably, cinnamic acid derivatives are not thiol traps, and therefore its mechanism of TRPA1 activation is presumably non-covalent (Avonto et al. 2011). Importantly, propolis is identified and also used as a neuroptotective compound (Shimazawa et al. 2005; Hata et al. 2012).

In general, the antioxidative and anti-inflammatory effects of spice constituents might also play a critical role in the genesis of age-related diseases, playing a role in what could be vaguely defined as "healthy ageing" (Prasad et al. 2012).

Finally, mention should be given to the recent interest in carvacrol, a constituent of the popular Mediterranean spices thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) and savory (*Origanum majorana* L.) as a treatment for traumatic brain injury. Carvacol activates TRPV3 and TRPA1 (Xu et al. 2006), and improves the recovery from brain injuries. This effect on the central nervous systems is connected to a still unknown TRPC1 sensitive mechanism, and inhibition of this channel by seemingly enhances recovery from brain injuries (Peters et al. 2012).

7.6 Antimicrobic Action of Spices

Spices exhibit antibacterial and antifungal activity. Microbiologists and food-product developers have conducted laboratory experiments to test the effect of spices against numerous foodborne bacteria, fungi, and yeasts. It is now clear that many spices have potent antimicrobial properties (Sherman and Billing 1999). All 30 tested spices inhibited or killed some tested bacterial species; 15 spices inhibited or killed at least 75 % of the species, and 4 spices (garlic, onion, allspice, and oregano) inhibited or killed all of the tested species. Garlic, onion, allspice, and oregano were found to be the most potent spices, which inhibited or killed every bacterium selected from the most frequent foodborne diseases (especially species of Clostridium, Escherichia, Listeria, Salmonella, Shigella, and Vibrio) (Billing and Sherman 1998).

Approximately 4.5 billion people host *Heliobacter pylori*, the microorganism that causes stomach ulcer and cancer. Similarly, infection with *Bacteroides fragilis* has been related to colon cancer. Its toxin, a metal protease, activates NF-κB and STAT3 (signal transducer and activator of transcription), triggering chronic inflammation, a prelude to cancer. *Escherischia coli* is a normally benign intestinal guest, whose metabolic activity is required for the generation of short-chain fatty acids, a

class of lipids which provide approximately 10 % of energy balance. All these bacteria might be affected by constituents of spices. Certain spices, like cinnamon, are rich of polyphenols, and especially procyanidins. These, along with other flavonoids, can affect the composition of the intestinal bacterial population in a way not different from the many other sources of polyphenolics, like apple or oranges (Rooks and Garrett 2011).

On the other hand, also some pungent compounds unique to spices can have a profound effect on the intestinal bacterial population (microbiota), that, with ten trillion cells, outnumber by an order of magnitude the number of human cells in a body. Thus, garlic (*Allium sativum* L.) was used by the phylantropic doctor (as well as organist and J. S. Bach scholar) Albert Schweitzer as an effective treatment for amoebic dysentery in West Africa. It has now been confirmed that garlic is also effective against *Salmonella*, *Escherichia coli* and *Giardia intestinalis*. Therefore, garlic is a safe, inexpensive, and common spice that could have public health implications in areas where infectious diarrhea is edemic (Harris et al. 2000, 2001; Beuchat et al. 2006).

Remarkably, the molecular mechanism(s) of the antibacterial activity of garlic is very similar to that of its sensory property, namely electrophilic thiol trapping (*S*-thiolation) from the thiosulfinate allicin (Rabinkov et al. 1998). It this case, it is not human TRPA1 that is involved, but two important classes of enzymes necessary for bacterial growth, namely cysteine proteinase, that provide infectious organisms with the means to damage and invade tissues, and alcohol dehydrogenases, essential for bacterial survival. The potency of allicin is in the range of 5–10 % the action of current microbial antibiotics and its sensory properties are an obvious burden to a mainstream pharmaceutical development (Fujisawa et al. 2009). On the other hand, crude garlic preparations outperform pure allicin in terms of antibacterial activity (Fujisawa et al. 2009), suggesting the need of further investigations. Allicin potentiates the antifungal activity of polyene antibiotics, a class of compounds that alter plasma membrane ion permeabilities by binding to ergosterol and show vacuole disruptive action. Allicin strongly inhibit ergosterol trafficking from plasma membrane to the vacuole, amplifying the activity of this class of antifungal agents (Ogita et al. 2012).

Thyme and thymol are potent antibacterial agents, with a remarkably wide spectrum of activity against antibiotic-resistant strains, also endowed with antifungal activity, especially against oral candidiasis. These TRP-ligands interfere with ergosterol biosynthesis and disrupt membrane integrity with a negligible toxicity on human cells (Ahmad et al. 2011). Interestingly, thyme was used by the ancient Egyptians in the complex mixtures used to preserve mummies. The activity of thymol on TRPA1 was blocked by the TRPA1 antagonist camphor (Xu et al. 2006; Lee et al. 2008) and it would be interesting to evaluate the effect of the addition of camphor on the antibacterial activity of this phenol.

Interestingly, also clove essential oil has been shown efficacious in models of vaginal Candidiasis (Giordani et al. 2004; Ahmad et al. 2005; Hersch-Martinez et al. 2005), a condition whose inflammatory status might be triggered by the fungal conversion of the anti-inflammatory eicosanoid andandamide, a mild TRPV1 agonist and cannabinoid receptors partial agonist, into the “pure” TRPV1 agonist

R 3-hydroxyanandamide, a compound devoid of significant affinity for cannabinoid receptors but equipotent to anandamide as a TRPV1 agonist (De Petrocellis et al. 2009).

Colonization of the gastrointestinal epithelium by *Helicobacter pylori* is associated to gastric ulcer and gastric cancer. Many spices, and especially chilli pepper, show activity in vitro against *H. pylori*, and clinical documentation of activity has been reported for broccoli sprouts, a good source of sulforafane. Consumption of various dosages (14, 28, or 56 g) of broccoli sprouts twice daily for 7 days led to complete eradication of the infection in three of the nine volunteers of the study (Galan et al. 2004). Thus, consumption of 100 g/day of broccoli, a source for isothiocyanates and sulforafane, for 7 days completely eradicate *H. pylori* in humans (O'Mahony et al. 2005).

7.7 Spices in Gastro-intestinal Diseases

Carminatives are herbs that relieve bloating and intestinal gas and are used against flatulence. Most are spices/culinary herbs, with aniseed, cinnamon, fennel seed, and peppermint being the archetypal examples. The mechanism of action of these spices is poorly known, as is their clinical documentation of activity. Several mechanisms are, in principle possible, like the promotion of carbohydrate digestion, the modulation of gas-producing enteric bacteria, and the decrease of visceral hypersensitivity to bloating. Clinical documentation of activity has been provided for capsaicin, that could improve bloating in patients with irritable bowel syndrome (Serra 2012).

Fennel in combination with chamomile and lemon balm improves infantile colic, presumably due to a spasmolitic effect. Clinical efficacy has been documented (Savino et al. 2005), but the mechanism of action is unclear. Fennel seems to be remarkably active against infant cholics, but shows mild estrogenic activity, and its prolonged used in children has been associated to premature thelarche (Turkyilmaz et al. 2008). The study of the estrogenic activity of fennel and anise had enormous impact in medicine, since it eventually led to the synthesis of diethylstilbestrol, the first non-steroid synthetic estrogen. Anethole, the major constituent of fennel and anise, is substantially devoid of estrogenic activity, and, surprisingly, the nature of the estrogenic principles of these plants is still poorly known, the current view being that its oligomers and photochemical reaction products are responsible for this action (Albert-Puleo 1980).

Spices are also used against LOSS OF APPETITE, as antiemetics and digestives. They include cinnamon, herbal liqueurs (digestives) such as anisette, crème de menthe, ginger (*Zingiber officinale* Rosc.), a home remedy against hyperemesis gravidarum, travel sickness, and chemotherapy-induced nausea and vomiting. Cinnamon is also an effective antiemetic and often preferred by children or those who find ginger to be "hot" or spicy. Powdered cinnamon probiotic-rich yogurt and given two to three times per day for minor cases of gastroenteritis

(see also the link cinnamon remedies). Considerable attention has been given to the anti-emetic activity of ginger, especially in the cancer supportive care and in pregnancy. The mechanism underlying the anti-emetic activity of ginger is unknown. The current view is that gingeroids (gingerols, and shogaols) inhibit 5-hydroxytryptamine (5-HT3) and NK1 receptors and show antihistaminic properties (Haniadka et al. 2012). A puzzling and so far unexplained variability in the prokinetic effects of ginger was observed during various clinical trials, even with standardized extracts. Given the very poor oral absorption of gingeroids, the possibility exists that the peripheral and not the central 5-HT3 and NK1 receptors are involved. It is also unclear to what extect inhibition of activity is due to direct inhibition, to downregulation of expression of the corresponding genes, or to a combination of both mechanisms (Qian et al. 2010).

Dietary TRPV1 agonists influence directly gastric secretion. Capsaicin and [6]-gingerol, pungent components of chilli pepper and ginger, not only play a role in gastric mucosal integrity in rats, but also, via peroral administration, inhibit gastric acid secretion and prevent harmful stomach acidification (Okumi et al. 2012). Stimulation of TRPV1 by capsaicinoids strongly improved safety and efficacy of swallow, and shortened the swallow response especially in older patients with oropharyngeal dysphagia (OD). Obviously, stimulation of TRPV1 might be a potential player in pharmacological strategies to treat OD (Rofes et al. 2012).

TRPV1 expressing sensory fibres have been reported to increase in the gastrointestinal tract of patients with FGID and visceral hypersensitivity. Chronic ingestion of natural capsaicin agonists or chili decrease dyspeptic and gastroesophageal reflux disease (GERD) symptoms. Therefore, the high prevalence of spicy food in Asia improves functional dyspepsia and GERD symptoms (Gonlachanvit 2010).

Rutaecarpine, an alkaloid found in certain herbs including *Evodia rutaecarpa*, a well-known Chinese herb called Wu-Chu-Yu, exerts hypotensive effects or protective effects against cardiac or gastrointestinal injury (Peng and Li 2010). Rice- and chilli – containing foods – are common in Asia. Rice is completely absorbed in the small bowel, produces little intestinal gas and has a low allergenicity. Rice/chili-based meals are well tolerated and may improve gastrointestinal symptoms in functional gastrointestinal disorders. Again, the active component of chili is capsaicin a TRPV1 activator!

Leaves and seeds from perilla [*Perilla frutescens* (L.) Britt.] are used as foods and play a role in traditional Chinese medicine for inducing diaphoresis and strengthening the stomach and digestion. Within the complex monoterpenoids produced by the plant, perillaldehyde and perillaketone activate TRPA1, and are responsible for the chemesthetic properties of this plant (Bassoli et al. 2009).

7.8 Do Spices Go Cardio-vascular?

A hot (capsaicin, pepper) based diet may lower cardiometabolic risks and prevalence of hypertension. TRPV1 activation by dietary capsaicin enhances endothelium-

dependent relaxation in wild-type mice, but not in TRPV1-ko's, an effect that is based on a TRPV1-mediated increase in NO production may represent a promising (Yang et al. 2010). In coronary vessels TRPV1 increases the cardiac flow. This is especially important in diabetes. Indeed, TRPV1 ($TRPV1^{-/-}$)-db/db diabetic mice develop defects in coronary function and diabetic cardiomyopathy. The capsaicin-mediated increase in blood flow was attenuated in db/db mice. TRPV1 clearly mediate coupling of myocardial blood flow to cardiac metabolism via a NO-dependent, BK channel-dependent pathway which is corrupted in diabetes (Guarini et al. 2012).

Dietary TRPV1 activation also reduced blood pressure and influences vascular dysfunction mediated by high-fat diet. Blood pressure, vascular relaxation, expressions of voltage-gated potassium-channel Kv1.4, and RhoA and Rho kinase in aorta were affected. Both endothelium dependent and independent aortic rings vasorelaxation in high-fat diet were significantly improved as compared with dietary capsaicin input. Dietary capsaicin also down-regulated the expression of RhoA and Rho kinase but up-regulated the expression of Kv1.4 in aorta. Thus, dietary capsaicin has the potential to ameliorate vasorelaxation dysfunction mediated by high-fat diet (Zhu et al. 2012). A new therapeutic intervention of hypertension: pass the chilli peppers, please (Sessa 2010; Yang et al. 2010)!

Daikenchuto (TU-100), a traditional Japanese herbal medicine increases the intestinal blood flow by inducing a vasodilatory effect. This effect is abolished by TRPA1-antagonists but not by TRPV1-antagonists. TU-100 and its ingredient [6]-shogaol (6SG) decrease dose-dependently the blood pressure, an effect again abrogated by TRPA1-antagonists. 6SG showed similar TRPA1-dependent vasodilatation in vivo. TRPA1 appears to be a promising target for the development of novel strategies for the treatment of various gastrointestinal disorders (Kono et al. 2013).

Evodiamine, a main bioactive component in the fruit of *Evodiae rutaecarpa*, is a TRPV1 activator. Chronic administration with evodiamine reduced dietary induces obesity, It also reduced the size of atherosclerotic lesions and alleviated the hyperlipidaemia and systemic inflammation, as well as hepatic macrovesicular steatosis, in $ApoE^{(-/-)}$ mice. Treating $ApoE^{(-/-)}$ mice with evodiamine enhanced hepatic cholesterol clearance, as revealed by upregulation of hepatic low-density lipoprotein receptor and ATP-binding cassette (ABC) transporters ABCG5, ABCG8 and cholesterol 7α-hydrolase. Genetic deletion of TRPV1 in $ApoE^{(-/-)}$ mice promoted the progression of atherosclerosis; elevated the serum levels of cholesterol, cytokines and chemokines; and exacerbated hepatic macrovesicular steatosis. Moreover, genetic deletion of TRPV1 abrogated the evodiamine-evoked atheroprotection effect in $ApoE^{(-/-)}$ mice. It is evident that evodiamine mediates a TRPV1-dependent atheroprotection and may in addition also confers a probably TRPV1-independent anti-obesity action (Wei et al. 2012). Despite all these beneficial cardiovascular effects, evodiamine is currently mainly used for its alleged thermogenic and slimming properties. A thriving market for Evodia-extracts exists, despite their very bitter taste, and with safety assumed essentially in the light of the long documentation of use of the Evodia fruits in the Chinese medicine.

Raw garlic, with its active electrophilic component, protects against the development of right ventricle hypertrophy, reduces right ventricular pressure in pulmonary diseases and also acts antihypertensive, probably by a protecting effect on the vascular endothelium. Boiled or aged garlic, which lack the active, electrophilic component allicin, have no effect. Garlic lowers blood cholesterol and lipids, lowers blood pressure and shows fibrinolytic activity. Raw garlic has been considered also as a tool against some cardiovascular diseases. Its active component allicin, protects against the development of right ventricle hypertrophy in monocrotaline-induced pulmonary hypertensive rats and reduces right ventricular pressure (Sun and Ku 2006). This protective effect is due to an action on vascular endothelium preventing endothelial cell dysfunction. Seen the remarkable activation of TRPA1 by garlic, it is intriguing to speculate that this channel might be involved in these beneficial effects. TRPA1 is expressed in the heart (Stokes et al. 2006). The effect of TRPA1 on endothelium can be explained by changes in endothelial $[Ca^{2+}]_i$ which regulate endothelium-dependent vasodilator pathways. TRPA1 activation induced dilation of pressurized vessels with myogenic tone. Dilation was attenuated by disruption of the endothelium and by application of the TRPA1 blocker HC-030031. TRPA1 is expressed on native endothelial cells and localized to endothelial cell membrane projections proximal to vascular smooth muscle cells. TRPA1 effects are independent of AITC-induced dilation was insensitive to nitric oxide synthase or cyclooxygenase inhibition but requires K^+ channels which mediate cell hyperpolarization. TRPA1 channels elicits vasodilation of cerebral arteries by a mechanism involving endothelial cell potassium channels SK, IK, Kir2.1 (Earley et al. 2009). Such a mechanism may explain the beneficial effect of TRPA1 activators on high blood pressure. TRPA1 agonists significantly increase the blood flow in skin. Also, a TRPA1-dependent relaxation exists in mesenteric arteries. Intravenously-injected TRPA1 agonists induced a transient hypotensive response accompanied by decreased heart rate. These effects are not much influenced by TRPV1, CGRP or substance P. The cholinergic antagonist atropine sulphate inhibited the depressor response and slowed heart rate caused by TRPA1 activation. Thus, TRPA1 is involved in mediating vasodilation, can influence changes in blood pressure possibly via the autonomic system reflexes and may contributes to the vasovagal/neurocardiogenic syncope disorders and arrhythmias (Pozsgai et al. 2010; Hazari et al. 2011). Let's consider this intriguing possible link between a TRP channel, TRPA1, and the beneficial effects of garlic as a challenge for new cooking!

7.9 TRPA1 and Cough

Stimulation of TRPV1 and TRPA1 induces cough (for a review see Nilius et al. 2012) and capsaicin is the provocative agent of choice to measure cough reflex sensitivity (Dicpinigaitis 2012). Hot spices should, obviously, be avoided by people under medication with drugs that induce cough, like ACE-inhibitors (McEwan and

Fuller 1989). The pulmonar toxicity of capsaicin might be due to a slower metabolization in the aerial pathways (Reilly and Yost 2006) and hot pepper can, indeed, induce fatal asthma crises. The pulmunar toxicity of hot pepper oleoresin is at the basis of the controversies regarding its use as a non-lethal weapon in self-defense spray devises (Clerici et al. 2012). Despite the huge literature on the respiratory hazard of inhaled isocyanates (Baur et al. 1994), the induction of asthma crises does not seem to have been documented for broccoli and cruciferous plants.

Thyme is a traditional medication for cough, also used for the synthomatic treatment of many airways disturbances (Gruenwald et al. 2005). Its major constituents, thymol and carvacrol, are both TRPV3 agonists (Vogt-Eisele et al. 2007), but it is unclear if modulation of this TRP channel, mainly expressed in the skin and neural tissues but also present in laryngeal epithelial cells (Hamamoto et al. 2008), could be involved in this activity.

Ursolic acid, a major non-volatile constituent of rosemary, shows TRPV1 antagonistic activity. Its low potency could be compensated by its high local concentration when extracts from the plant are used for mouth washes or gargles (Zhang et al. 2011). In a study on cough remedies from the traditional Chinese medicine, it was found that the activity of ursolic acid was improved by the steroid alkaloids peimine and peiminine, per se inactive as TRPV1 antagonists, and a combination of these compounds occurs in a traditional Chinese cough medication that combines the ursolic acid-rich leaves of loquat [*Eryobotria japonica* (Thunb) Lindl.], with the bulbs of *Fritillaria thunbergii* Miq, that contain steroid alkaloids. Loquat leaves are used as a tea in the Japanese beverage Biwa Cha, a product increasingly popular also in Europe and USA.

7.10 A Spicy Pancreas Connection?

Interestingly and worth to follow are the reported effects of spices on age-coupled diseases such as type 2 diabetes. Therapeutic interventions for its treatment are in great demand and justify the search for novel therapeutic approaches or alternative solutions, including dietary supplementation. TRPV1 has a role in diabetes. Beneficial effects of dietary capsaicin, an agonist of TRPV1 receptors, were identified for improving glucose, insulin and glucagon-like peptide-1 levels. Recent findings regarding TRPV1 receptors in association with whole body metabolism including glucose homeostasis will be reviewed in this article (for a detailed review see Zsombok 2013).

Cinnamon increases insulin receptor sensitivity and/or directly stimulates insulin-producing cells (Verspohl et al. 2005). Intake of 1 g/day of cinnamon reduced serum blood glucose by 20 %. In general, insulin secretion can also be stimulates by electrophilic compounds in a TRPA1-dependent mechanism. TRPA1 is largely expressed in pancreatic islets, where it mediates cell depolarization coupled to the L-type voltage-dependent Ca^{2+} channel and ultimately insulin secretion (Numazawa et al. 2012). Activation of TRPA1 induces a depolarization which supports closing of KATP channels and potentiates insulin release (Cao et al. 2012).

The clinical literature on the anti-diabetic activity of cinnamon, where also procyanidins are seemingly involved, is contrasting, as highlighted by a recent Cochrane review (Leach and Kumar 2012), that highlighted the need of larger randomized studies before hailing cinnamon as the "spice of diabetes".

Also dietary capsaicin affects glucose homeostasis through TRPV1-mediated glucagon-like peptide-1 (GLP-1) secretion. Dietary capsaicin not only improved glucose tolerance and increased insulin levels, but also lowered daily blood glucose profiles, increasing plasma GLP-1 levels in mice, an effect not observed in *TRPV1*$^{(-/-)}$ mice. In *db*/*db* (dysfunctional leptin receptor) mice, TRPV1 activation by dietary capsaicin ameliorated abnormal glucose homeostasis and increased GLP-1 levels in the plasma and ileum (Wang et al. 2012a).

Taurine, 2-aminoethanesulfonic acid, is naturally taken up in our food, especially in seafood and meat, and can be transformed into *N*-acyl taurines, i.e. *N*-arachidonoyl taurine and *N*-oleoyl taurine. Both compounds induce a high frequency of calcium oscillations in pancreas β-cells via activation of TRPV1, which mediates an increase in insulin secretion. TRPV1 is probably involved in insulin secretion in response to *N*-arachidonoyl taurine and *N*-oleoyl taurine treatment (Waluk et al. 2012).

7.11 An Action of Spicy Channel Activators in the Brain?

The role of TRP channels in the brain is still somewhat elusive. In a recent study, capsaicin has been shown to act neuroprotective against stress-induced impairment. It mitigates cognitive and Alzheimer Disease-like pathological alterations in rats, which were exposed to cold-water stress. The animals fed with capsaicin had significantly diminished stress-induced spatial memory impairment. The stress-induced dendritic regression was attenuated and they showed enhanced expression of several memory-associated proteins, such as synapsin I and PSD93. Thus, some hope is expressed that activation of TRPV1 can mitigate Alzheimer Disease-like neuropathological alterations and cognitive impairment (Jiang et al. 2013). It has strongly been suggested that cucurmin from turmeric contributes to the low prevalence of Alzheimer's Disease in India (Gomez-Pinilla 2008). Electrophiles like MO and cinnamon also activate in mM concentration magnocellular neurosecretory cells (MNCs) that produce vasopressin in the supraoptic nucleus (SON). TRPA1 is involved in this activation. TRPA1 plant-derived agonists increased the frequency of miniature excitatory postsynaptic currents. TRPA1 is expressed exist at presynaptic terminals to the MNCs and enhance glutamate release in the SON (Yokoyama et al. 2011). Eugenol, which is contained in several plants including clove, has anesthetic effects and produces sedation and the reduction of convulsion threshold. Eugenol affects synaptic transmission in the CNS, especially, enhances spontaneous glutamatergic excitatory transmission in substantia gelatinosa (SG; lamina II) neurons of adult rats is increased. This effect is via TRPA1 activation. Thus, we may consider also neuronal TRP channels as targets for electrophiles (Inoue et al. 2012). Possible neuroprotective effects of spices have

been already mentioned. The anti-anxiety and anti-depressive activity of incense fumes have been related to the inhalation of incensol acetate, a cembrane diterpenoid that activates TRPV3 (Moussaieff et al. 2008). This landmark study should trigger further investigations aimed at a better understanding of the role, if any, of aromatherapy in medicine, suggesting that nasal (and retro-nasal) absorption could play a role in the biological profile of spices.

7.12 A Bone Connection?

Curcumin has been already mentioned several times. It also has a low affinity to the vitamin D receptor (VDR) and interacts with the retinoid X receptor (RXR) to form a heterodimer that binds to the vitamin D responsive elements in the region of genes directly controlled by 1,25D. Curcumin activated VDR-RXR complexes modulate the transcription of genes encoding proteins which regulate bone remodeling and Ca2+ homeostasis such as RANKL, SPP1 (osteopontin), and BGP (osteocalcin), TRPV6, CaBP(9k), and claudin 2; and TRPV5, and klotho. The potential exists, therefore, that curcumin may delay the insurgence of osteoporosis (Haussler et al. 2012).

[6]-Gingerol is a major constituent of ginger, and is already widely considered to have several health beneficial effects. However, there seems to be a complication with the skeletal system. [6]-Gingerol has stimulatory effects on osteoclast differentiation of bone marrow macrophages but had no effect on osteoblasts, an effect attenuated by TRPV1 blockers. [6]-Gingerol also stimulates the Ca^{2+} influx in osteoclasts. Daily feeding of 6-gingerol caused an increase in trabecular osteoclast number, but causes also microarchitectural erosion at all trabecular sites and loss of vertebral stiffness. Bone formation was unaffected. Daily feeding of 6-gingerol to skeletally mature female mice caused, indeed, trabecular osteopenia. The mechanism appeared to be activation of osteoclast formation via the TRPV1 channel (Khan et al. 2012).

7.12.1 Bioavailability: The Gray Side of Spices

There is, undoubtedly, an impressive amount of pre-clinical documentation highlighting the beneficial effects of spices. However, most of the correlations between the consumption of spices and its specific beneficial health effects are indirect, that is, they are only suggested by epidemiological studies, supported by studies in animal models, or extrapolated from studies in vitro, with lillte clinical validation. Curcumin exemplifies nicely the biomedical status of many spice constituents. This compound has over 5,150 entries (as to January 31, 2013) in PubMed, almost 10 % of the entries of aspirin, the best known drug. Yet, there are very few clinical studies on curcumin, and the compound has not yet entered the mainstream pharmaceutical market. One major reason for the discrepancy between

the pre-clinical and the clinical status of spice constituents is the very poor bioavailability of many of these compounds in non-food matrixes like pharmaceutical formulations (Shen and Ji 2012). Thus, curcumin is sparingly soluble both in water and in organic solvents, and, just like many polyphenolics, tends to self-aggregates and resist intestinal absorption. On the other hand, the culinary elaboration of turmeric involves its mixing with fatty ingredients like palm oil or egg yolk (turmeric egg) containing surfactants like lecithin, a known booster of the absorption of poorly soluble phytochemicals (Cuomo et al. 2011). A low oral absorption has also been observed for capsaicin, whose "dietary" plasma concentrations require the intake of large amounts of chilli peppers, unrealistical in culinary terms for most Westeners. In a recent study, the time-dependent response to intragastric administration of capsaicin (15 mg/kg body weight, Wistar rats) was measured by Mass spectrometry. The plasma concentrations of capsaicin reached a peak of 10 ng/ml, corresponding to ~30 nM, at the first hour, and then declined rapidly. The Cmax observed in this study is sufficient to activate TRPV1, but, when translated into a human context, corresponds, depending on the way the approximative comparison is done (body surface or body weight), to a dosage of 700 mg to 1 g capsaicin, that is, at least 100 g of the fierest varieties of hot peppers. The oral absorption of capsaicin is only approximately 1 % of the ingested dosage (Yang et al. 2010), and similar low values have been reported also for gingerols and shogahols from ginger (Yu et al. 2011). The science of pharmacokinetics focuses on dietarily artificial matrixes and ways of administration, and not on culinary elaborations of food ingredients. Therefore, data obtained on pure compounds in pharmaceutical formulations can be poorly predictive of the absorption of a compound in a meal, especially for lipophilic compounds like spice ingredients whose octanol-water partition coefficient log P is substantially higher than the optimal value (<5) for absorption in a pharmaceutical formulation. The presence of fats and, in general, of compounds that stimulates the production of bile, is expected to improve the absorption of water-insoluble compounds like the spice constituents. If pharmacokinetics is already complicated due to the genetic heterogeneity of humans, the translation of its data in a dietary setting adds a further layer of complexity, since the absorption is expected to be "menu-specific" (Yu et al. 2011).

7.12.2 Spices as Double-Edged Swords: Do They Have a Toxic Dark Side?

Just like there are beneficial epidemiological correlations between the consumption of spices and a reduced incidence of several chronic diseases, so there are correlations between the culinary spiciness of a cuisine and an increased incidence of certain diseases. The same caution used to evaluate the beneficial links should also be used for the negative ones. The best know example of "bad" epidemiological link involving spices is the one between capsaicin consumption and stomach or gall bladder cancer. After decades of studies, the current view is that contamination

with pesticides and environmental polluters of the fatty ingredients associated with the culinary elaboration of hot pepper significantly blurs the study of this epidemiological link. Pollutants are mainly lipophilic, and tends to concentrate in the Capsicum oleoresin used in many of these studies, with pure capsaicin having, indeed, a very low genotoxic and carcinogenic potential (Bley et al. 2012). Capsaicin and hot peppers are, nevertheless, counter-indicated for asthmatic patients and in people receiving medication with ACE-inhibitors or other "tussigenic" drugs, due to the relatively slow and incomplete metabolic inactivation of capsaicin at pulmonary level (Reilly and Yost 2006), an unfortunate event since capsaicin has, per se, anti-hypertensive properties (Sessa 2010; Yang et al. 2010). Another often quoted case is the gastro-intestinal discomfort associated to curcumin. Actually, most references to this side-effect are drawn from studies where mega-dosages (>4–5 g) of curcumin, of little, if any, dietary relevance, were used (Fan et al. 2012).

The most controversial toxicity issues associated to spices involve, however, pesto, the popular Italian green sauce. The traditional Genovese recipe for the preparation of this sauce recommends the use of young basil leaves. Although dainty, the young leaves contain high concentrations of methyleugenol (Miele et al. 2001), a compound toxified by the xenobiotic-metabolizing system into a genotoxic 1′-sulfo-oxygenated derivative. As basil grows, methyleugenol is transformed into eugenol, a non-genotoxic phenylpropanoid, that, due to the presence of a phenolic hydroxyl, undergoes harmless conjugative metabolization and not oxidative bio-toxification. The potentially genotoxic young leaves were evidently selected for their different and more pleasant flavor, a sensory cue that, at first view, seems a toxicological blunder, and replacement with the Neapolitan variety of basil is of no avail, since this variety contains estragol, another genotoxic phenylpropanoid. Apparently, the only "safe" pesto is the one prepared from old leaves of basil, despite their inferior sensory properties. The methyleugenol/pesto issue highlights one important and often overlooked issue in nutritional science, namely the failure of a reductionist approach to consider the entourage effect. In short, people do not eat pills containing a specific compound, but a food matrix containing, in addition of the compound under study, also a host of other phytochemicals that can exert modulatory activity on its pharmacokinetic and pharmacodynamic profile. In the case of basil, its leaves contain the polymethoxylated flavonoid nevadensin, an inhibitor of the sulfotransferase enzymes involved in the bio-toxification of phenylpropanoids (Alhusainy et al. 2010). When co-administered with dosages of estragol inducing hepatic tumors in vivo and in the same ratio with estragol commonly occurring in basil (ca. 100:6), nevadensic completely blocked the formation of the ultimate carcinogenic metabolite 1′-sulfo-oxyestragole and, therefore, the tumor-inducing properties of estragol (Alhusainy et al. 2010). Such strong matrix effects could, in principle, also be negative, tempering or even reverting the beneficial effects associated to spicy constituents, but this possibility has so far been largely neglected in systematic studies. The growing tendency to replace spices with standardized extracts will surely foster studies on the role of the food matrix and the entourage effect in the activity of spice constituents.

8 Concluding Remarks

Spice-derived compounds not only satisfy our crave for the improvement of flavor and taste of food, but also mediate a host of potentially beneficial effects in humans, many of which are mediated by sensory TRP receptors. Our selection of food following a spicy cue has a long-reaching cultural history, maybe somewhat lost in the modern world. In human history, eating has always had a fundamental role as a positive aggregating element, cogently testified by the frequency of eating scenes and banquets in Western art, from Leonardo's Last Supper to Caravaggio's Supper at Emmaus, to Rembrandt's Belshazar Feast, to Tiepolo's Cleopatra's banquet. Nowadays, food has acquired a negative role, and is associated to the loss of physical shape and the development of obesity and metabolic diseases. All these effects are mediated by primary food constituents like fats and sugars. Secondary metabolites, the hallmark of spices, have been reduced in many modern variety of food plants because of their sub-optimal sensory properties (even visual: potatoes, corn, and carrots used to be blue because of the presence of anthocyanins), but have the potential to reverse this perverse equation, and this review offers some hopefully surprising and positive views on this issue.

For a long time, the quality of food was ealuated in a "fodder-type" style, making reference only to macronutriente (proteins, lipids, carbohydrates) and essential micronutriente (vitamins and mineral). There is growing awareness that secondary plant metabolites from food plants, commercially known as nutraceuticals, play also a critical role (Ryan and Seeley, 2013), and spices, containing them in concentrated form, are ideal probes to investigate these ideas.

In this context, new players might soon emerge. Thus, plant-derived microRNAs (miRNA) can accumulate in the serum of plant feeding animals, reaching the cell interior after food intake and exerting biological activity. For instance, the low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) was identified as a target of MIR168a, a plant miRNA, in human sera. This miRNA can reduce the expression of LDLRAP1 mRNA, and mice fed with rice, a plant rich in MIR168a, show a reduction of LDLRAP1 mRNA expression in the liver, with a decreased clearance of LDL from plasma. Human sera contain this miRNA, that, unsurprisingly because of its abundance in rise, is one of the most abundant exogenous miRNAs in Chinese people (Zhang et al. 2012). Plant derived miRNAs from food plants might account for many still unexplained effects of nutrition (Jiang et al. 2012a). There will be, of course, many novel and healthy additions to the science of food beyond savory spiciness, but in the beginning, it was the spice!

Note. When this MS was under proof, an exciting paper has been published showing that in type II taste receptor cells the non-selective, voltage-activated calcium homeostasis modulator 1 (CALHM1) channel functions as the ATP release channel to transmit sweet, umami, bitter taste information from type II cells to the surrounding sensory nerve fibers (Taruno et al 2013).

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References

Abdoul-Azize S, Dramane G, Subramanium S, Simonin AM, Besnard P, Akhtar Khan N (2012) STIM1 is indispensable for the lingual CD36-mediated Ca(2+) signaling and spontaneous preference for fat. Med Sci (Paris) 28:1053–1055

Aggarwal BB (2011) Healing spices. Sterling, New York

Aggarwal BB, Sethi G, Ahn KS, Sandur SK, Pandey MK, Kunnumakkara AB, Sung B, Ichikawa H (2006) Targeting signal-transducer-and-activator-of-transcription-3 for prevention and therapy of cancer: modern target but ancient solution. Ann N Y Acad Sci 1091:151–169

Aggarwal BB, Van Kuiken ME, Iyer LH, Harikumar KB, Sung B (2009) Molecular targets of nutraceuticals derived from dietary spices: potential role in suppression of inflammation and tumorigenesis. Exp Biol Med 234:825–849

Ahmad N, Alam MK, Shehbaz A, Khan A, Mannan A, Hakim SR, Bisht D, Owais M (2005) Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. J Drug Target 13:555–561

Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N (2011) Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. Eur J Clin Microbiol Infect Dis 30:41–50

Albert-Puleo M (1980) Fennel and anise as estrogenic agents. J Ethnopharmacol 2:337–344

Alhusainy W, Paini A, Punt A, Louisse J, Spenkelink A, Vervoort J, Delatour T, Scholz G, Schilter B, Adams T, van Bladeren PJ, Rietjens IM (2010) Identification of nevadensin as an important herb-based constituent inhibiting estragole bioactivation and physiology-based biokinetic modeling of its possible in vivo effect. Toxicol Appl Pharmacol 245:179–190

Alqareer A, Alyahya A, Andersson L (2006) The effect of clove and benzocaine versus placebo as topical anesthetics. J Dent 34:747–750

Al-Saffar FJ, Ganabadi S, Fakurazi S, Yaakub H, Lip M (2010) Chondroprotective effect of zerumbone on monosodium iodoacetate induced osteoarthritis in rats. J Appl Sci 10:248–260

Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB (2008) Biological activities of curcumin and its analogues (Congeners) made by man and mother nature. Biochem Pharmacol 76:1590–1611

Appendino G, Spagliardi P, Cravotto G, Pocock V, Milligan S (2002) Daucane phytoestrogens: a structure-activity study. J Nat Prod 65:1612–1615

Appendino G, Minassi A, Pagani A, Ech-Chahad A (2008) The role of natural products in the ligand deorphanization of TRP channels. Curr Pharm Des 14:2–17

Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, Spiegelman BM (2008) HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. Nature 451:1008–1012

Artaria C, Maramaldi G, Bonfigli A, Rigano L, Appendino G (2011) Lifting properties of the alkamide fraction from the fruit husks of *Zanthoxylum bungeanum*. Int J Cosmet Sci 33:328–333

Avonto C, Taglialatela-Scafati O, Pollastro F, Minassi A, Di Marzo V, De Petrocellis L, Appendino G (2011) An NMR spectroscopic method to identify and classify thiol-trapping agents: revival of Michael acceptors for drug discovery? Angewandte Chemie 50:467–471

Bartoshuk LM, Lee CH, Scarpellino R (1972) Sweet taste of water induced by artichoke (*Cynara scolymus*). Science 178:988–989

Bartoshuk LM, Duffy VB, Miller IJ (1994) PTC/PROP tasting: anatomy, psychophysics, and sex effects. Physiol Behav 56:1165–1171

Bassoli A, Borgonovo G, Caimi S, Scaglioni L, Morini G, Moriello AS, Marzo VD, Petrocellis LD (2009) Taste-guided identification of high potency TRPA1 agonists from *Perilla frutescens*. Bioorg Med Chem 17:1636–1639

Baur X, Marek W, Ammon J, Czuppon AB, Marczynski B, Raulf-Heimsoth M, Roemmelt H, Fruhmann G (1994) Respiratory and other hazards of isocyanates. Int Arch Occup Environ Health 66:141–152

Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED, Julius D, Jordt SE, Zygmunt PM (2005) Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci USA 102:12248–12252

Bautista DM, Sigal YM, Milstein AD, Garrison JL, Zorn JA, Tsuruda PR, Nicoll RA, Julius D (2008) Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. Nature Neurosci 11:772–779

Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, Lee CH, Smith AB, Breslin PA (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 437:45–46

Bennett SM, Hayes JE (2012) Differences in the chemesthetic subqualities of capsaicin, ibuprofen, and olive oil. Chem Senses 37:471–478

Beuchat LR, Ryu JH, Adler BB, Harrison MD (2006) Death of *Salmonella*, *Escherichia coli* O157: H7, and *Listeria monocytogenes* in shelf-stable, dairy-based, pourable salad dressings. J Food Prot 69:801–814

Bhutani M, Pathak AK, Nair AS, Kunnumakkara AB, Guha S, Sethi G, Aggarwal BB (2007) Capsaicin is a novel blocker of constitutive and interleukin-6-inducible STAT3 activation. Clin Cancer Res 13:3024–3032

Billing J, Sherman PW (1998) Antimicrobial functions of spices: why some like it hot. Q Rev Biol 73:3–49

Birerdinc A, Jarrar M, Stotish T, Randhawa M, Baranova A (2012) Manipulating molecular switches in brown adipocytes and their precursors: a therapeutic potential. Prog Lipid Res 52:51–61

Bley K, Boorman G, Mohammad B, McKenzie D, Babbar S (2012) A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. Toxicol Pathol 40:847–873

Brillat-Savarin JA (1826) Physiologie du Goût, ou Méditations de Gastronomie Transcendante; ouvrage théorique, historique et à l'ordre du jour, dédié aux Gastronomes parisiens, par un Professeur, membre de plusieurs sociétés littéraires et savantes, Paris

Bundy R, Walker AF, Middleton RW, Wallis C, Simpson HC (2008) Artichoke leaf extract (*Cynara scolymus*) reduces plasma cholesterol in otherwise healthy hypercholesterolemic adults: a randomized, double blind placebo controlled trial. Phytomedicine 15:668–675

Cao DS, Zhong L, Hsieh TH, Abooj M, Bishnoi M, Hughes L, Premkumar LS (2012) Expression of transient receptor potential ankyrin 1 (TRPA1) and its role in insulin release from rat pancreatic beta cells. PLoS One 7:e38005

Cettour-Rose P, Bezencon C, Darimont C, le Coutre J, Damak S (2013) Quinine controls body weight gain without affecting food intake in male C57BL6 mice. BMC Physiol 13:5

Chandrashekar J, Kuhn C, Oka Y, Yarmolinsky DA, Hummler E, Ryba NJ, Zuker CS (2010) The cells and peripheral representation of sodium taste in mice. Nature 464:297–301

Chanet A, Milenkovic D, Manach C, Mazur A, Morand C (2012) Citrus flavanones: what is their role in cardiovascular protection? J Agric Food Chem 60:8809–8822

Chang RB, Waters H, Liman ER (2010) A proton current drives action potentials in genetically identified sour taste cells. Proc Natl Acad Sci USA 107:22320–22325

Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR (2013) tmc-1 encodes a sodium-sensitive channel required for salt chemosensation in *C. elegans*. Nature 494:95–99

Chaudhari N, Roper SD (2010) The cell biology of taste. J Cell Biol 1906:285–296

Chen BY, Lin DP, Su KC, Chen YL, Wu CY, Teng MC, Tsai YT, Sun CY, Wang SR, Chang HH (2011a) Dietary zerumbone prevents against ultraviolet B-induced cataractogenesis in the mouse. Mol Vis 17:723–730

Chen BY, Lin DP, Wu CY, Teng MC, Sun CY, Tsai YT, Su KC, Wang SR, Chang HH (2011b) Dietary zerumbone prevents mouse cornea from UVB-induced photokeratitis through inhibition of NF-kappaB, iNOS, and TNF-alpha expression and reduction of MDA accumulation. Mol Vis 17:854–863

Cherno M (1963) Feuerbach's "Man is what he eats": a rectification. J Hist Ideas 24:397–406

Choi MJ, Jin Z, Park YS, Rhee YK, Jin YH (2011) Transient receptor potential (TRP) A1 activated currents in TRPV1 and cholecystokinin-sensitive cranial visceral afferent neurons. Brain Res 1383:36–42

Chu KM, Ngan MP, Wai MK, Yeung CK, Andrews PL, Percie du Sert N, Rudd JA (2009) Olvanil: a non-pungent trpv1 activator has anti-emetic properties in the ferret. Neuropharmacology 459:369–375

Cioffi DL (2007) The skinny on TRPV1. Circ Res 100:934–936

Cizza G, Rother KI (2011) Was Feuerbach right: are we what we eat? J Clin Invest 121:2969–2971

Clark AA, Liggett SB, Munger SD (2012) Extraoral bitter taste receptors as mediators of off-target drug effects. FASEB J 26:4827–4831

Clerici CA, Pelettii G, Veneroni L, de Micheli A (2012) Spray for self-defense against subjects with aggressive behavior: review of the scientific literature on the use of oleoresin capsicum. G Ital Med Lav Ergon 34:151–157

Cuomo J, Appendino G, Dern AS, Schneider E, McKinnon TP, Brown MJ, Togni S, Dixon BM (2011) Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. J Nat Prod 74:664–669

Czifra G, Varga A, Nyeste K, Marincsak R, Toth BI, Kovacs I, Kovacs L, Biro T (2009) Increased expressions of cannabinoid receptor-1 and transient receptor potential vanilloid-1 in human prostate carcinoma. J Cancer Res Clin Oncol 135:507–514

Darvesh AS, Aggarwal BB, Bishayee A (2011) Curcumin and liver cancer: a review. Curr Pharm Biotechnol 13:218–228

De Petrocellis L, Deva R, Mainieri F, Schaefer M, Bisogno T, Ciccoli R, Ligresti A, Hill K, Nigam S, Appendino G, Di Marzo V (2009) Chemical synthesis, pharmacological characterization, and possible formation in unicellular fungi of 3-hydroxy-anandamide. J Lipid Res 50:658–666

Dehkordi O, Rosem JE, Fatemi M, Allard JS, Balan KV, Young JK, Fatima S, Millis RM, Jayam-Trouth A (2012) Neuronal expression of bitter taste receptors and downstream signaling molecules in the rat brainstem. Brain Res PMID:22836012

Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JS, Liggett SB (2012) Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nat Med 16:1299–1304

Desimone JA, Phan TH, Ren Z, Mummalaneni S, Lyall V (2012a) Changes in taste receptor cell [Ca2+]i modulate chorda tympani responses to bitter, sweet, and umami taste stimuli. J Neurophysiol 108:3221–3232

Desimone JA, Ren Z, Phan TH, Heck GL, Mummalaneni S, Lyall V (2012b) Changes in taste receptor cell [Ca2+]i modulate chorda tympani responses to salty and sour taste stimuli. J Neurophysiol 108:3206–3220

Dewis ML, Phan TH, Ren Z, Meng X, Cui M, Mummalaneni S, Rhyu MR, Desimone JA, Lyall V (2012) N-geranylcyclopropylcarboximide (NGCC) modulates salty and umami taste in humans and animal models. J Neurophysiol 23221408

Dias AG, Rousseau D, Duizer L, Cockburn M, Chiu W, Nielsen D, El-Sohemy A (2012) Genetic variation in putative salt taste receptors and salt taste perception in humans. Chem Senses PMID:23118204

Dicpinigaitis PV (2012) Review: effect of drugs on human cough reflex sensitivity to inhaled capsaicin. Cough 8:10

Drahl c (2011) Behind olive's bite. www.cen-online.org, 21 March, pp 46–47
Drahl C (2012) cis-8-methyl-N-vanillyl-6-nonenamide becomes "zucapsaicin". Chemi Eng News 1012:36–37
Dramane G, Abdoul-Azize S, Hichami A, Vogtle T, Akpona S, Chouabe C, Sadou H, Nieswandt B, Besnard P, Khan NA (2012) STIM1 regulates calcium signaling in taste bud cells and preference for fat in mice. J Clin Invest 122:2267–2282
Duke J (2010) Reductionist's rant: principles of spice therapy. J Am Herbalists Guild 9:60–61
Dus M, Min S, Keene AC, Lee GY, Suh GS (2011) Taste-independent detection of the caloric content of sugar in *Drosophila*. Proc Natl Acad Sci USA 108:11644–11649
Earley S, Gonzales AL, Crnich R (2009) Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca2+−activated K+ channels. Circ Res 104:987–994
Eisenberg DM, Harris ES, Littlefield BA, Cao S, Craycroft JA, Scholten R, Bayliss P, Fu Y, Wang W, Qiao Y, Zhao Z, Chen H, Liu Y, Kaptchuk T, Hahn WC, Wang X, Roberts T, Shamu CE, Clardy J (2011) Developing a library of authenticated traditional Chinese medicinal (TCM) plants for systematic biological evaluation–rationale, methods and preliminary results from a Sino-American collaboration. Fitoterapia 82:17–33
Fan X, Zhang C, Liu DB, Yan J, Liang HP (2012) The clinical applications of curcumin: current state and the future. Curr Pharmaceut Des PMID:23116310
Farrelly C (2012) 'Positive biology' as a new paradigm for the medical sciences. Focusing on people who live long, happy, healthy lives might hold the key to improving human well-being. EMBO Rep 13:186–188
Feeney E, O'Brien S, Scannell A, Markey A, Gibney ER (2011) Genetic variation in taste perception: does it have a role in healthy eating? Proc Nutr Soc 70:135–143
Fernholz E, Moore ML (1939) The isolation of a-spinasterol from alfalfa. J Am Chem Soc 61:2467–2468
Finger TE, Bottger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL (2003) Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. Proc Natl Acad Sci USA 100:8981–8986
Freedman P (2008) Out of the east: spices and the medieval imagination. Yale University Press, New Heaven
Fujisawa H, Watanabe K, Suma K, Origuchi K, Matsufuji H, Seki T, Ariga T (2009) Antibacterial potential of garlic-derived allicin and its cancellation by sulfhydryl compounds. Biosci Biotechnol Biochem 73:1948–1955
Galan MV, Kishan AA, Silverman AL (2004) Oral broccoli sprouts for the treatment of *Helicobacter pylori* infection: a preliminary report. Dig Dis Sci 49:1088–1090
Galgani JE, Ryan DH, Ravussin E (2010) Effect of capsinoids on energy metabolism in human subjects. Br J Nutr 103:38–42
Galindo MM, Voigt N, Stein J, van Lengerich J, Raguse JD, Hofmann T, Meyerhof W, Behrens M (2012) G protein-coupled receptors in human fat taste perception. Chem Senses 37:123–139
Garami A, Pakai E, Oliveira DL, Steiner AA, Wanner SP, Almeida MC, Lesnikov VA, Gavva NR, Romanovsky AA (2011) Thermoregulatory phenotype of the Trpv1 knockout mouse: thermoeffector dysbalance with hyperkinesis. J Neurosci 31:1721–1733
Garcia de Vinuesa A, Sancho R, Garcia-Limones C, Behrens A, Peter Ten Dijke P, Calzado MA, Munoz E (2012) Vanilloid receptor-1 regulates neurogenic inflammation in colon and protects mice from colon cancer. Cancer Res 72:1705–1716
Gauthier ML, Beaudry F,Vachon P (2012) Intrathecal [6]-gingerol administration alleviates peripherally induced neuropathic pain in male sprague–dawley rats. Phytother Res PMID:22972597
Gilbertson TA, Fontenot DT, Liu L, Zhang H, Monroe WT (1997) Fatty acid modulation of K+ channels in taste receptor cells: gustatory cues for dietary fat. Am J Physiol 272:C1203–C1210
Gilbertson TA, Yu T, Shah BP (2010) Gustatory mechanisms for fat detection. In: Montmayeur JP, le Coutre J (eds) Fat detection: taste, texture, and post ingestive effects. CRC Press, Boca Raton

Giordani R, Regli P, Kaloustian J, Mikail C, Abou L, Portugal H (2004) Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytother Res 18:990–995

Glendinning JI, Gillman J, Zamer H, Margolskee RF, Sclafani A (2012a) The role of T1r3 and Trpm5 in carbohydrate-induced obesity in mice. Physiol Behav 107:50–58

Glendinning JI, Simons YM, Youngentob L, Youngentob SL (2012b) Fetal ethanol exposure attenuates aversive oral effects of TrpV1, but not TrpA1 agonists in rats. Exp Biol Med (Maywood) 237:236–240

Gomez-Pinilla F (2008) Brain foods: the effects of nutrients on brain function. Nat Rev Neurosci 9:568–578

Gonlachanvit S (2010) Are rice and spicy diet good for functional gastrointestinal disorders? J Neurogastroenterol Motil 16:131–138

Grant J (2012) Tachykinins stimulate a subset of mouse taste cells. PLoS One 7:e31697

Grosjean Y, Rytz R, Farine J-P, Abuin L, Cortot J, Jefferis GSXE, Benton R (2011) An olfactory receptor for food-derived odours promotes male courtship in *Drosophila*. Nature 478:236–240

Gruenwald J, Graubaum HJ, Busch R (2005) Efficacy and tolerability of a fixed combination of thyme and primrose root in patients with acute bronchitis. A double-blind, randomized, placebo-controlled clinical trial. Arzneimittelforschung 55:669–676

Guarini G, Ohanyan VA, Kmetz JG, Dellostritto DJ, Thoppil RJ, Thodeti CK, Meszaros JG, Damron DS, Bratz IN (2012) Disruption of TRPV1-mediated coupling of coronary blood flow to cardiac metabolism in diabetic mice: role of nitric oxide and BK channels. Am J Physiol Heart Circ Physiol 303:H216–H223

Gulbransen BD, Clapp TR, Finger TE, Kinnamon SC (2008) Nasal solitary chemoreceptor cell responses to bitter and trigeminal stimulants in vitro. J Neurophysiol 99:2929–2937

Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB (2012a) Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxid Redox Signal 16:1295–1322

Gupta SC, Kim JH, Kannappan R, Reuter S, Dougherty PM, Aggarwal BB (2012b) Role of nuclear factor kappaB-mediated inflammatory pathways in cancer-related symptoms and their regulation by nutritional agents. Exp Biol Med (Maywood) 236:658–671

Haedrick DR (2010) Power over peoples: technology, environments, and western imperialism, 1400 to the present. Princeton University Press, Princeton

Hamamoto T, Takumida M, Hirakawa K, Takeno S, Tatsukawa T (2008) Localization of transient receptor potential channel vanilloid subfamilies in the mouse larynx. Acta Otolaryngol 128:685–693

Hamlyn DW (1968) Aristotle De Anima, Books II and III. Clarendon, Oxford

Han EH, Kim HG, Choi JH, Jang YJ, Lee SS, Kwon KI, Kim E, Noh K, Jeong TC, Hwang YP, Chung YC, Kang W, Jeong HG (2012) Capsaicin induces CYP3A4 expression via pregnane X receptor and CCAAT/enhancer-binding protein beta activation. Mol Nutr Food Res 56:797–809

Haniadka R, Rajeev AG, Palatty PL, Arora R, Baliga MS (2012) *Zingiber officinale* (ginger) as an anti-emetic in cancer chemotherapy: a review. J Altern Complement Med 18:440–444

Hansen HS, Rosenkilde MM, Holst JJ, Schwartz TW (2012) GPR119 as a fat sensor. Trends Pharm Sci 33:374–381

Harris JC, Plummer S, Turner MP, Lloyd D (2000) The microaerophilic flagellate *Giardia intestinalis*: *Allium sativum* (garlic) is an effective antigiardial. Microbiology 146:3119–3127

Harris JC, Cottrell SL, Plummer S, Lloyd D (2001) Antimicrobial properties of *Allium sativum* (garlic). Appl Microbiol Biotechnol 57:282–286

Hata T, Tazawa S, Ohta S, Rhyu MR, Misaka T, Ichihara K (2012) Artepillin C, a major ingredient of Brazilian propolis, induces a pungent taste by activating TRPA1 channels. PLoS One 7:e48072

Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka PW (2012) Molecular mechanisms of vitamin D action. Calcified Tissue Int PMID:22782502

Hayes JE, Bartoshuk LM, Kidd JR, Duffy VB (2008) Supertasting and PROP bitterness depends on more than the TAS2R38 gene. Chem Senses 33:255–265

Hazari MS, Haykal-Coates N, Winsett DW, Krantz QT, King C, Costa DL, Farraj AK (2011) TRPA1 and sympathetic activation contribute to increased risk of triggered cardiac arrhythmias in hypertensive rats exposed to diesel exhaust. Environ Health Perspect 119:951–957

Hegde VL, Nagarkatti PS, Nagarkatti M (2011) Role of myeloid-derived suppressor cells in amelioration of experimental autoimmune hepatitis following activation of TRPV1 receptors by cannabidiol. PLoS One 6:e18281

Hempenstall K, Rice AS (2002) Current treatment options in neuropathic pain. Curr Opin Investig Drugs 3:441–448

Hempenstall K, Nurmikko TJ, Johnson RW, A'Hern RP, Rice AS (2005) Analgesic therapy in postherpetic neuralgia: a quantitative systematic review. PLoS Med 2:e164

Hersch-Martinez P, Leanos-Miranda BE, Solorzano-Santos F (2005) Antibacterial effects of commercial essential oils over locally prevalent pathogenic strains in Mexico. Fitoterapia 76:453–457

Heyman E, Gamelin FX, Aucouturier J, Di Marzo V (2012) The role of the endocannabinoid system in skeletal muscle and metabolic adaptations to exercise: potential implications for the treatment of obesity. Obes Rev 13:1110–1124

Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoeur C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffeis C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Korner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Levy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, Froguel P (2012) Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature 483:350–354

Iida T, Moriyama T, Kobata K, Morita A, Murayama N, Hashizume S, Fushiki T, Yazawa S, Watanabe T, Tominaga M (2003) TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. Neuropharmacology 44:958–967

Inoue M, Fujita T, Goto M, Kumamoto E (2012) Presynaptic enhancement by eugenol of spontaneous excitatory transmission in rat spinal substantia gelatinosa neurons is mediated by transient receptor potential A1 channels. Neuroscience 210:403–415

Inoue-Choi M, Yuan J-M, Yang CS, Van Den Berg DJ, Lee M-J, Gao Y-T, Yu MC (2010) Genetic association between the COMT genotype and urinary levels of tea polyphenols and their metabolites among daily green tea drinkers. Int J Mol Epidemiol Gen 1:114–123

Ishii S, Kurokawa A, Kishi M, Yamagami K, Okada S, Ishimaru Y, Misaka T (2012) The response of PKD1L3/PKD2L1 to acid stimuli is inhibited by capsaicin and its pungent analogs. FEBS J 279:1857–1870

Ito N, Ruegg UT, Kudo A, Miyagoe-Suzuki Y, Takeda S (2012) Activation of calcium signaling through Trpv1 by nNOS and peroxynitrite as a key trigger of skeletal muscle hypertrophy. Nat Med 19:101–106

Iwasaki Y, Tanabe M, Kayama Y, Abe M, Kashio M, Koizumi K, Okumura Y, Morimitsu Y, Tominaga M, Ozawa Y, Watanabe T (2009) Miogadial and miogatrial with alpha, beta-unsaturated 1,4-dialdehyde moieties-novel and potent TRPA1 agonists. Life Sci 85:60–69

Janssen S, Laermans J, Verhulst P-J, Thijs T, Tack J, Depoortere I (2011) Bitter taste receptors and a-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. Proc Natl Acad Sci (USA) 108:2094–2099

Ji HF, Li XJ, Zhang HY (2009) Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Rep 10:194–200

Jiang M, Sang X, Hong Z (2012a) Beyond nutrients: food-derived microRNAs provide cross-kingdom regulation. BioEssays: News Rev Mol Cell Dev Biol 34:280–284

Jiang P, Josue J, Li X, Glaser D, Li W, Brand JG, Margolskee RF, Reed DR, Beauchamp GK (2012b) Major taste loss in carnivorous mammals. Proc Natl Acad Sci USA 109:4956–4961

Jiang X, Jia LW, Li XH, Cheng XS, Xie JZ, Ma ZW, Xu WJ, Liu Y, Yao Y, Du LL, Zhou XW (2013) Capsaicin ameliorates stress-induced Alzheimer's disease-like pathological and cognitive impairments in rats. J Alzheimers Dis 35:91–105

Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430

Josse AR, Sherriffs SS, Holwerda AM, Andrews R, Staples AW, Phillips SM (2010) Effects of capsinoid ingestion on energy expenditure and lipid oxidation at rest and during exercise. Nutr Metab (Lond) 7:65

Kanetkar P, Singhal R, Kamat M (2007) *Gymnema sylvestre*: a memoir. J Clin Biochem Nutr 41:77–81

Kang JH, Tsuyoshi G, Han IS, Kawada T, Kim YM, Yu R (2009) Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice Fed a high-fat diet. Obesity (Silver Spring) 18:780–787

Kaske S, Krasteva G, Konig P, Kummer W, Hofmann T, Gudermann T, Chubanov V (2007) TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. BMC Neurosci 8:49

Katsumata T, Nakakuki H, Tokunaga C, Fujii N, Egi M, Phan TH, Mummalaneni S, Desimone JA, Lyall V (2008) Effect of maillard reacted peptides on human salt taste and the amiloride-insensitive salt taste receptor (TRPV1t). Chem Senses 33:665–680

Kawashima M, Imura K, Sato I (2012) Topographical organization of TRPV1-immunoreactive epithelium and CGRP-immunoreactive nerve terminals in rodent tongue. Eur J Histochem 56:e21

Keller KL (2012) Genetic influences on oral fat perception and preference. Presented at the symposium "The taste for fat: new discoveries on the role of fat in sensory perception, metabolism, sensory pleasure and beyond" held at the Institute of Food Technologists 2011 Annual Meeting, New Orleans, 12 June 2011. J Food Sci 77:S143–147

Khan K, Singh A, Mittal M, Sharan K, Singh N, Dixit P, Sanyal S, Maurya R, Chattopadhyay N (2012) [6]-Gingerol induces bone loss in ovary intact adult mice and augments osteoclast function via the transient receptor potential vanilloid 1 channel. Mol Nutr Food Res 56:1860–1873

Kim JH, Gupta SC, Park B, Yadav VR, Aggarwal BB (2012) Turmeric (*Curcuma longa*) inhibits inflammatory nuclear factor (NF)-kappaB and NF-kappaB-regulated gene products and induces death receptors leading to suppressed proliferation, induced chemosensitization, and suppressed osteoclastogenesis. Mol Nutr Food Res 56:454–465

Kingwell K (2011) Dementia: overweight or obesity during midlife is associated with late-life dementia. Nat Rev Neurol 7:299

Kinnamon SC (2011) Taste receptor signaling– from tongues to lungs. Acta physiologica 204:158–168

Kinnamon SC, Reynolds SD (2009) Cell biology. Using taste to clear the air(ways). Science 325:1081–1082

Klein AH, Sawyer CM, Zanotto KL, Ivanov MA, Cheung S, Iodi Carstens M, Furrer S, Simons CT, Slack JP, Carstens E (2011) A tingling sanshool derivative excites primary sensory neurons and elicits nocifensive behavior in rats. J Neurophysiol 105:1701–1710

Knaapila A, Hwang LD, Lysenko A, Duke FF, Fesi B, Khoshnevisan A, James RS, Wysocki CJ, Rhyu M, Tordoff MG, Bachmanov AA, Mura E, Nagai H, Reed DR (2012) Genetic analysis of chemosensory traits in human twins. Chem Senses 37:869–881

Kobata K, Todo T, Yazawa S, Iwai K, Watanabe T (1998) Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 sweet of pepper (*Capsicum annuum* L.). J Agric Food Chem 46:1695–1697

Kobata K, Sutoh K, Todo T, Yazawa S, Iwai K, Watanabe T (1999) Nordihydrocapsiate, a new capsinoid from the fruits of a nonpungent pepper, capsicum annuum. J Nat Prod 62:335–336

Kobayashi M, Watanabe K, Yokoyama S, Matsumoto C, Hirata M, Tominari T, Inada M, Miyaura C (2012) Capsaicin, a TRPV1 ligand, suppresses bone resorption by inhibiting the prostaglandin E production of osteoblasts, and attenuates the inflammatory bone loss induced by lipopolysaccharide. ISRN Pharmacol 2012:439860

Kokrashvili Z, Mosinger B, Margolskee RF (2009) T1r3 and alpha-gustducin in gut regulate secretion of glucagon-like peptide-1. Ann N Y Acad Sci 1170:91–94

Kono T, Kaneko A, Omiya Y, Ohbuchi K, Ohno N, Yamamoto M (2013) Epithelial transient receptor potential ankyrin 1 (TRPA1)-dependent adrenomedullin up-regulates blood flow in rat small intestine. Am J Physiol Gastrointest Liver Physiol 304:G428–G436

Koo JY, Jang Y, Cho H, Lee CH, Jang KH, Chang YH, Shin J, Oh U (2007) Hydroxy-alpha-sanshool activates TRPV1 and TRPA1 in sensory neurons. Eur J Neurosci 26:1139–1147

Krasteva G, Hartmann P, Papadakis T, Bodenbenner M, Wessels L, Weihe E, Schutz B, Langheinrich AC, Chubanov V, Gudermann T, Ibanez-Tallon I, Kummer W (2012) Cholinergic chemosensory cells in the auditory tube. Histochem Cell Biol 137:483–497

Kyriazis GA, Soundarapandian MM, Tyrberg B (2012) Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci USA 109:E524–E532

Lang Y, Kisaka H, Sugiyama R, Nomura K, Morita A, Watanabe T, Tanaka Y, Yazawa S, Miwa T (2009) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in *Capsicum annuum* cv. CH-19 sweet. Plant J 59:953–961

Laugerette F, Passilly-Degrace P, Patris B, Niot I, Febbraio M, Montmayeur JP, Besnard P (2005) CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. J Clin Invest 115:3177–3184

Leach MJ, Kumar S (2012) Cinnamon for diabetes mellitus. Cochrane Database Syst Rev 9: CD007170

Leamy AW, Shukla P, McAlexander MA, Carr MJ, Ghatta S (2011) Curcumin ((E, E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) activates and desensitizes the nociceptor ion channel TRPA1. Neurosci Lett 503:157–162

Lee SP, Buber T, Yang Q, Cerne R, Cortés RY, Sprous DG, Bryant RW (2008) Thymol and related alkyl phenols activate the hTRPA1 channel. Brit J Pharmacol 153:1739–1749

Lee MS, Kim CT, Kim IH, Kim Y (2011) Effects of capsaicin on lipid catabolism in 3T3-L1 adipocytes. Phytother Res: PTR 25:935–939

Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, Kennedy DW, Beauchamp GK, Doulias PT, Ischiropoulos H, Kreindler JL, Reed DR, Cohen NA (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. J Clin Invest 122:4145–4159

Li F, Zhou M (2012) Depletion of bitter taste transduction leads to massive spermatid loss in transgenic mice. Mol Hum Reprod 18:289–297

Li L, Chen J, Ni Y, Feng X, Zhao Z, Wang P, Sun J, Yu H, Yan Z, Liu D, Nilius B, Zhu Z (2012) TRPV1 activation prevents nonalcoholic fatty liver through UCP2 upregulation in mice. Pflugers Archiv: Eur J Physiol 463:727–732

Liebler DC, Guengerich FP (2005) Elucidating mechanisms of drug-induced toxicity. Nat Rev Drug Discov 4:410–420

Liu P, Shah BP, Croasdell S, Gilbertson TA (2011) Transient receptor potential channel type M5 is essential for fat taste. J Neurosci 31:8634–8642

Ludy MJ, Mattes RD (2012) Comparison of sensory, physiological, personality, and cultural attributes in regular spicy food users and non-users. Appetite 58:19–27

Ludy MJ, Moore GE, Mattes RD (2012) The effects of capsaicin and capsiate on energy balance: critical review and meta-analyses of studies in humans. Chem Senses 37:103–121

Luo Z, Ma L, Zhao Z, He H, Yang D, Feng X, Ma S, Chen X, Zhu T, Cao T, Liu D, Nilius B, Huang Y, Yan Z, Zhu Z (2012) TRPV1 activation improves exercise endurance and energy metabolism through PGC-1alpha upregulation in mice. Cell Res 22:551–564

Lutsey PL, Steffen LM, Stevens J (2008) Dietary intake and the development of the metabolic syndrome: the atherosclerosis risk in communities study. Circulation 117:754–761

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Desimone JA (2005) A novel vanilloid receptor-1 (VR-1) variant mammalian salt taste receptor. Chem Senses 30(Suppl 1):i42–i43

Lyall V, Phan TH, Ren Z, Mummalaneni S, Melone P, Mahavadi S, Murthy KS, Desimone JA (2010) Regulation of the putative TRPV1t salt taste receptor by phosphatidylinositol 4, 5-bisphosphate. J Neurophysiol 103:1337–1349

Maillet EL, Margolskee RF, Mosinger B (2009) Phenoxy herbicides and fibrates potently inhibit the human chemosensory receptor subunit T1R3. J Med Chem 52:6931–6935

Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, Shirazi-Beechey SP (2007) T1R3 and gustducin in gut sense sugars to regulate expression of Na+−glucose cotransporter 1. Proc Natl Acad Sci USA 104:15075–15080

Martin C, Passilly-Degrace P, Chevrot M, Ancel D, Sparks SM, Drucker DJ, Besnard P (2012) Lipid-mediated release of GLP-1 by mouse taste buds from circumvallate papillae: putative involvement of GPR120 and impact on taste sensitivity. J Lipid Res 53:2256–2265

Mattes RD (2011) Fat taste in humans: is it a primary? In: Montmayeur JP, le Coutre J (eds) Fat detection: taste, texture, and post ingestive effects. CRC Press, Boca Raton

Mattes RD (2012) Spices and energy balance. Physiol Behav 107:584–590

Mattson MP (2008) Dietary factors, hormesis and health. Ageing Res Rev 7:43–48

Mauer L, El-Sohemy A (2012) Prevalence of cilantro (*Coriandrum sativum*) disliking among different ethnocultural groups. Flavour 1:8

McEwan JR, Fuller RW (1989) Angiotensin converting enzyme inhibitors and cough. J Cardiovasc Pharmacol 13(Suppl 3):S67–S69

McGee H (2001) On food and cooking: the science and lore of the kitchen. Scribner, New York

Menozzi-Smarrito C, Riera CE, Munari C, Le Coutre J, Robert F (2009) Synthesis and evaluation of new alkylamides derived from alpha-hydroxysanshool, the pungent molecule in Szechuan pepper. J Agric Food Chem 11:1982–1989

Merigo F, Benati D, Cristofoletti M, Osculati F, Sbarbati A (2011) Glucose transporters are expressed in taste receptor cells. J Anat 219:243–252

Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M (2012) The molecular receptive ranges of human TAS2R bitter taste receptors. Chem Senses 35:157–170

Miele M, Dondero R, Ciarallo G, Mazzei M (2001) Methyleugenol in *Ocimum basilicum* L. Cv. genovese gigante. J Agri Food Chem 49:517–521

Miranda M (2009) Johannes Vermeer and Anthon van Leeuwenhoek: Delft art and science together during the golden Dutch century. Rev Méd Chile 137:567–574

Morera E, De Petrocellis L, Morera L, Schiano Moriello A, Nalli M, Di Marzo V, Ortar G (2012) Synthesis and biological evaluation of [6]-gingerol analogues as transient receptor potential channel TRPV1 and TRPA1 modulators. Bioorg Med Chem Lett 22:1674–1677

Morice AH, Bennett RT, Chaudhry MA, Cowen ME, Griffin SC, Loubani M (2011) Effect of bitter tastants on human bronchi. Nat Med 17:775

Motter AL, Ahern GP (2008) TRPV1-null mice are protected from diet-induced obesity. FEBS Lett 582:2257–2262

Moussaieff A, Gross M, Nesher E, Tikhonov T, Yadid G, Pinhasov A (2008) Incensole acetate reduces depressive-like behavior and modulates hippocampal BDNF and CRF expression of submissive animals. J Psychopharmacol 26:1584–1593

Nakamura Y, Miyoshi N (2010) Electrophiles in foods: the current status of isothiocyanates and their chemical biology. Biosci Biotechnol Biochem 74:242–255

Nakamura E, Hasumura M, Gabriel AS, Uneyama H, Torii K (2010) New frontiers in gut nutrient sensor research: luminal glutamate-sensing cells in rat gastric mucosa. J Pharmacol Sci 112:13–18

Narukawa M, Koizumi K, Iwasaki Y, Kubota K, Watanabe T (2010) Galangal pungent component, 1′-acetoxychavicol acetate, activates TRPA1. Biosci Biotechnol Biochem 74:1694–1696

Negri R, Di Feola M, Di Domenico S, Scala MG, Artesi G, Valente S, Smarrazzo A, Turco F, Morini G, Greco L (2012) Taste perception and food choices. J Pediatr Gastroenterol Nutr 54:624–629

Niki M, Yoshida R, Takai S, Ninomiya Y (2010) Gustatory signaling in the periphery: detection, transmission, and modulation of taste information. Biol Pharm Bull 33:1772–1777
Nilius B (2007) TRP channels in disease. Biochim Biophys Acta 1772:805–812
Nilius B, Appendino G (2011) Tasty and healthy TR(i)Ps. The human quest for culinary pungency. EMBO Rep 12:1094–1101
Nilius B, Owsianik G, Voets T, Peters JA (2007) Transient receptor potential channels in disease. Physiol Rev 87:165–217
Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Archiv: Eur J Physiol 464:425–458
Numazawa S, Takase M, Ahiko T, Ishii M, Shimizu S, Yoshida T (2012) Possible involvement of transient receptor potential channels in electrophile-induced insulin secretion from RINm5F cells. Biol Pharm Bull 35:346–354
O'Mahony R, Al-Khtheeri H, Weerasekera D, Fernando N, Vaira D, Holton J, Basset C (2005) Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori*. World J Gastroenterol: WJG 11:7499–7507
Oehler B, Scholze A, Schaefer M, Hill K (2012) TRPA1 is functionally expressed in melanoma cells but is not critical for impaired proliferation caused by allyl isothiocyanate or cinnamaldehyde. Naunyn Schmiedeberg's Arch Pharmacol 385:555–563
Ogita A, Fujita K, Tanaka T (2012) Enhancing effects on vacuole-targeting fungicidal activity of amphotericin B. Front Microbiol 3:100
Ogura T, Bekkerman M, Lin W (2008) TRPM5-expressing solitary chemosensory cells in mouse vomeronasal organ. Chem Senses 33:S105
Ohkawara S, Tanaka-Kagawa T, Furukawa Y, Nishimura T, Jinno H (2010) Activation of the human transient receptor potential vanilloid subtype 1 by essential oils. Biol Pharm Bull 33:1434–1437
Okumi H, Tashima K, Matsumoto K, Namiki T, Terasawa K, Horie S (2012) Dietary agonists of TRPV1 inhibit gastric acid secretion in mice. Planta Med 78:1801–1806
Ooi SX, Lee PL, Law HY, Say YH (2010) Bitter receptor gene (TAS2R38) P49A genotypes and their associations with aversion to vegetables and sweet/fat foods in Malaysian subjects. Asia Pac J Clin Nutr 19:491–498
Pathak AK, Bhutani M, Nair AS, Ahn KS, Chakraborty A, Kadara H, Guha S, Sethi G, Aggarwal BB (2007) Ursolic acid inhibits STAT3 activation pathway leading to suppression of proliferation and chemosensitization of human multiple myeloma cells. Mol Cancer Res 5:943–955
Paul M, Jauch J (2012) Efficient preparation of incensole and incensole acetate, and quantification of these bioactive diterpenes in *Boswellia papyrifera* by a RP-DAD-HPLC method. Nat Prod Commun 7:283–288
Peng J, Li YJ (2010) The vanilloid receptor TRPV1: role in cardiovascular and gastrointestinal protection. Eur J Pharmacol 627:1–7
Peters M, Trembovler V, Alexandrovich A, Parnas M, Birnbaumer L, Minke B, Shohami E (2012) Carvacrol together with TRPC1 elimination improve functional recovery after traumatic brain injury in mice. J Neurotrauma 29:2831–2834
Peyrot des Gachons C, Uchida K, Bryant B, Shima A, Sperry JB, Dankulich-Nagrudny L, Tominaga M, Smith AB 3rd, Beauchamp GK, Breslin PA (2011) Unusual pungency from extra-virgin olive oil is attributable to restricted spatial expression of the receptor of oleocanthal. J Neurosci 31:999–1009
Pietras CO, James A, Konradsen JR, Nordlund B, Soderhall C, Pulkkinen V, Pedroletti C, Daham K, Kupczyk M, Dahlen B, Kere J, Dahlen SE, Hedlin G, Melen E (2012) Transcriptome analysis reveals upregulation of bitter taste receptors in severe asthmatics. Eur Respir J PMID: 23222870
Pozsgai G, Bodkin JV, Graepel R, Bevan S, Andersson DA, Brain SD (2010) Evidence for the pathophysiological relevance of Trpa1 receptors in the cardiovascular system in vivo. Cardiovasc Res 87:760–768
Pramod K, Ansari SH, Ali J (2006) Eugenol: a natural compound with versatile pharmacological actions. Nat Prod Commun 5:1999–2006

Prasad S, Sung B, Aggarwal BB (2012) Age-associated chronic diseases require age-old medicine: role of chronic inflammation. Prev Med 54(Suppl):S29–S37
Qian QH, Yue W, Chen WH, Yang ZH, Liu ZT, Wang YX (2010) Effect of gingerol on substance P and NK1 receptor expression in a vomiting model of mink. Chin Med J 123:478–484
Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D, Weiner L (1998) The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim Biophys Acta 1379:233–244
Rech JC, Eckert WA, Maher MP, Banke T, Bhattacharya A, Wickenden AD (2011) Recent advances in the biology and medicinal chemistry of TRPA1. Future Med Chem 2:843–858
Reilly CA, Yost GS (2006) Metabolism of capsaicinoids by P450 enzymes: a review of recent findings on reaction mechanisms, bio-activation, and detoxification processes. Drug Metab Rev 38:685–706
Riera CE, Vogel H, Simon SA, le Coutre J (2007) Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors. Am J Physiol Regul Integr Comp Physiol 293:R626–R634
Riera CE, Vogel H, Simon SA, Damak S, le Coutre J (2008) The capsaicin receptor participates in artificial sweetener aversion. Biochem Biophys Res Commun 376:653–657
Riera CE, Vogel H, Simon SA, Damak S, le Coutre J (2009) Sensory attributes of complex tasting divalent salts are mediated by TRPM5 and TRPV1 channels. J Neurosci 29:2654–2662
Rodrigues MR, Kanazawa LK, Neves TL, Silva CF, Horst H, Pizzolatti MG, Santos AR, Baggio CH, Werner MF (2011) Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of salvia officinalis in mice. J Ethnopharmacol 139:519–526
Rofes L, Arreola V, Martin A, Clave P (2012) Natural capsaicinoids improve swallow response in older patients with oropharyngeal dysphagia. Gut PMID:22722616
Rooks MG, Garrett WS (2011) Bacteria, food, and cancer. F1000 Biol Rep 3:12
Roth HJ (2005) Friedrich von Schiller, ein Schnüffler? Deutsche Apotheker Zeitung 145:63–65
Ruiz C, Gutknecht S, Delay E, Kinnamon S (2006) Detection of NaCl and KCl in TRPV1 knockout mice. Chem Senses 31:813–820
Ryan KR, Seeley RJ (2013) Food as a hormone. Science 339:918–919
Sachs J (2011) Aristotle's on the soul and on memory and recollection. Green Lion Press, London
Saito M, Toyoda H, Kawakami S, Sato H, Bae YC, Kang Y (2012) Capsaicin induces theta-band synchronization between gustatory and autonomic insular cortices. J Neurosci 32:13470–13487
Santoni G, Caprodossi S, Farfariello V, Liberati S, Gismondi A, Amantini C (2012) Antioncogenic effects of transient receptor potential vanilloid 1 in the progression of transitional urothelial cancer of human bladder. ISRN Urol 2012:458238
Savino F, Cresi F, Castagno E, Silvestro L, Oggero R (2005) A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants. Phytother Res: PTR 19:335–340
Scheiper R (1993) Hot spice. Haarman and Reimer, Springfield
Schiffman SS, Booth BJ, Sattely-Miller EA, Graham BG, Gibes KM (1999) Selective inhibition of sweetness by the sodium salt of +/−2-(4-methoxyphenoxy)propanoic acid. Chem Senses 24:439–447
Schivelbusch W (1990) Das paradies, der geschmack and die vernunft. Eine Geschichte der Genussmittel, Fischer, Geschichte
Scimemi A (2013) A TRP among the astrocytes. J Physiol 591:9–15
Sclafani A (2007) Sweet taste signaling in the gut. Proc Natl Acad Sci USA 104:14887–14888
Sclafani A, Zukerman S, Glendinning JI, Margolskee RF (2007) Fat and carbohydrate preferences in mice: the contribution of alpha-gustducin and Trpm5 taste-signaling proteins. Am J Physiol Regul Integr Comp Physiol 293:R1504–R1513
Serra J (2012) Intestinal gas: has diet anything to do in the absence of a demonstrable malabsorption state? Curr Opin Clin Nutr Metab Care 15:489–493

Sessa WC (2010) A new way to lower blood pressure: pass the chili peppers please! Cell Metab 12:109–110
Setty AR, Sigal LH (2005) Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. Semin Arthritis Rheum 34:773–784
Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ (2009) Motile cilia of human airway epithelia are chemosensory. Science 325:1131–1134
Shah BP, Liu P, Yu T, Hansen DR, Gilbertson TA (2011) TRPM5 is critical for linoleic acid-induced CCK secretion from the enteroendocrine cell line, STC-1. Am J Physiol Cell Physiol 302:C210–C219
Shelton P, Jaiswal AK (2013) The transcription factor NF-E2-related factor 2 (Nrf2): a protooncogene? FASEB J 27:414–423
Shen L, Ji HF (2012) The pharmacology of curcumin: is it the degradation products? Trends Mol Med 18:138–144
Shepherd GM (2006) Smell images and the flavour system in the human brain. Nature 444:316–321
Shepherd GM (2012) Neurogastronomy. Columbia University Press, New York
Sherman PW, Billing J (1999) Darwinian gastronomy: why we use spices. Bioscience 49:453–463
Shimazawa M, Chikamatsu S, Morimoto N, Mishima S, Nagai H, Hara H (2005) Neuroprotection by Brazilian green propolis against in vitro and in vivo ischemic neuronal damage. Evid Based Complement Altern Med: eCAM 2:201–207
Shin DH, Nam JH, Lee ES, Zhang Y, Kim SJ (2012) Inhibition of Ca(2+) release-activated Ca(2+) channel (CRAC) by curcumin and caffeic acid phenethyl ester (CAPE) via electrophilic addition to a cysteine residue of Orai1. Biochem Biophys Res Commun 428:56–61
Shindo Y, Morishita K, Kotake E, Miura H, Carninci P, Kawai J, Hayashizaki Y, Hino A, Kanda T, Kusakabe Y (2011) FXYD6, a Na, K-ATPase regulator, is expressed in type II taste cells. Biosci Biotechnol Biochem 75:1061–1066
Singh N, Vrontakis M, Parkinson F, Chelikani P (2011) Functional bitter taste receptors are expressed in brain cells. Biochem Biophys Res Commun 406:146–151
Smith KR, Treesukosol Y, Paedae AB, Contreras RJ, Spector AC (2012) The contribution of the TRPV1 channel to salt taste quality in mice as assessed by conditioned taste aversion generalization and chorda tympani nerve responses. Am J Physiol Regul Integr Comp Physiol 303:R1195–R1205
Snitker S, Fujishima Y, Shen H, Ott S, Pi-Sunyer X, Furuhata Y, Sato H, Takahashi M (2009) Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. Am J Clin Nutr 89:45–50
Son HJ, Kim MJ, Park JH, Ishii S, Misaka T, Rhyu MR (2012a) Methyl syringate, a low-molecular-weight phenolic ester, as an activator of the chemosensory ion channel TRPA1. Arch Pharm Res 35:2211–2218
Son HJ, Kim Y, Misaka T (2012b) Activation of the chemosensory ion channels TRPA1 and TRPV1 by hydroalcohol extract of kalopanax pictus leaves. Biomol Therapeut 20:550–555
Sothilingam V, Hass N, Breer H (2011) Candidate chemosensory cells in the stomach mucosa of young postnatal mice during the phases of dietary changes. Cell Tissue Res 344:239–249
Stock K, Kumar J, Synowitz M, Petrosino S, Imperatore R, Smith ES, Wend P, Purfurst B, Nuber UA, Gurok U, Matyash V, Walzlein JH, Chirasani SR, Dittmar G, Cravatt BF, Momma S, Lewin GR, Ligresti A, Petrocellis LD, Cristino L, Marzo VD, Kettenmann H, Glass R (2012) Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1. Nat Med PMID:22820645
Stokes A, Wakano C, Koblan-Huberson M, Adra CN, Fleig A, Turner H (2006) TRPA1 is a substrate for de-ubiquitination by the tumor suppressor CYLD. Cell Signal 18:1584–1594
Straub I, Mohr F, Stab J, Konrad M, Philipp S, Oberwinkler J, Schaefer M (2012) Citrus fruit and fabacea secondary metabolites potently and selectively block TRPM3. Brit J Pharmacol 23190005

Sun X, Ku DD (2006) Allicin in garlic protects against coronary endothelial dysfunction and right heart hypertrophy in pulmonary hypertensive rats. Am J Physiol Heart Circ Physiol 291: H2431–H2438

Sundram V, Chauhan SC, Ebeling M, Jaggi M (2012) Curcumin attenuates beta-catenin signaling in prostate cancer cells through activation of protein kinase D1. PLoS One 7:e35368

Sung B, Prasad S, Yadav VR, Aggarwal BB (2012) Cancer cell signaling pathways targeted by spice-derived nutraceuticals. Nutr Cancer 64:173–197

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolskee RF, Nilius B (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438:1022–1025

Talavera K, Ninomiya Y, Winkel C, Voets T, Nilius B (2007) Influence of temperature on taste perception. Cell Mol Life Sci: CMLS 64:377–381

Tamura Y, Iwasaki Y, Narukawa M, Watanabe T (2012) Ingestion of cinnamaldehyde, a TRPA1 agonist, reduces visceral fats in mice fed a high-fat and high-sucrose diet. J Nutr Sci Vitaminol (Tokyo) 58:9–13

Tanaka Y, Munetaka H, Miwa T, Watanabe T, Yazawa S (2010) Newly mutated putative aminotransferase in nonpungent pepper (*Capsicum annuum*) results in biosynthesis of capsinoids, capsaicinoid analogues. J Agric Food Chem 58:1761–1767

Taruno A, Vingtdeux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A, Adrien L, Zhao H, Leung S, Abernethy M, Koppel J, Davies P, Civan MM, Chaudhari N, Matsumoto I, Hellekant G, Tordoff MG, Marambaud P, Foskett JK (2013) CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. Nature 495:223–226

Tizzano M, Finger TE (2013) Chemosensors in the nose: guardians of the airways. Physiology 28:51–60

Tizzano M, Cristofoletti M, Sbarbati A, Finger TE (2011) Expression of taste receptors in solitary chemosensory cells of rodent airways. BMC Pulm Med 11:3

To Quynh CT, Iijima Y, Kubota K (2010) Influence of the isolation procedure on coriander leaf volatiles with some correlation to the enzymatic activity. J Agric Food Chem 58:1093–1099

Touwaide A (2012) When Hippocrates had a headache. New Sci 213:30–31

Treesukosol Y, Lyall V, Heck GL, DeSimone JA, Spector AC (2007) A psychophysical and electrophysiological analysis of salt taste in Trpv1 null mice. Am J Physiol Regul Integr Comp Physiol 292:R1799–R1809

Trevisan G, Rossato MF, Walker CI, Klafke JZ, Rosa F, Oliveira SM, Tonello R, Guerra GP, Boligon AA, Zanon RB, Athayde ML, Ferreira J (2012) Identification of the plant steroid a-spinasterol as a novel transient receptor potential vanilloid 1 antagonist with antinociceptive properties. J Pharmacol Exp Ther 343:258–269

Turkyilmaz Z, Karabulut R, Sonmez K, Can Basaklar A (2008) A striking and frequent cause of premature thelarche in children: Foeniculum vulgare. In J Pediatr Surg 43:2109–2111

Turner J (2004) Spices. History of a temptation. Harper Collins, New York

Ursu D, Knopp K, Beattie RE, Liu B, Sher E (2010) Pungency of TRPV1 agonists is directly correlated with kinetics of receptor activation and lipophilicity. Eur J Pharmacol 641:114–122

Verspohl EJ, Bauer K, Neddermann E (2005) Antidiabetic effect of *Cinnamomum cassia* and *Cinnamomum zeylanicum* in vivo and in vitro. Phytother Res: PTR 19:203–206

Vogt-Eisele AK, Weber K, Sherkheli MA, Vielhaber G, Panten J, Gisselmann G, Hatt H (2007) Monoterpenoid agonists of TRPV3. Br J Pharmacol 151:530–540

Waluk DP, Vielfort K, Derakhshan S, Aro H, Hunt MC (2012) N-acyl taurines trigger insulin secretion by increasing calcium flux in pancreatic beta-cells. Biochem Biophys Res Commun PMID: 23159632

Wang P, Yan Z, Zhong J, Chen J, Ni Y, Li L, Ma L, Zhao Z, Liu D, Zhu Z (2012a) Transient receptor potential vanilloid 1 activation enhances Gut glucagon-like peptide-1 secretion and improves glucose homeostasis. Diabetes 61:2155–2165

Wang Y, Wang X, Yang Z, Zhu G, Chen D, Meng Z (2012b) Menthol inhibits the proliferation and motility of prostate cancer DU145 cells. Pathol Oncol Res: POR 18:903–910

Wanner SP, Garami A, Pakai E, Oliveira DL, Gavva NR, Coimbra CC, Romanovsky AA (2012) Aging reverses the role of the transient receptor potential vanilloid-1 channel in systemic inflammation from anti-inflammatory to proinflammatory. Cell Cycle 11:343–349

Watanabe T, Ohnuki K, Kobata K (2011) Studies on the metabolism and toxicology of emerging capsinoids. Expert Opin Drug Metab Toxicol 7:533–542

Wei J, Ching LC, Zhao JF, Shyue SK, Lee HF, Kou YR, Lee TS (2012) Essential role of transient receptor potential vanilloid type 1 in evodiamine-mediated protection against atherosclerosis. Acta Physiol PMID:23025809

Whiting S, Derbyshire E, Tiwari BK (2012) Capsaicinoids and capsinoids – a potential role for weight management? A systematic review of the evidence. Appetite 59:341–348

Wrangham R (2009) Catching fire: how cooking made us human. Basic Books, New York

Xu H, Delling M, Jun JC, Clapham DE (2006) Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 9:628–635

Yang D, Luo Z, Ma S, Wong WT, Ma L, Zhong J, He H, Zhao Z, Cao T, Yan Z, Liu D, Arendshorst WJ, Huang Y, Tepel M, Zhu Z (2010) Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension. Cell Metab 12:130–141

Yassaka RT, Inagaki H, Fujino T, Nakatani K, Kubo T, Mikoshiba K (2010) Enhanced activation of the transient receptor potential channel TRPA1 by ajoene, an allicin derivative. Neurosci Res 66:99–105

Yasumatsu K, Ogiwara Y, Takai S, Yoshida R, Iwatsuki K, Torii K, Margolskee RF, Ninomiya Y (2012) Umami taste in mice uses multiple receptors and transduction pathways. J Physiol 590:1155–1170

Ye L, Kleiner S, Wu J, Sah R, Gupta RK, Banks AS, Cohen P, Khandekar MJ, Bostrom P, Mepani RJ, Laznik D, Kamenecka TM, Song X, Liedtke W, Mootha VK, Puigserver P, Griffin PR, Clapham DE, Spiegelman BM (2012) TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. Cell 151:96–110

Yeon KY, Kim SA, Kim YH, Lee MK, Ahn DK, Kim HJ, Kim JS, Jung SJ, Oh SB (2012) Curcumin produces an antihyperalgesic effect via antagonism of TRPV1. J Dent Res 89:170–174

Yokoyama T, Ohbuchi T, Saito T, Sudo Y, Fujihara H, Minami K, Nagatomo T, Uezono Y, Ueta Y (2011) Allyl isothiocyanates and cinnamaldehyde potentiate miniature excitatory postsynaptic inputs in the supraoptic nucleus in rats. Eur J Pharmacol 655:31–37

Yoshida R, Ninomiya Y (2010) New insights into the signal transmission from taste cells to gustatory nerve fibers. Int Rev Cell Mol Biol 279:101–134

Yoshioka M, St-Pierre S, Drapeau V, Dionne I, Doucet E, Suzuki M, Tremblay A (1999) Effects of red pepper on appetite and energy intake. Brit J Nutr 82:115–123

Young RL, Sutherland K, Pezos N, Brierley SM, Horowitz MK, Rayner CK, Blackshaw LA (2009) Expression of taste receptor molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes. Gut 58:337–346

Yu Y, Zick S, Li X, Zou P, Wright B, Sun D (2011) Examination of the pharmacokinetics of active ingredients of ginger in humans. AAPS J 13:417–426

Yu T, Shah BP, Hansen DR, Park-York M, Gilbertson TA (2012) Activation of oral trigeminal neurons by fatty acids is dependent upon intracellular calcium. Pflugers Archiv: Eur J Physiol 464:227–237

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301

Zhang LL, Yan Liu D, Ma LQ, Luo ZD, Cao TB, Zhong J, Yan ZC, Wang LJ, Zhao ZG, Zhu SJ, Schrader M, Thilo F, Zhu ZM, Tepel M (2007a) Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. Circ Res 100:1063–1070

Zhang Z, Zhao Z, Margolskee R, Liman E (2007b) The transduction channel TRPM5 is gated by intracellular calcium in taste cells. J Neurosci 27:5777–5786

Zhang Y, Sreekrishna K, Lin Y, Huang L, Eickhoff D, Degenhardt C, Xu T (2011) Modulation of transient receptor potential (TRP) channels by Chinese herbal extracts. Phytother Res: PTR 25:1666–1670

Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, Yin Y, Wang C, Zhang T, Zhu D, Zhang D, Xu J, Chen Q, Ba Y, Liu J, Wang Q, Chen J, Wang J, Wang M, Zhang Q, Zhang J, Zen K, Zhang CY (2012) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 22:107–126

Zhong J, Pollastro F, Prenen J, Zhu Z, Appendino G, Nilius B (2011) Ligustilide: a novel TRPA1 modulator. Pflugers Archiv: Eur J Physiol 462:841–849

Zhu Z, Luo Z, Ma S, Liu D (2010) TRP channels and their implications in metabolic diseases. Pflugers Archiv: Eur J Physiol 461:211–223

Zhu ZY, Zhang LL, Wang PJ, Ma LQ, Wang LJ, Liu DY, Zhu ZM (2012) Activation of transient receptor potential vanilloid 1 inhibits RhoA/Rho kinase and improves vasorelaxation dysfunction mediated by high-fat diet in mice. Acta Academiae Medicinae Sinicae 33:600–605

Zsombok A (2013) Vanilloid receptors-do they have a role in whole body metabolism? Evidence from TRPV1. J Diabet Its Complications PMID: 23332888

Zweig S (1938) Conqueror of the seas: the story of Magellan (original title: Magellan. Der Mann und seine Tat). Fischer Verlag, Wien

Free Fatty Acid Receptors and Their Role in Regulation of Energy Metabolism

Takafumi Hara, Ikuo Kimura, Daisuke Inoue, Atsuhiko Ichimura, and Akira Hirasawa

Abstract The free fatty acid receptor (FFAR) is a G protein-coupled receptor (GPCR) activated by free fatty acids (FFAs), which play important roles not only as essential nutritional components but also as signaling molecules in numerous physiological processes. In the last decade, FFARs have been identified by the GPCR deorphanization strategy derived from the human genome database. To date, several FFARs have been identified and characterized as critical components in various physiological processes. FFARs are categorized according to the chain length of FFA ligands that activate each FFAR; FFA2 and FFA3 are activated by short chain FFAs, GPR84 is activated by medium-chain FFAs, whereas FFA1 and GPR120 are activated by medium- or long-chain FFAs. FFARs appear to act as physiological sensors for food-derived FFAs and digestion products in the gastrointestinal tract. Moreover, they are considered to be involved in the regulation of energy metabolism mediated by the secretion of insulin and incretin hormones and by the regulation of the sympathetic nerve systems, taste preferences, and inflammatory responses related to insulin resistance. Therefore, because FFARs can be considered to play important roles in physiological processes and various pathophysiological processes, FFARs

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have been targeted in therapeutic strategies for the treatment of metabolic disorders including type 2 diabetes and metabolic syndrome. In this review, we present a summary of recent progress regarding the understanding of their physiological roles in the regulation of energy metabolism and their potential as therapeutic targets.

Contents

1 Introduction

Metabolic diseases, such as diabetes and obesity, are a threat to global health and are one of the most important health problems that impact our quality of life at great expense to society. Obesity is associated with chronic inflammation and induces insulin resistance which in turn causes type 2 diabetes. Diabetes is as a metabolic disorder, the incidence of which has reached epidemic proportions: 150 million people worldwide are estimated to be afflicted by diabetes, and this number is expected to double within 20 years (Wild et al. 2004). Type 2 diabetes results from the body's inability to produce sufficient amounts of insulin and from resistance of the body's fat and muscle cells to insulin. Elevated blood glucose levels are a hallmark of diabetes. There is currently no cure for diabetes, although treatment

options using medicines and dietary approaches are available that can help patients to prevent symptoms. Unfortunately, these approaches have many limitations; therefore, the development of alternative therapeutic targets and new anti-diabetic agents is desirable.

Free fatty acids (FFAs) are an important energy source for most body tissues, and are categorized by the length of their aliphatic tails; short-chain fatty acids (SCFAs) have less than 6 carbons, medium-chain fatty acids have 6–12 carbons, and long-chain fatty acids have 12 or more carbons. In addition to functioning as an energy source, FFAs exhibit a broad range of function such as modulation of receptor signaling, gene expression and whole body fuel energy homeostasis under various physiological conditions (Hara et al. 2011). As physiological sensors of FFAs, members of the intracellular or nuclear lipid binding protein families including fatty acid binding proteins and peroxisome proliferator-activated receptors (PPARs) are considered functional receptors that contribute to the regulation of numerous physiological and pathophysiological processes (Chawla et al. 2001). However, because some of the physiological observations induced by FFAs cannot be explained by functions that are mediated through these sensors, other mechanisms, perhaps involving plasma membrane receptors, may be expected to mediate some of the biological processes of FFAs.

The human genome contains 800 G protein-coupled receptors (GPCRs) that belong to large gene families. GPCRs are seven-transmembrane receptors that activate heterotrimeric G proteins and thus provide a wide variety of therapeutic targets for numerous diseases (Lagerstrom and Schioth 2008). To date, approximately 350 GPCRs, with the exception of the olfactory receptors, have been characterized in the human genome using bioinformatics analyses based on their sequence similarities. Approximately 260 of these GPCRs have already been characterized as deorphaned receptors after identification of the respective endogenous ligand; however, approximately 70 orphan GPCRs remain (Civelli et al. 2013). Therefore, these receptors have yet to be explored as potential drug targets. Several groups, including our own, have identified a series of orphan receptor, which are activated by FFAs and its derivatives. To date, five free fatty acid receptors (FFARs) have received considerable attention as a result of their physiological importance in various biological processes (Table 1 and Fig. 1). Among the FFARs that have been identified, FFA1 (GPR40) and GPR120 are activated by medium- and long-chain FFAs, and GPR84 is activated by medium-chain FFAs. In contrast, FFA2 (GPR43) and FFA3 (GPR41) are activated by SCFAs (Hara et al. 2012; Ichimura et al. 2009). Therefore, each FFAR may act as a sensor for FFAs with selectivity for a particular FFA carbon chain length that is derived from food or food-derived metabolites. Physiological functions of FFARs have been reported to include the secretion of insulin and incretin hormones, adipocyte differentiation, anti-inflammatory effect, nerve activation, and taste preferences. Accordingly, these physiological functions of FFARs could be considered to regulate energy metabolism. Therefore, FFARs have received considerable attention as potential therapeutic targets for energy metabolism disorders. In this review, we focus on recent advances regarding the understanding of FFARs in conjunction with our observations.

Table 1 Characteristics of FFARs

Nomenclature	Natural agonist	Synthetic agonist	Antagonist	G-protein coupling	Expression	Physiological functions	Chromosomal location	mRNA and protein (human)
FFA1 (GPR40)	Medium to long FFAs	GW9508	GW1100	Gq/11	Pancreatic β-cell	Insulin secretion	19q13.1	NM_005303
	Conjugate linoleic acid	MEDICA16			Intestine	Incretin secretion		NP_005294
		Thiazolidinediones			Tongue	Fat preferences		(300 aa)
		TAK-875						
		AMG837, AMG1638, AMG6226,						
		TUG424, Compound 7						
FFA2 (GPR43)	Short chain FFAs	Propiolic acid	Compound 3 and 4 (orthosteric antagonist)	Gq/11	Adipose tissue	Leptin production	19q13.1	NM_005306
		Angelic acid		Gi/o		Adipogenesis		NP_005297
		Compound 1 and 2 (orthosteric agonist)				Lipolysis		(330 aa)
		Phenylacetamides 1 and 2 (allosteric agonist)			Intestine	Incretin secretion		
						Intestinal motility		
					Immune cells (neutrophil)	Inflammatory response		

FFA3 (GPR41)	Short chain FFAs	Compound 5	Compound 6	Gi/o	Intestine	PYY, GLP-1	19q13.1	NM_005304
			β-hydroxybutyrate	Gβγ	Sympathetic nerve system	Sympathetic activation		NP_005295 (346 aa)
GPR84	Medium chain FFAs	Diindolylmethane		Gi/o	Adipose tissue	Adiponectin	12q13.13	NM_020370
					Macrophage Microglia			NP_065103 (396 aa)
GPR120	Medium to long FFAs	NCG21		Gq/11	Intestine	GLP-1 secretion	10q23.33	NM_181745
	Grifolic acid (partial agonist)	Compound 8		β-arrestin	Adipocytes	Differentiation		NP_859529
		Compound 9			Macrophage Tongue	Anti-inflammatory Fat preferences		(377 aa)

Molecular, biochemical and functional characteristics of FFARs are shown

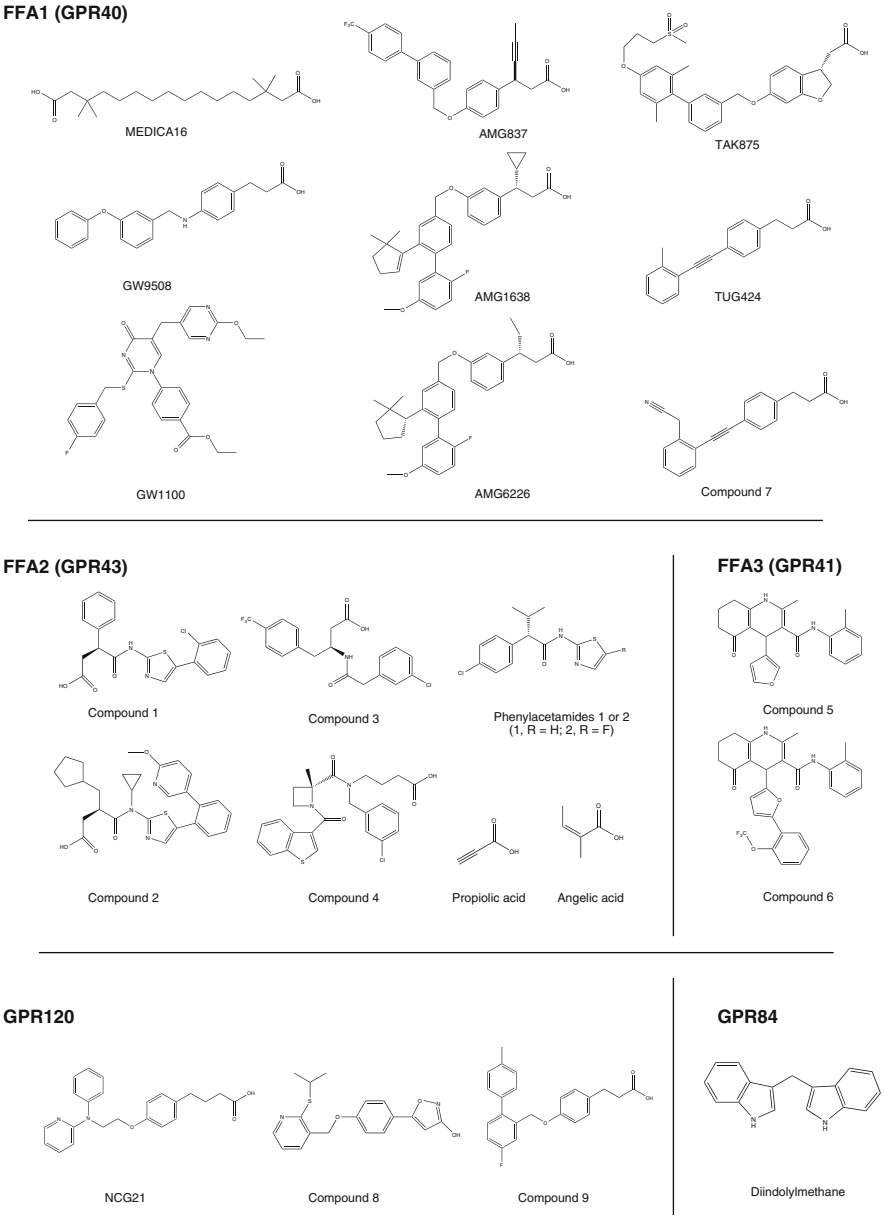

Fig. 1 Chemical structures of synthetic FFARs ligands. The name of each synthetic compound is also described in Table 1

2 FFA2 (GPR43)

2.1 Ligands

FFA2 was identified as a receptor for SCFAs and was de-orphaned in 2003. During a routine ligand bank screening with bioactive compounds, termed ligand fishing, it emerged that FFA2 was activated by acetate using Ca^{2+} mobilization assays in transfected mammalian cells (Brown et al. 2003; Nilsson et al. 2003). FFA2 was activated in vitro by physiological concentrations (micromolar range) of other SCFAs such as formate, propionate, butyrate, and pentanoate. The rank order of potencies with respect to activation of FFA2 was as follows: acetate = propionate > butyrate, whereas that of FFA3 was as follows: propionate > butyrate > acetate (Brown et al. 2003; Le Poul et al. 2003). The 100-fold lower activation potency of acetate to FFA3 can be used diagnostically to distinguish FFA3 from FFA2 (Brown et al. 2003). Interestingly, SCFA ligands showed different rank order of potency between species orthologs of FFA2 and FFA3 (Hudson et al. 2012). Acetate is selective for human FFA2 compared to human FFA3 however, this selectivity was not observed among the mouse orthologs. In addition, although propionate did not show selectivity between human orthologs, this ligand selectively activated mouse FFA3 compared to mouse FFA2. These results might be caused by high constitutive activity of human FFA3 and mouse FFA3 which was monitored by [^{35}S]-GTPγS binding assay. The molecular basis analysis suggested that the single negatively charged residues that were not conserved between species might regulate constitutive activity of FFA2 and FFA3.

2.2 Selective Compound

According to the physiological function of FFA2 (as described under Sect. 2.4), activation of colonic and adipose FFA2 by SCFA or pharmacological compounds is considered a promising therapeutic target for the treatment of obesity. Several selective agonists such as propiolic acid and angelic acid that showed 10–100-fold selectivity for human FFA2 compared to FFA3 were reported (Schmidt et al. 2011). A series of compounds that showed agonistic or antagonistic activities were reported as the patented compounds (Brantis et al. 2011; Hoveyda et al. 2010; Saniere et al. 2012; Ulven 2012). Compound 1 dose dependently increased glucose uptake in 3T3-L1 adipocyte cell line. On the other hand, Compound 2 increased GLP-1 secretion from NCI-H716 cell line. Further Compound 3 and 4 showed antagonistic activity for FFA2, with IC_{50} values of 20 and 100 nM, respectively. Since radiolabeled Compound 3 was displaced by natural agonist propionate, these synthetic compounds were considered to act as orthosteric ligands. Also, Phenylacetamide 1 and 2 are novel synthetic allosteric agonists for FFA2 that have pharmaceutical potential. Lee et al. (2008) demonstrated greater activation potency by phenylacetamides 1 and 2 compared with SCFAs by high-throughput screening

and showed that these compounds activate both G_q and $G_{i/o}$ pathways (Lee et al. 2008). These compounds may serve as useful tools for further elucidation of the physiological functions of the receptor and its involvement in various diseases. Results from in vivo studies using these compounds are eagerly awaited.

2.3 Signal Transduction

Although FFA2 and FFA3 have similar endogenous ligands, their respective G-protein signaling mechanisms differ: The pertussis toxin-sensitive $G_{i/o}$ pathway is activated by FFA2 and FFA3, whereas the $G_{q/11}$ pathway is activated by FFA2 (Le Poul et al. 2003). Stimulation of FFA2 by SCFAs resulted in inhibition of cAMP production; activation of the extra cellular regulated kinase 1/2 (ERK1/2) cascade through interactions with the $G_{i/o}$ family of G proteins; elevation of $[Ca^{2+}]_i$ and activation of the ERK1/2 cascade via interactions with the G_q family of G proteins. However, the physiological significance of this dual-coupled signaling mechanism associated with FFA2 is still unclear.

2.4 Expression and Physiological Functions

The expression of FFA2 in adipose and gastrointestinal tissues suggests that FFA2 may be involved in energy regulation (Sleeth et al. 2010). Reverse transcription polymerase chain reaction (RT-PCR) anatomical profiling in murine tissue revealed that *FFA2* was expressed in white adipose tissue and in the gastrointestinal tract (Hong et al. 2005). The presence of FFA2 in adipose tissues may imply a role in obesity and energy accumulation because *FFA2* mRNA is expressed in white adipose tissues such as subcutaneous, perirenal, epididymal, 3T3-L1-derived adipocytes, and mature adipocytes (Hong et al. 2005). The presence of FFA2 in the intestine may imply a role in appetite regulation because *FFA2* mRNA is expressed in both rat whole-wall and separated mucosa from the distal ileum and colon (Karaki et al. 2006). These findings were confirmed by Dass et al. (2007) and also reported for whole-walls of the human colon (Karaki et al. 2008). Moreover, recent quantitative real-time PCR anatomical profiling in murine tissue revealed that *FFA2* was abundantly expressed in immune cells such as neutrophils and in immune tissues such as the spleen and bone marrow (Maslowski et al. 2009). Therefore, FFA2 may be involved in various physiological processes (Fig. 2).

2.4.1 Adipose Tissues

FFA2 may be involved in obesity and related disorders such as metabolic syndrome. Metabolic syndrome is a combination of medical disorders and increases the risk of developing obesity-related cardiovascular disease and diabetes, impaired

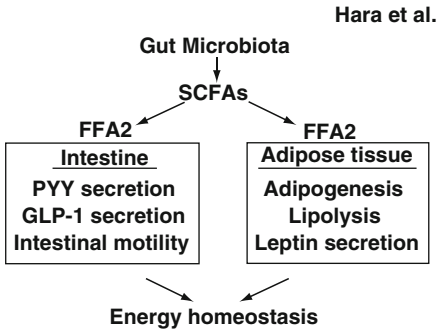

Fig. 2 Schematic diagram of the metabolic regulation by FFA2 via gut microbiota. SCFAs are produced in gut by bacterial fermentation of dietary fiber followed by the stimulation of FFA2 expressed in intestine and adipose tissues. FFA2 regulates incretin secretion (such as PYY and GLP-1) and motility in intestine. On the other hand, FFA2 also regulates adipogenesis, lipolysis and leptin secretion in adipose tissues. These functions induced by the FFA2 stimulation contribute to the energy homeostasis of the whole body

glucose tolerance, dyslipidemia, and hypertension (Grundy et al. 2004). Excess fat mass, characterized by both adipocyte hyperplasia and hypertrophy, is cause to exhibit the various symptoms of the metabolic syndrome. Adipose tissue is considered not only an energy reservoir for lipids and glucose but also a complex endocrine organ. It secretes hormones and inflammatory cytokines, some of which act as peripheral sensors for energy balance (Tilg and Moschen 2006).

2.4.2 Adipogenesis

Based on the *FFA2* expression in adipose tissues and adipocytes, Hong et al. (2005) performed a series of studies to elucidate the physiological role of FFA2 in adipocytes. They first showed that expression of *FFA2* in adipose tissues from high-fat diet-induced obesity mice was significantly greater than that of normal chow-fed mice. After induction of adipogenesis in 3T3-L1 cells, the expression of mRNA for *FFA2* and *Pparγ2* (a marker of mature adipocytes) was increased by treatment with the SCFAs, acetate and propionate. A reduction of *FFA2* mRNA by small interfering RNA (siRNA) in 3T3-L1 cells blocked adipogenesis. Collectively, these results suggested that activation of FFA2 by SCFA may be critical for the differentiation of adipocytes, resulting in promotion of fat accumulation.

2.4.3 Lipolysis

Following the ingestion of a meal, insulin stimulates the uptake of nutrients such as glucose into specialized tissues, and also potently inhibits lipolysis in adipocytes. However, cells that have become insulin resistant do not respond to this cue,

resulting in an outflow of lipids to plasma and ensuing dyslipidemia. FFA2 may exhibit the protective effect against this metabolic disruption by inhibiting lipid release from adipocytes. In 3T3-L1-derived adipocytes, acetate and propionate suppressed isoproterenol-induced lipolysis in a dose-dependent manner (Hong et al. 2005). Similarly, it was reported that the SCFAs, acetate and propionate, produced by long term intervention of resistant starch rich diet suppressed release of non-esterified fatty acid and glycerol from subcutaneous abdominal adipose tissues (Robertson et al. 2005). Moreover, Ge et al. (2008) demonstrated that these effects were mediated by FFA2 using *FFA2* knockout (KO) mice. It was reported that acetate produced a dose-dependent suppression of lipolysis and the release of glycerol in adipocytes from wild-type (WT) mice in vitro. Furthermore, intraperitoneal injection of sodium acetate to WT mice elicited a simultaneous peak in plasma acetate concentration, and instantly reduced plasma non-esterified fatty acids after injection in vivo (Ge et al. 2008). These effects were abolished in *FFA2* KO mice. Thus activation of FFA2 by SCFAs directly leads to inhibition of lipolysis and suppression of plasma FFAs.

2.4.4 Leptin Secretion

Activation of FFA2 by SCFAs potentially leads to the promotion of adipogenesis and inhibition of lipolysis, which may lead to the improvement of metabolic syndrome. This may partly explain the beneficial effects of dietary fiber supplementation on glucose control and dyslipidemia that have been observed in some studies (Chandalia et al. 2000). Another significant finding of SCFA receptor function in adipose tissues was reported by several groups. Brown et al. (2003) demonstrated the presence of FFA3 mRNA in human adipose tissue by quantitative RT-PCR. Xiong et al. (2004) reported that murine FFA3 was expressed in white adipose tissue and that propionate increased the concentration of leptin in the culture medium of primary white adipose tissue from mice. Further, leptin secretion was increased according to the overexpression of exogenous FFA3 and was decreased by siRNA-mediated knockdown of FFA3 in adipocytes. However, contradictory reports state that FFA3 expression has not been confirmed in murine adipose tissue (Hong et al. 2005; Kimura et al. 2011). Thus, Hong et al. (2005) reported that they could not detect FFA3 mRNA in differentiated 3T3-L1 cells nor in murine subcutaneous, perirenal, mesenteric, or epididymal fat pads, despite using the same PCR primers as those used in the study by Xiong et al. (2004). We were also unable to detect FFA3 mRNA expression in murine adipose tissues by quantitative RT-PCR or by in situ hybridization analysis (Kimura et al. 2011). In contrast, Hong et al. (2005) demonstrated the presence of FFA2 mRNA in murine adipose tissues, and Zaibi et al. (2010) reported that FFA2, rather than FFA3, was expressed in murine adipose tissue. They showed that acetate, rather than butyrate, stimulated leptin secretion in mesenteric adipocytes from WT mice but not in adipocytes from FFA3 KO mice, suggested that this effect was mediated by FFA2, whose expression was reduced in FFA3 KO mice compared with WT mice. Nevertheless, in the

presence of adenosine deaminase to suppress $G_{\alpha\ (i/o)}$ signaling through the adenosine A1 receptor, SCFA stimulated leptin secretion by adipocytes from WT but not FFA3 KO mice (Zaibi et al. 2010). Pertussis toxin prevented stimulation of leptin secretion by propionate in epididymal adipocytes, thus implicating $G_{\alpha\ (i/o)}$ signaling mediated by FFA2 in SCFA-stimulated leptin secretion (Al-lahham et al. 2010). Leptin is secreted from white adipose tissue and is a potent anorexigenic hormone and a long-term dynamic marker of body adiposity. This suggests that SCFA supplementation may also act on appetite through an FFA2-mediated response. Furthermore, to investigate the relationships between FFA3 expression and the effect of SCFAs on leptin secretion in adipose tissues, additional experiments using an FFA3-specific modulator, and/or adipose tissue-specific FFA3 KO mice are required.

2.4.5 Intestine

The intestines play a critical role in energy homeostasis because they are associated with nutrient absorption, and secretion of gut hormones that are involved in appetite control. Karaki et al. (2006) demonstrated that FFA2-expressing cells completely co-localize with peptide YY (PYY)-expressing enteroendocrine L cells of the gastrointestinal tract by immunohistochemistry (Karaki et al. 2006). Although both neural and hormonal factors stimulate the release of PYY, it is also likely that nutrients in the luminal environment induce secretion of this peptide and other peptides from L cells; for example, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) encoded by the proglucagon gene (Greeley et al. 1989). The expression of FFA2 in L cells in both rats and humans may suggest that activation of FFA2 by SCFA regulates PYY and GLP-1 secretion (Karaki et al. 2006). As the source of the SCFA, gut microbiota played important role in producing SCFA through fermentation processes (Owira and Winter 2008). In addition, colonic fermentation-derived SCFAs might regulate body weight and incidence of diabetes (Sleeth et al. 2010).

2.4.6 PYY Secretion

FFA2 which is reported to be expressed in enteroendocirne L cells, contributed to the secretion of PYY (Karaki et al. 2006, 2008).

2.4.7 GLP-1 Secretion

Enteroendocrine L cells are a major source of GLP-1 and GLP-2. These peptides are co-stored and co-secreted with PYY from enteroendocrine L cells (Kim et al. 2005). Therefore, SCFA may also stimulate L cell secretion of GLP-1 and other products of the pre-proglucagon gene through FFA2. Incretins are intestinal

hormones that act to increase insulin secretion. GLP-1 is a potent anorexigenic hormone and incretin. GLP-1 secretion by ileal L cells is dependent on the presence of nutrients in the lumen of the small intestine (Elliott et al. 1993). Acute intracerebroventricular administration of GLP-1 to fasted rats is associated with a decline in short-term energy intake (Turton et al. 1996) and a significant reduction in body weight in response to repeated administration (Davis et al. 1998). Moreover, GLP-1 as an incretin promotes insulin secretion through the GLP-1 receptor, which is expressed in pancreatic islets (Bullock et al. 1996). Studies of non-digestive fiber supplementation have indicated that administration of SCFA was associated with increased colonic proglucagon mRNA expression and increased circulating plasma GLP-1 levels (Delzenne et al. 2005; Keenan et al. 2006). It was reported that SCFAs resulted in the secretion of GLP-1 through FFA2 in mixed colonic cultures and in vivo. Quantitative PCR revealed enriched expression of FFA2 and FFA3 in GLP-1-secreting L cells, and SCFAs raised cytosolic Ca^{2+} through G_q signaling pathways in L cells in primary culture. Mice lacking FFA2 or FFA3 exhibited reduced SCFA-triggered GLP-1 secretion in vitro and in vivo and a parallel impairment of glucose tolerance (Tolhurst et al. 2012). Thus, the potential for dietary or pharmacological manipulation to increase satiety could be used in the treatment of obesity. Furthermore, with specific relevance to GLP-1, incretin action may be useful for potentiating insulin secretion in patients with type 2 diabetes. The anorexigenic neural circuits involving PYY and GLP-1 are subsequently activated to reduce food intake and increase energy expenditure to restore the body back to neutral energy balance. Hence, regulation of PYY and GLP-1 secretion through FFA2 may control energy intake and could be applied to the treatment of obesity and metabolic syndrome.

2.4.8 Intestinal Motility

Gastrointestinal motility enhances digestion and nutrient absorption, resulting in increased energy intake. Moreover, gastrointestinal motility may be partially controlled by FFA2. Recent findings have indicated that FFA2 may also affect the release of gastrointestinal 5-hydroxytryptamine (5-HT or serotonin). 5-HT, or serotonin, is a neurotransmitter in the central nervous system (CNS) known to modulate mood, behavior and appetite (Berger et al. 2009) because of co-localization with PYY. 5-HT is found peripherally in the gastrointestinal tract, primarily in enterochromaffin cells but also in 5-HT-containing mucosal mast cells (Kim and Camilleri. 2000). It is released in a dose-dependent manner in response to mechanical and chemical stimulation, including SCFA, during nutrient ingestion in the gut (Zhu et al. 2001; Fukumoto et al. 2003). It was reported that FFA2-expressing cells co-localize with 5-HT-containing mucosal mast cells of the rat distal ileum and colon and human colon (Karaki et al. 2006, 2008). Therefore, SCFA activation of FFA2 may mediate the release of 5-HT in the gut, thus constituting gastric motility-mediated appetite regulation that is independent of PYY.

3 FFA3 (GPR41)

3.1 Ligands

The FFA3 gene was found downstream of CD22 on human chromosomal locus 19q13.1 by PCR using degenerate primers based on conserved sequences in encoding transmembrane domains of the rat galanin receptor 1 (GALR1), GALR2, and human GALR1 (Sawzdargo et al. 1997). Several groups have performed screenings to identify ligands for FFA3. In 2003, two distinct groups reported that FFA3 can be activated by SCFAs, particularly by propionate, butyrate, and valerate (Brown et al. 2003; Le Poul et al. 2003). On the other hand, several synthetic compounds are reported as FFA3 agonist (Compound 5) or antagonists (Compound 6) (Leonard et al. 2006).

3.2 Signal Transduction

Brown et al. (2003) measured SCFA-induced GTPγS binding using HEK293T cells expressing human or rat FFA3. Le Poul et al. (2003) examined SCFA-mediated cAMP inhibition in CHO-K1 cells expressing human FFA3. The following year, Xiong et al. (2004) confirmed these results using human FFA3-expressing *Xenopus melanophore* cells. Le Poul et al. (2003) also showed that SCFAs induced the release of $[Ca^{2+}]_i$ and the phosphorylation of ERK1/2. Both these responses were completely abolished by pertussis toxin (PTX) treatment, suggesting the unique coupling of FFA3 to $G_{\alpha(i/o)}$.

3.3 Expression and Physiological Functions

3.3.1 Adipose Tissue

The involvement of FFA3, which was expressed in adipose tissue, in the release of leptin is still a lot of debate. To understand the physiological function of FFA3 related to leptin secretion, the study using the adipose tissue-specific FFA3 KO mice might be required (refer to Sect. 2.4.4 in paragraph of FFA2).

3.3.2 Intestine

FFA3 is reported to regulate host energy absorption by modulating gut motility. Samuel et al. (2008) showed that quantitative RT-PCR was used to demonstrate that FFA3 mRNA is expressed in the distal small intestine and colon of the mouse.

Tazoe et al. (2009) found that human FFA3 is also expressed in PYY-containing enteroendocrine cells, and Samuel et al. (2008) used in situ hybridization analysis to demonstrate that FFA3 mRNA is expressed in enteroendocrine cells. Samuel et al. (2008) showed that the body weight and fat pad weight of FFA3 KO mice were significantly lower than those of WT mice and that these differences were abolished in germ free mice. In addition, fasting plasma leptin level was reduced in FFA3 KO mice compared to WT mice raised in conventional condition. Co-colonization of human gut-derived *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii* in germ-free mice led to significantly increased levels of circulating PYY, which regulates gut motility. This effect was significantly suppressed in FFA3 KO littermates, although FFA3 deletion did not affect food intake. These results indicated that the function of FFA3 depends on SCFAs produced by bacterial fermentation. Also, the intestinal transit rate was significantly faster in FFA3 KO mice compared with WT littermates, and this effect was abolished in germ-free mice. The fecal content of SCFA in FFA3 KO mice was significantly higher than of WT mice. These results led the authors to suggest that the deceased PYY levels in FFA3 KO mice increased gut motility, leading to reduced SCFA absorption as an energy source, which resulted in the lean phenotype (Samuel et al. 2008). In contrast, Bellahcene et al. (2012) reported that male but not female FFA3 KO mice have a higher body fat mass than their WT littermates when they are fed with either a low- or high-fat diet. Genotype had no effect on the amount of food intake by either sex regardless of maintenance on a low- or high-fat diet. This included mice of the same age (10 weeks) as those used in the report by Samuel and co-workers (2008). This observation was supported by Zaibi et al. (2010) stated that FFA3 KO mice exhibit obesity in an unpublished observation. In the high-fat-fed condition, male but not female FFA3 KO mice had lower energy expenditure than WT mice (Bellahcene et al. 2012). Low fat diet-fed male FFA3 KO mice also displayed lowered liver TG and plasma FFA concentrations, elevated plasma adiponectin and impaired glucose tolerance. On the other hand, HFD-fed male FF3 KO mice displayed elevated plasma glucose and leptin and reduced lean body mass. The differences in the effects of sex hormones on adipose tissue distribution and on the central regulation of metabolism could explain why female FFA3 KO mice have comparable energy expenditure to WT mice. Nevertheless, we cannot exclude the possibility that reduced SCFA absorption by increased gut motility alleviated obesity in FFA3 KO mice used in the study by Bellahcene et al. (2012). Further, energy expenditure was reduced in male but not female FFA3 KO mice compared to WT mice, which might be caused by reduced sympathetic activity (refer to Sect. 3.3.3). Therefore, the obese phenotype in male FFA3 KO mice could be explained by reduced energy expenditure. Consistent with reports by Samuel et al. (2008) and Tazoe et al. (2009), Tolhurst et al. (2012) demonstrated FFA3 mRNA in intestinal L cells, which secrete PYY and GLP-1. Glucose-stimulated GLP-1 secretion was reduced in primary colonic cultures obtained from FFA3 KO mice compared with cultures from WT mice. Consistent with these findings, oral glucose tolerance was impaired in FFA3 KO mice (Tolhurst et al. 2012). Blunted GLP-1 secretion in response to SCFA in colonic cultures from FFA3 KO mice was

suggested to result from the reduced expression of FFA2 because SCFAs stimulated the release of GLP-1 even when $G_{i/o}$ protein signaling was inhibited by pretreatment with PTX.

3.3.3 Sympathetic Nervous System (SNS)

FFA3 regulates host energy balance by modulating sympathetic nerve activity. In 2011, we reported that murine FFA3 mRNA was abundantly expressed in sympathetic ganglia in murine tissues from the embryonic to the adult stage and in human sympathetic ganglia. This was confirmed by in situ hybridization and quantitative RT-PCR analysis (Kimura et al. 2011). FFA3 KO mice exhibited a significantly reduced density of sympathetic innervation and a reduced level of tyrosine hydroxylase (TH; rate limiting enzyme for catecholamine biosynthesis) protein in the heart, suggesting that FFA3 is involved in sympathetic nerve growth. However, further studies are needed to clarify the role of FFA3 in sympathetic nerve differentiation and growth. In adult WT or FFA2 KO mice, energy expenditure was reflected by propionate-induced increase in heart rate and oxygen consumption, whereas these effects were abolished in FFA3 KO mice. The effect of propionate on heart rate is inhibited by pretreatment with the β-adrenergic receptor blocker propranolol but not by the nicotinic acetylcholine receptor blocker hexamethonium. This indicates that propionate activates SNS through FFA3 at the ganglionic level (Kimura et al. 2011). This function of FFA3 in sympathetic ganglia is consistent with the lower energy expenditure and obese phenotype of FFA3 KO mice reported by Bellahcene et al. (2012). We further clarified the signaling mechanism for sympathetic activation using primary cultured murine sympathetic ganglion neurons. Pharmacological and knockdown experiments showed that propionate increased the release of tritium-labeled noradrenaline from sympathetic neurons through the FFA3-$G_{\beta\gamma}$-phospholipase C (PLC) β3-ERK1/2-synapsin 2 pathway (Kimura et al. 2011; Inoue et al. 2012). The synapsins are a family of synaptic vesicle-associated phosphoproteins. Serine 426 of synapsin 2b is phosphorylated by activated ERK1/2 in response to propionate stimulation in murine sympathetic neurons (Inoue et al. 2012).

During assessment of the effects of SCFAs and ketone bodies in FFA3-expressing HEK293 cells, we found that β-hydroxybutyrate (β-HB) had a potent antagonistic effect on FFA3-mediated ERK1/2 phosphorylation and cAMP inhibition (Kimura et al. 2011). β-HB is a ketone body, which can be produced in the liver under ketogenic conditions such as fasting, low-carbohydrate dietary intake, and diabetes. In contrast, another major ketone body, acetoacetate, had no significant effect. We further showed that β-HB suppressed propionate-induced sympathetic activation in primary cultured sympathetic neurons, and in mice (Kimura et al. 2011; Inoue et al. 2012). These findings suggest that FFA3 functions as an energy sensor in sympathetic ganglia to maintain energy homeostasis (Fig. 3). Under fed conditions, SCFAs are produced in the gut by bacterial fermentation of dietary fiber. SCFAs activate SNS by stimulating FFA3, leading to an increase in energy expenditure to consume excess

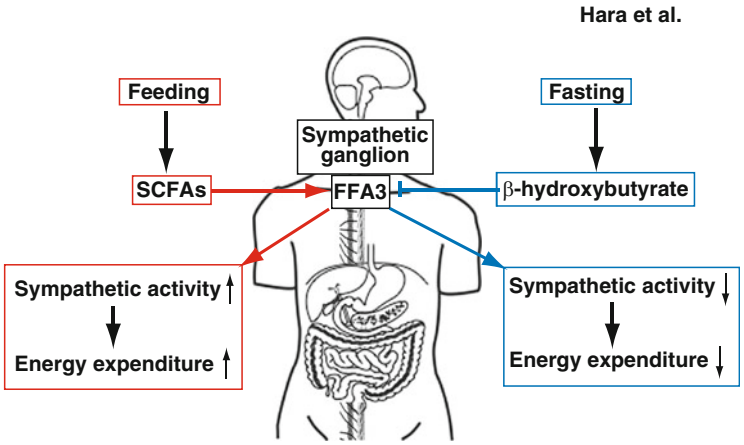

Fig. 3 Schematic diagram of the metabolic regulation by FFA3 on sympathetic ganglion. Under fed condition, SCFAs are produced in gut by bacterial fermentation of dietary fiber followed by the increase of sympathetic activity through FFA3 expressed in sympathetic ganglion. Sympathetic activation leads to the increase in energy expenditure. On the other hand, under fasting condition, β-hydroxybutyrate is produced as energy sources in liver and decreases sympathetic activity through inhibition of FFA3 in sympathetic ganglion. Accordingly, β-hydroxybutyrate suppresses energy expenditure through FFA3

energy. In contrast, β-HB is produced in the liver under fasted conditions. β-HB suppresses SNS by inhibiting FFA3, leading to a reduction in energy expenditure to preserve energy. Thus, these monocarboxylic metabolites appear to control energy balance by directly regulating FFA3-mediated sympathetic activation at the ganglionic level.

4 FFA1 (GPR40)

4.1 Ligands

In 2003, three groups almost simultaneously reported that medium to long-chain FFAs activated FFA1 by monitoring either $[Ca^{2+}]_i$ or by reporter assay (Briscoe et al. 2003; Itoh et al. 2003; Kotarsky et al. 2003). Since FFAs are involved in various physiological properties, stably expressing cell lines were primarily used in high-throughput screening to examine the effects of FFAs on FFA1. Numerous FFAs were reported to act as agonists for FFA1 in the micromolar concentration range, and the rank order of the agonistic activities of FFA ligands measured by $[Ca^{2+}]_i$ response were as follows: docosahexaenoic acid (DHA, C22:6) > α-linolenic acid (α-LA) (C18:3) = oleic acid (C18:1) > palmitic acid (C16) = lauric acid (C12) > capric acid (C10) > caprylic acid (C8). These results indicated

that both saturated and unsaturated FFAs could be agonists for FFA1. Carbon chain length was important for agonistic activities of saturated fatty acids but not for unsaturated fatty acids (Itoh et al. 2003). Interestingly, SCFA such as acetic acid (C2), butyric acid (C4), caproic acid (C6), and methyl linoleate did not show any response, suggesting that carbon chain length and the presence of carboxylate group in the structure appeared to be critical for the activation of FFA1. In addition to the essential FFAs, several compounds of clinical interest such as conjugated linoleic acid, which is known as a dietary component associated with anticarcinogenic effects, and 9-hydroxyoctadecadienoic acid (9-HODE), which is associated with arteriosclerosis, were revealed to activate FFA1 in a dose-dependent manner.

4.2 Selective Ligands

The physiological functions of FFARs appear to be strongly related to metabolic energy regulation such as glucose homeostasis, and secretion of insulin and incretins; thus, studies to develop potent and selective agonists are of global interest. Several research groups including pharmaceutical companies have reported novel series of FFA1 agonists, which were evaluated by in vitro or in vivo studies (Bharate et al. 2009; Garrido et al. 2006; Hu et al. 2009; Humphries et al. 2009; Zhang et al. 2010; Zhou et al. 2010). In particular, an FFA1 selective ligand, GW9508, was used in various studies as a reference compound for FFA1. GW9508 showed 100-fold more potent agonistic activity for FFA1 compared with GPR120. In addition, we reported that PPARγ agonists (thiazolidinediones), also known as antidiabetic drugs, and the experimental anti-obesity compound MEDICA16 could be FFA agonists (Briscoe et al. 2006; Hara et al. 2009b). Furthermore, we developed a synthetic ligand, NCG75, by *in silico* docking simulation using the FFA1 homology model that showed strong activation of ERK1/2 and $[Ca^{2+}]_i$ response. NCG75 promoted insulin secretion from MIN6 (murine insulinoma) cells, which express endogenous FFA1. The potent synthetic compound, TAK-875, has recently entered clinical trials (refer to Sect. 4.8). Results from in vitro and in vivo studies showed that TAK-875 enhanced glucose-stimulated insulin secretion (GSIS) in a glucose-dependent manner in β-cells. The stimulatory effect of TAK-875 was correlated with the $[Ca^{2+}]_i$ response without stimulation of glucagon secretion. Therefore, TAK-875 may be useful as a treatment to control plasma glucose levels without the risk of developing hypoglycemia and β-cell toxicity (Yashiro et al. 2012; Tsujihata et al. 2011). As a partial agonist, AMG837 was identified by optimization of a series of β-substituted phenylpropanoic acids with an EC_{50} value of approximately 0.1 μM (Houze et al. 2012). TAK-875 and AMG837 could act as antihyperglycemic agents, however these two compounds did not improve incretin levels in vivo. Therefore, to investigate novel class of agonist which might improve both insulin and incretin level, chemical modification of AMG837 was conducted and led to the discovery of the potent full agonist AM1638 and AM6226 (Luo et al. 2012). These compounds

stimulated GLP-1 and GIP secretion and increase GSIS. On the other hand, known FFA1 ligands showed lipophilicity responsible for the poor pharmacokinetic properties and toxicity. Christiansen et al. (2013) reported that a novel compound (Compound 7) was developed based on the structure of TUG424 reported as potent agonist with moderate metabolic stability in vitro (Christiansen et al. 2008). This compound showed low lipophylicity, high selectivity, marked bioavailability and efficacy on glucose tolerance. As a result, these compounds are expected to be useful in the future clinical trials and the investigation of FFA1.

4.3 Signal Transduction

Various reports have confirmed that FFAR stimulation promoted the release of $[Ca^{2+}]_i$ and ERK1/2 phosphorylation in cells that both transiently and stably expressed FFA1 (Itoh et al. 2003). The increase in $[Ca^{2+}]_i$ was examined in MIN6 cells and also in primary pancreatic β-cells. Furthermore, since the $[Ca^{2+}]_i$ and ERK1/2 responses did not promote cAMP accumulation, FFA1 could be coupled to a G-protein α-subunit of the G_q family but not from the $G_{i/o}$ or G_s family. Moreover, an endogenous agonist such as linoleic acid promoted PLC activation by the G_q protein through FFA1 expressed in HEK293 cells (Salehi et al. 2005). In contrast, agonist-induced inhibition of voltage-gated K^+ current in pancreatic β-cells could be mediated by cAMP signaling leading to an increase in excitability (Fujiwara et al. 2005; Zhao et al. 2008). Recently, the precise mechanisms of FFA-induced GSIS were further analyzed using islets from FFA1 KO mice. Oleate-induced GSIS in an FFA1-dependent manner and rapid F-actin remodeling was observed in islets from WT but not from KO mice. Moreover, protein kinase D (PKD) phosphorylation induced by oleate stimulation was also observed in WT but not in KO mice. Furthermore, pharmacological inhibition of PKD1 prevented oleate-induced GSIS and F-actin depolymerization. Hence, these results indicated that the signaling pathways leading to insulin secretion in pancreatic islets could be involved in F-actin depolymerization and PKD1 activation (Ferdaoussi et al. 2012). Two different groups reported mechanisms that could regulate the expression levels of FFA1 in pancreatic islet. The paired box 6 (PAX6) protein, a known transcription factor in pancreatic α-cells, could interact with the promoter region of the FFA1 gene which contribute to regulate the expression of FFA1 (Gosmain et al. 2012). On the other hand, pancreatic duodenal homeobox-1 (PDX-1) is involved in glucose-induced FFA1 gene transcription in pancreatic β-cells. Interestingly, PI3K-dependent O-GlcNAcylation of PDX-1 mediated by the association of O-GlcNAc transferase to phosphatidylinositol 3,4,5-triphosphate (PIP_3) is crucial process of regulating genes related to glucose metabolism including FFA1. Therefore, as the compensation mechanism, stimulation of FFA1 gene expression might enhance FFA1 signaling under the condition of excess nutrients, which contribute to normalize blood glucose level (Kebede et al. 2012).

In our previous reports, we mentioned a potential discrepancy between agonist-induced intracellular signaling (ERK1/2 phosphorylation or [Ca] response) and effects on insulin secretion. The selective agonists, NCG75, GW9508, and TAK-875 have been described as potent because they can activate intracellular signalings at lower concentrations compared with the endogenous ligands such as oleic acid, linoleic acid and α-LA however, the effects of these compounds on insulin secretion in the cells that express endogenous FFA1 (INS-1 or MIN6 cells) were similar to those of endogenous ligand (Tsujihata et al. 2011; Briscoe et al. 2006; Takeuchi et al. 2013). In previous studies, insulin secretion was shown to be regulated by the multiple signaling pathways including Ca and ERK (Longuet et al. 2005; Selway et al. 2012). Therefore, FFAs-induced insulin secretion was appeared to be activated by multiple pathways. Together, these discrepancies may be explained by contribution of another signaling mechanism, which could regulate insulin secretion. Further research to address the relationship between signaling pathways and physiological functions are required to investigate the precise mechanisms of FFA1.

4.4 Expression and Physiological Functions

The expression profiles of FFA1 were determined in various tissues by reverse-transcription polymerase chain reaction, immunohistochemistry, and in situ hybridization.

4.4.1 Pancreas

Expression analysis of FFA1 by RT-PCR and immunohistochemistry revealed high levels of expression in insulin-producing pancreatic islet cells (Itoh et al. 2003; Tomita et al. 2006). Itoh et al. reported that the activation of FFA1 by FFA ligands enhanced GSIS from pancreatic β-cells. Several research groups reported a protective effect of FFA1 on β-cells against GSIS using KO and/or transgenic (TG) mice. In addition, FFA1 protein was detected in the periphery of murine islet ⟨-cells and hamster glucagonoma cells (Flodgren et al. 2007). The FFA1 KO mice fed with a regular chow diet did not show any differences in body weight, fasting glucose level, and insulin, triglyceride, and glucose tolerance compared with WT mice. However, KO mice and WT littermates who were fed with high fat diet (HFD) showed fasting hyperglycemia, obesity, glucose intolerance, and insulin resistance. The levels of insulin secretion in response to a mixture of mostly unsaturated fatty acids were reduced by approximately 50 %. Furthermore, although the effects of glucose stimulation on insulin secretion in isolated islets from KO mice were unchanged, fatty acid stimulated-induced insulin secretion was significantly reduced (Lan et al. 2008; Kebede et al. 2008; Latour et al. 2007). Moreover, knockout of FFA1 in mice did not contribute to glucose intolerance,

hyperglycemia, and hypertriglyceridemia. Several groups reported the results of FFA1 TG mice studies. FFA1 overexpression under the mouse PDX-1 promoter exhibited impaired β-cell function (Steneberg et al. 2005). However, other groups could not confirm these observations. FFA1 overexpression under the mouse insulin II promoter did not impair metabolic status, whereas the levels of fasting blood glucose was lower than that of WT mice. Also, insulin secretion and oral glucose tolerance were improved in FFA1 TG mice without affecting insulin tolerance (Nagasumi et al. 2009). These discrepancies were partly explained by differences of the expression levels of FFA1 in each TG mouse and of the promoter subjected to construct TG mice (Alquier and Poitout 2009). Together, these findings indicate that FFA1 contributes to GSIS and the regulation of basal energy metabolism.

4.4.2 Intestine

Edfalk et al. (2008) reported that FFA1 is expressed in endocrine cells of the gastrointestinal tract, including cells expressing the incretin hormones GLP-1 and GIP such as the intestinal L and K cells, respectively. Since FFA1 mediates FFA-induced incretin secretion, the effects of FFAs on insulin secretion are possibly mediated by both direct (through the activation of FFA1 expressed on β-cells) and indirect (through the release of incretin hormone) pathways. Furthermore, FFA1 expression was also confirmed in I cells in the intestine that secretes cholecystokinin (CCK) (Liou et al. 2011). Therefore, FFA1 modulates FFA-induced insulin secretion from β-cells directly and indirectly through regulation of incretin secretion (Edfalk et al. 2008; Latour et al. 2007) (Fig. 4).

4.4.3 Taste Buds

Immunohistochemistry of circumvallate papillae (CV) section using antibodies against FFA1 and GPR120 showed positive signals in type I and type II taste bud cells, respectively. These signals were eliminated in taste tissue from GPR40 KO and GPR120 KO mice (Cartoni et al. 2010). Studies using both FFA1 and GPR120 KO mice revealed that taste nerve responses to fatty acids and preferences for fatty acids, evaluated by short-access tests using a lick meter comparing linoleic acid and xanthan gum, were inhibited in KO mice. There was no difference in preference for other tastes such as bitter, sweet, sour, salty, or umami by FFA1 KO and GPR120 KO mice compared with WT mice. However, recent publication did not confirm the expression of FFA1 in CV, fungiform papillae and taste cell-free lingual epithelium by RT-PCR (Galindo et al. 2012). Matsumura et al. (2007) also reported that GPR120 but not FFA1 could detect in the epithelium of the circumvallate papillae. Therefore, the expression of FFA1 in sensory taste cells is quite controversial. Further research should be necessary to clarify the precise expression profiles of FFA1 and GPR120 in sensory taste cells.

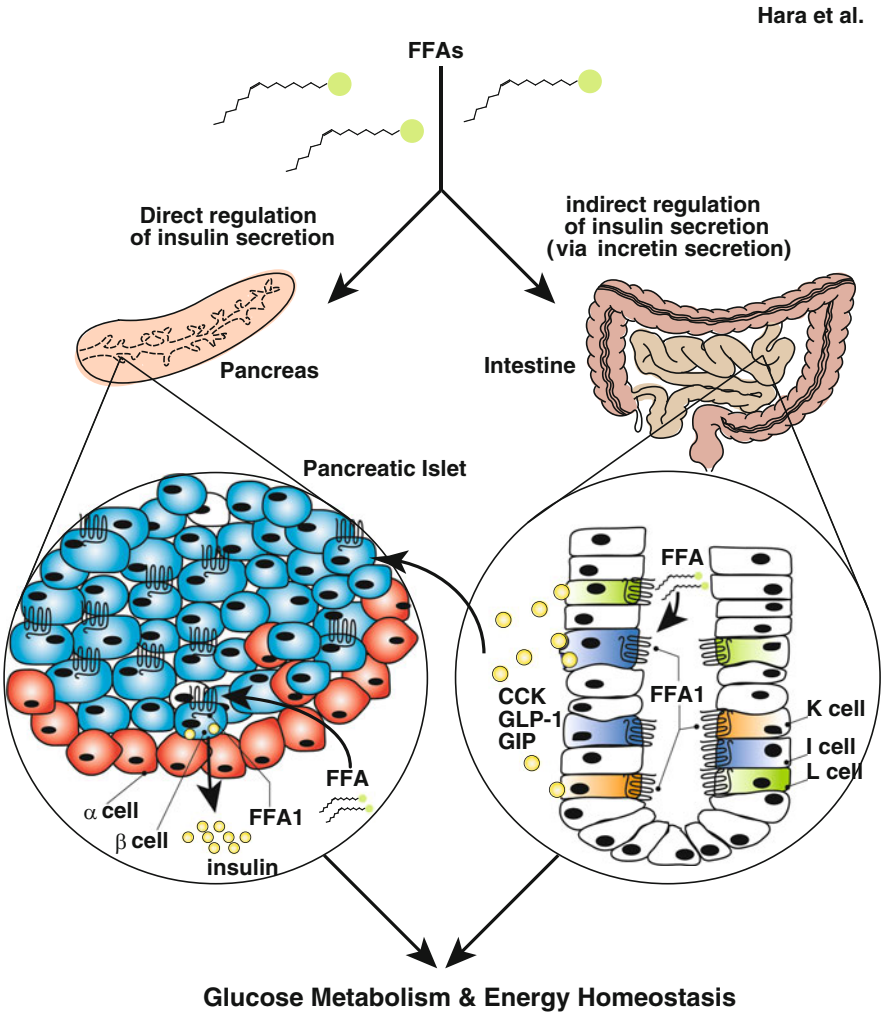

Fig. 4 Schematic diagram of physiological functions of FFA1 in glucose metabolism and energy homeostasis. FFAs stimulate FFA1 expressed in pancreatic β-cells and promote insulin secretion in direct mechanisms. FFAs also stimulate FFA1 expressed in incretin secreting cells and promote insulin secretion in indirect mechanisms. FFA1 partly modulates FFAs-induced insulin secretion from β-cells not only directly but also indirectly mechanisms

4.4.4 Brain

Ma et al. confirmed the expression and distribution profiles of FFA1 in the central nervous system of adult monkeys by immunoblotting and immunohistochemistry. Furthermore, FFAs such as docosahexaenoic acid that could activate FFA1 may play an important role in the regulation of adult hippocampal neurogenesis in primates (Yamashita 2008; Ma et al. 2010).

4.4.5 Mammary Gland

Yonezawa et al. detected FFA1 mRNA in MCF-7 human breast cancer cells, bovine mammary epithelial cells, and bovine mammary gland. These were activated by oleate and linoleate and may be involved in a proliferative effect through $[Ca^{2+}]_i$ response, ERK1/2, and Akt kinase (Yonezawa et al. 2008).

4.5 Polymorphism

Genetic polymorphisms that alter protein expression or function can modify the risk of disease. Polymorphisms have been reported for FFA1: in humans, several single nucleotide polymorphisms identified in the FFA1 coding region are reportedly associated with the function of FFA1. The Gly180Ser polymorphism that was identified in a screening analysis of 734 subjects showed significantly lower levels of insulin secretion in Gly/Gly carriers. In addition, Gly/Ser carriers showed lower levels of plasma insulin and C-peptide in response to a lipid load in a case-control study. Furthermore, these results are supported by an in vitro study in which the Gly/Ser mutant expressed in HeLa cells showed a lower $[Ca^{2+}]_i$ response induced by oleic acid compared with the Gly/Gly mutant (Vettor et al. 2008). The His211Arg polymorphism is considered to be associated with the altered insulin secretion capability and metabolic parameters in Danish Caucasian subjects and Japanese men. In Danish Caucasian subjects, the Arg211His polymorphism showed a similar allele frequency between type 2 diabetic patients and middle-aged glucose-tolerant subjects. Moreover, the levels of insulin release determined by oral glucose tolerance test showed no significant difference between these carriers. Similarly, allele frequencies of this variant in Japanese subjects showed no difference between type 2 diabetic patients and healthy subjects. However, Arg/Arg homozygotes showed a significantly lower homeostasis model of insulin resistance (HOMA-IR) and ®-cell function (HOMA-b) together with serum insulin levels compared to His/His genotypes. In addition, a rare mutation, Asp175Asn was identified during the analysis of Danish Caucasian subjects. The effects of these two variants, Arg211His and Asp175Asn, on inositol phosphate production in response to 5,8,11-eicosatriynoic acid were similar to each other; however, Asp175Asn, but not Arg211His, showed lower maximal efficacy compared to the WT. Further studies addressing the relationships between FFA1 and other genes that control metabolic energy regulation within different ethnicities are required to understand the contribution of these polymorphisms to diabetes and modulation of the function of the FFA1 protein (Hamid et al. 2005; Ogawa et al. 2005).

4.6 Structure–Activity Relationships (SARs)

FFA1 is reportedly involved in the activation of insulin release and as such is considered an attractive therapeutic target for the treatment of diabetes. Thus, numerous groups have engaged in the development of potential lead compounds as ligands for the receptor. A deep understanding of the molecular details of ligand recognition and receptor activation is useful for the identification and rational design of ligands that show biological activity and selectivity for a specific receptor. Numerous groups including our own have attempted to develop candidate compounds using docking simulation with homology models for FFA1. Findings have included information concerning the chemical structure of compounds and also the binding mode in the binding cavity of FFA1. The ionic lock at the cytoplasmic surface is known to play an important role in the activation mechanism of class A GPCRs including FFARs. However, the contribution of agonist binding to alteration of the ionic locks remains unclear. The ionic locks in FFA1 are predicted by homology modeling to be located between the second extracellular loop of FFA1 (Glu145 and Glu172) and transmembrane domain residues Arg183 and Arg258. Agonist interaction-induced dissociation of the ionic interactions between Glu–Arg was predicted by simulation of molecular dynamics. Furthermore, the constitutive activation of FFA1 induced by the breakage of these interactions was observed in mutagenesis studies. Therefore, these ionic locks may act as a molecular switch toward receptor activation (Sum et al. 2009). Among the FFA1 agonists, linoleic acid known as a natural polyunsaturated FFA ligand and GW9508 were subjected to examine the molecular determinants of agonist binding to FFA1. Hydrophobic, aromatic and hydrophilic/positively charged amino acid residues were predicted to be potential binding residues in the binding cavity of FFA1. A mutagenesis study revealed that Arg183, Asn244, and Arg258 contributed to the interaction between the carboxylate group present in the structures of linoleic acid and GW9508. In addition, His86, Tyr91, and His137 contributed to the aromatic or hydrophobic interactions in GW9508 binding. Moreover, His147 and His86 may contribute to GW9508-induced receptor activation. Hence, these results may explain why GW9508 could strongly activate FFA1 signaling compared with other FFA ligands (Tikhonova et al. 2007; Sum et al. 2007). Our group also conducted *in silico* docking simulations to search for selective agonists and to investigate the binding modes of these ligands. Consistent with other reports, Arg183 and Arg258 were found to be important for agonist recognition and activation of FFA1. In contrast, our synthetic agonist, NCG75 (refer to section titled “Selective ligand”), and α-LA, a natural agonist, showed a different binding profile compared with GW9508. Thus, Arg 258 and not a combination of Arg183 and Arg258, was deemed to be important for FFA1 activation. This would explain the prediction that the hydrogen bond distance between the ligand and the amino acid residues may reflect agonistic potency. In addition, we confirmed the contribution of other amino acid residues to ligand interaction; His86, Phe87, and Tyr240 formed interactions with pyridine and phenyl ring of

NCG75; Val141, Ala146, and Ala173 stabilized binding through hydrophobic interactions. These findings concerning the critical roles of these residues for ligand interaction are useful for investigating the pharmacology of FFA1 and for further development of potent and selective ligands (Takeuchi et al. 2013).

4.7 Binding Assay

Experimental systems that can monitor direct interactions between ligands and FFARs have not yet been developed because of the relatively weak affinity of natural ligands for their receptors and the lack of potent ligands. Therefore, an experimental system was established using flow cytometry that could detect direct interactions between ligands and FFA1 (Hara et al. 2009a). We designed a fluorescent-labeled ligand (BODIPY-labeled FFA) as a probe, which could activate FFA1. However, we were forced to isolate the FFA1 protein by immunoprecipitation as a result of non-specific binding of the fluorescent probe to cells overexpressing FFA1. Sf9 cells expressing FLAG-tagged FFA1 were solubilized. The lysates containing FFA1 protein were immobilized using immunomagnetic beads and were detected by flow cytometry as the cells that express FFA1. Flow cytometry-based binding assays revealed that fluorescent-labeled FFA specifically interacted with its binding site located on FFA1 in a saturable manner. In addition to the FFA ligands, synthetic ligands including GW9508, MEDICA16, and thiazolidinediones competed with the fluorescent-labeled ligand and bound to the FFA1 protein in a dose-dependent manner. A novel agonist that shows agonistic activity at nanomolar concentrations has recently been used to monitor direct interactions in a conventional radioligand binding assay. This radiolabeled compound is currently unavailable commercially. Therefore, further efforts to develop potent ligands could benefit from successful monitoring of direct interaction with FFA1 or other FFARs. Moreover, these ligands will be useful for further investigation of the physiological and pharmacological functions of FFA1.

4.8 Clinical Trials

The effect of a single oral dose of TAK-875 was evaluated for safety, tolerability, pharmacokinetics, and pharmacodynamics in a phase I, double-blind, placebo-controlled study in healthy volunteers (Naik et al. 2012). The results indicated that TAK-875 appears to be safe and tolerated in healthy subjects after a single oral administration. Two phase II, multicenter, randomized, double-blind, parallel group studies were conducted in Japanese patients with type 2 diabetes. Efficacy and tolerability were demonstrated after administration of TAK-875 for 2 weeks (Araki et al. 2012). In addition, the results of a 12-week dose-ranging study of TAK-875 indicated that TAK-875 showed effective antihyperglycemic properties without severe adverse effects in type 2 diabetes patients whose symptoms were not

adequately controlled by diet and exercise (Kaku et al. 2013). Furthermore, a phase II, randomized, double-blind, placebo-controlled, and active comparator-controlled trial was conducted in type 2 diabetes patients who were non-responders to diet or metformin treatment (Burant et al. 2012). The effect of once daily administration of TAK-875 for 12 weeks revealed an improvement in glycemic control in patients with minimal risk of hypoglycemia. These clinical trials strongly suggest that FFA1 is a crucial drug target, and that FFA1 agonists could be a novel class of therapeutic drugs for the treatment of type 2 diabetes.

5 GPR84

5.1 Ligands

GPR84 was activated by medium-chain FFAs (C9-C14) (Wang et al. 2006). In contrast, neither short- nor long-chain FFAs did not activate GPR84. Among these medium-chain FFA ligands, capric acid (C10:0), undecanoic acid (C11:0) and lauric acid (C12:0) showed the most potent activity for GPR84. The EC_{50} values for these three FFAs were approximately 5–10 μM. As a small molecule for GPR84, diindolylmethane was reported to activate GPR84 with greater potency than FFA agonists. The EC_{50} value of this compound measured by [^{35}S]-GTPγS binding assay was approximately 0.5 μM.

5.2 Signal Transduction

Medium-chain FFAs dose-dependently increased $[Ca^{2+}]_i$ in CHO cells transiently express GPR84. In addition, forskolin-stimulated cAMP production was inhibited by medium-chain FFAs. GPR84 dependent inhibition of cAMP production was reduced by pretreatment with pertussis toxin. Further, medium-chain FFAs increased [^{35}S]-GTPγS incorporation. Therefore, GPR84 was activated by medium-chain FFAs and coupled to $G_{i/o}$ pathway (Wang et al. 2006).

5.3 Expression and Physiological Functions

GPR84 was identified by using an expressed sequence tag (EST) data mining strategy (Wittenberger et al. 2001). GPR84 is reported to be express in various tissues such as heart, lung, kidney, liver and leucocytes. Especially, as related to inflammatory cells, granulocytes, splenic T and B cells express GPR84 (Yousefi et al. 2001; Venkataraman and Kuo 2005). Also, GPR84 is induced by the

stimulation of lipopolysaccharide (LPS) in monocytes (Wang et al. 2006). Furthermore, Nagasaki et al. (2012) reported that adipose tissue and 3T3-L1 adipocyte cell line express GPR84. Bouchard et al. (2007) reported that microglia express GPR84 in a strong and sustained manner.

5.3.1 Immune System

The functional study of GPR84 showed that CD3 antibody-induced IL-4, but not IFN-γ and IL-2 production was increased in GPR84 deficient mice compared to WT mice. However, the stimulation effects of various mitogen on the proliferation of T and B cells were not changed between GPR84-deficient and WT mice. Further, the levels of IL-4, but not IFN-γ mRNA was also increased in response to antibody stimulations of CD28 together with CD3. Also, the expression level of GPR84 mRNA was increased in monoyotes or differentiated into macrophages after stimulation of LPS. In addition, GPR84 expressed in microglia was induced by proinflammatory cytokines such as TNF-α and IL-1. The expression of GPR84 was potent observed in not only in mice suffering from endotoxemia, but also during experimental autoimmune encephalomyelitis (Bouchard et al. 2007). Hence, GPR84 might contribute to regulate neuroimmunological processes.

5.3.2 Adipose Tissue

GPR84 is reported to be expressed in adipose tissue and 3T3-L1 adipocytes. In HFD supplemented mice, GPR84 expression was detected in fat pads. Also, 3T3-L1 adipocytes co-cultured with a macrophage cell line RAW264, significantly induced GPR84 expression. On the other hand, medium-chain fatty acids reduced mRNA expression level of adiponectin in 3T3-L1 cell line through GPR84. This report suggested that macrophages that infiltrated into adipose tissue and secreted inflammatory cytokines such as TNF-α, contributed to enhance the expression of GPR84 mRNA (Nagasaki et al. 2012). Accordingly, GPR84 might play important physiological roles in the regulation of insulin sensitivity under inflammatory condition such as type 2 diabetes.

6 GPR120

6.1 Ligands

We previously isolated the GPR120 gene from genomic DNA. Medium to long-chain FFAs were identified as endogenous ligands of GPR120 using a receptor internalization assay (Fukunaga et al. 2006). GPR120 was activated by saturated

FFAs (C14–18) and unsaturated FFAs (C16–22). Various polyunsaturated fatty acids, regardless of ω-3 or ω-6 type, were found to act as agonists of GPR120 in the micromolar concentration range, with α-linolenic acid being the most potent (Hirasawa et al. 2005). The ligand profiles were similar to those for FFA1; however, the amino acid homology between GPR120 and FFA1 is only 10 %. The similarity in ligand specificity may be the result of convergent evolution. In addition, we synthesized a series of carboxylate group-containing compounds that were based on the structure of the thiazolidinedione (PPAR-γ) agonists. The relative ERK1/2 phosphorylation of these compounds examined in FFA1 and GPR120 expressing cells were correlated well with the calculated hydrogen bonding energy based on FFA1 and GPR120 homology model, respectively (Fig. 5a). The selective agonist NCG21 was developed (Suzuki et al. 2008) using a homology model and docking simulation for GPR120 (Fig. 5b) (Sun et al. 2010). To identify other natural ligands of GPR120, we screened for and identified a selective partial agonist from a series of natural compounds; grifolic acid, derived from the fruiting bodies of *Albatrellus ovinus* (Hara et al. 2009b). Grifolic acid activated GPR120 in GPR120 over-expressing cells and also in STC-1 cells, which express endogenous GPR120. Further Hashimoto et al. (2010) reported a synthetic compound (Compound 8) as a patented compound. More recently, Shimpukade et al. (2012) reported a potent and selective GPR120 agonist (Compound 9), which showed high potency on both human and murine GPR120. These compounds may be useful tools to monitor the physiological effects of GPR120 and may be useful for the development of novel drug candidates for the treatment of type 2 diabetes, obesity, and metabolic diseases.

6.2 Signal Transduction

Polyunsaturated fatty acids and synthetic ligands induced a rise in cytosolic free Ca^{2+} in GPR120 over-expressing HEK293 cells through GPR120, but they did not promote cAMP production. This suggested that GPR120 is coupled to the G_q protein family, similarly to FFA1, but not to the G_s or $G_{i/o}$ families (Hirasawa et al. 2005). GPR120 can also induce the activation of ERK1/2 under certain conditions, and the activation of PI3-kinase and the serine/threonine protein kinase Akt in GPR120-expressing cells (Katsuma et al. 2005). Oh et al. showed that the ω-3 FFAs, DHA and eicosapentaenoic acid (EPA) exert anti-inflammatory effects through GPR120. The underlying mechanism involved inhibition of TGF-β-activated kinase 1 (TAK1) phosphorylation related to the toll-like receptor (TLR), and tumor necrosis factor-α (TNF-α) inflammatory pathways through β-arrestin 2 signaling in monocytic RAW264.7 cells and primary intraperitoneal macrophages (Oh et al. 2010). Recently, Shah et al. showed that linoleic acid leads to activation of monovalent cation-specific transient receptor potential channel type M5 (TRPM5) in STC-1 cells (Shah et al. 2012). Polyunsaturated fatty acid-induced depolarization is significantly reduced by blockade of G proteins and PLC, and

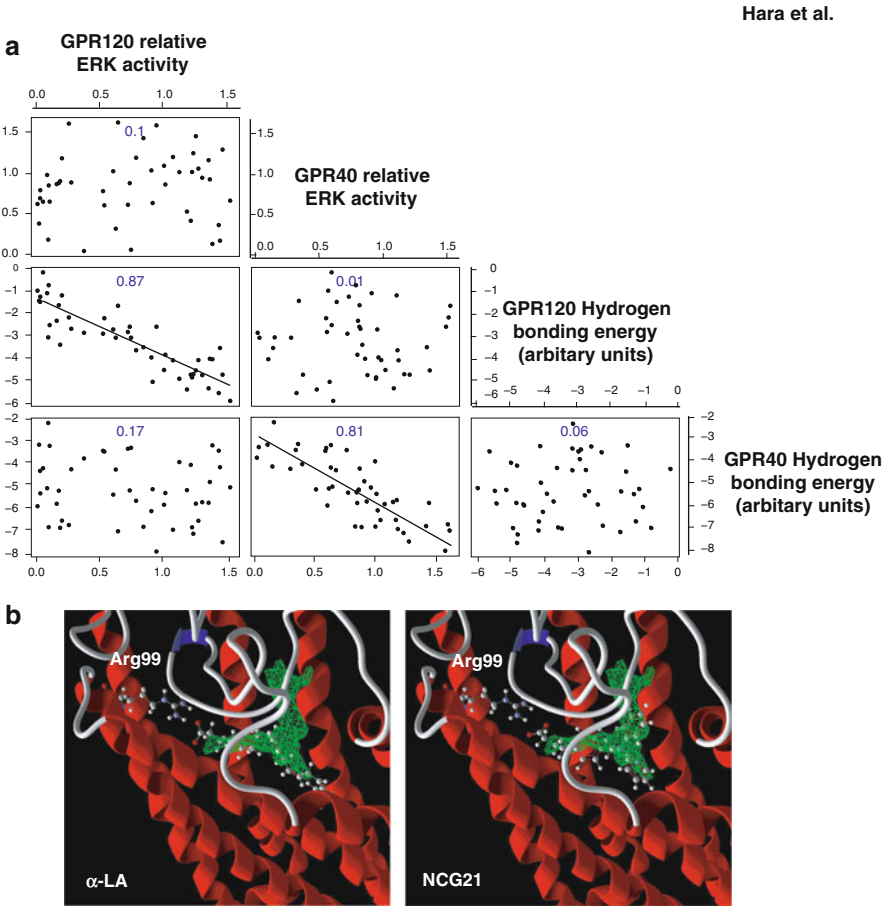

Fig. 5 Docking simulation of each compound in homology models. (**a**) The relative ERK activity versus the calculated energy of interaction based on each modeling was plotted. The coefficient of determination ($R^2 = 0.81$, FFA1 and 0.87, GPR120) reflects a high correlation between the hydrogen bonding energy and relative ERK activity. (**b**) GPR120 homology model docked with α-LA and NCG21. α-LA and NCG21 were docked into the binding pocket of GPR120. Red balls: oxygen atoms of carboxylate group; green: the predicted binding pocket by Molegro cavity detection algorithm

siRNA transfection against TRPM5 resulted in a significant reduction of α-LA-induced intracellular calcium rise as well as CCK secretion from STC-1 cells, suggesting that TRPM5 plays a crucial role in GPR120 signaling.

6.3 Expression and Physiological Functions

6.3.1 Intestine

Endogenous expression of GPR120 was demonstrated in the intestines of humans and mice. Furthermore, the enteroendocrine cell line STC-1 expressed endogenous GPR120. Our previous study showed that GPR120-expressing cells are located in the GLP-1-expressing enteroendocrine cells in the large intestine (Hirasawa et al. 2005; Tanaka et al. 2008; Miyauchi et al. 2009). Stimulation by FFAs induced GLP-1 and CCK secretion in murine enteroendocrine STC-1 cells (Sidhu et al. 2000), and siRNA directed against GPR120 inhibited the FFA-induced effect on incretin secretion and $[Ca^{2+}]_i$ response. The effect of FFAs on plasma levels of GLP-1 and insulin were examined by the administration of FFAs into murine colon (Hirasawa et al. 2005). These reports tempt us to speculate about the physiological function of GPR120 on GLP-1 secretion in vivo. In addition, K cells that express GPR120 and also synthesize GIP were located in the intestinal tract (Parker et al. 2009). Moreover, recent reports suggest that GPR120 may play a role in the lipid-sensing cascade in ghrelin cells (Lu et al. 2012).

6.3.2 Macrophages

Oh et al. (2010) demonstrated endogenous expression of GPR120 in monocytic RAW 264.7 cells and in primary proinflammatory M1-like macrophages. Stimulation of GPR120 with ω-3 FFAs caused broad anti-inflammatory effects in these cells, all of which were abrogated by siRNA against GPR120. In vitro experiments revealed the molecular mechanism underlying ω-3 FFA-mediated anti-inflammatory effects. Stimulation of GPR120 specifically inhibited TAK1 phosphorylation and activation, providing a common mechanism for the inhibition of both TLR and TNF-α signaling. In vivo experiments showed that ω-3 FFA treatment inhibited inflammation and enhanced systemic insulin sensitivity in WT mice; however, these effects by ω-3 FFA were not observed in GPR120-deficient mice. These results showed that GPR120 is a functional ω-3 FFA receptor and that it mediates potent insulin sensitizing and antidiabetic effects in vivo by repressing macrophage-induced tissue inflammation.

6.3.3 Adipocytes

Gotoh et al. (2007) reported that adipose tissue expressed GPR120 and that the mRNA expression level in adipocytes was higher than in stromal-vascular cells. GPR120 expression was increased during adipocyte differentiation of 3T3-L1 cells. Small interfering RNA against GPR120 inhibited this effect on adipocyte differentiation (Gotoh et al. 2007). These findings suggested that GPR120 may play important

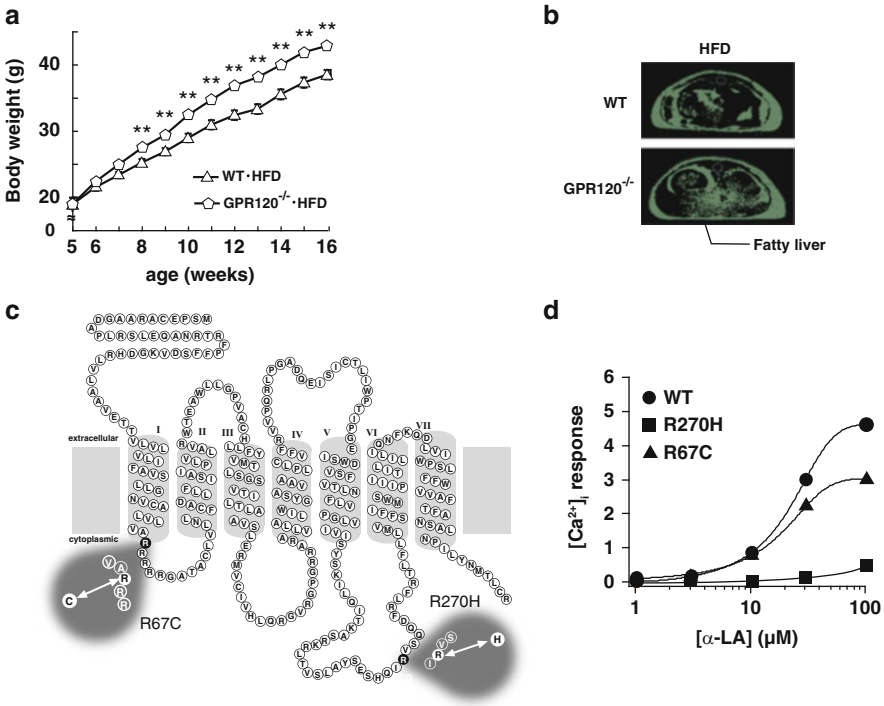

Fig. 6 Obesity and hepatic steatosis in HFD-fed GPR120-deficient mice and pharmacological characterization of two non-synonymous variants of GPR120. (**a**) Body weight changes of WT and GPR120-deficient mice fed HFD. Data represent mean ± s.e.m. $^{**}p < 0.01$ versus the corresponding WT data. (**b**) Representative cross-sectional images of WT and GPR120-deficient mice subjected to microcomputed tomography analysis of the in situ accumulation of fat. Fat depots are demarcated (green) for illustration. The fatty liver in HFD-fed GPR120-deficient mouse was indicated as black line. (**c**) Schematic diagram of two-dimensional topology of GPR120 receptor. Two non-synonymous variants p.R67C and p.R270H were shown. (**d**) α-LA-induced $[Ca^{2+}]_i$ responses in cells expressing WT GPR120 or a p.R67C or p.R270H variant

roles in differentiation, and also in the maturation processes of adipocytes. Moreover, we recently reported that dysfunctional GPR120 led to obesity in both mice and humans (Ichimura et al. 2012). We found that GPR120-deficient mice fed HFD developed obesity and fatty liver with decreased adipocyte differentiation and lipogenesis, and enhanced hepatic lipogenesis (Fig. 6a and b). Insulin resistance in such mice was associated with reduced insulin signaling and enhanced inflammation in adipose tissue. We showed that GPR120 expression in human adipose tissue was significantly higher in obese individuals than in lean controls. GPR120 exon

sequencing in obese subjects revealed two non-synonymous mutation p. R270H and p. R67C (Fig. 6c). The p. R270H variant that inhibited GPR120 signaling activity (Fig. 6d) might be significantly associated with obesity.

Further, since HFD-fed GPR120-deficient mice showed fat liver and obesity, the molecular basis of the metabolic changes in adipose tissues and livers of HFD-fed GPR120-deficient mice and WT mice were examined by using gene expression analysis (Fig. 7a and b). Approximately 1,600 and 600 differentially expressed genes were identified in adipose tissues and livers, respectively. Notably, adipocyte differentiation (*Fabp4*), lipogenesis (*Scd1*) and insulin signal (*Irs2* and *Insr*) related genes were depressed in adipose tissues, whereas these genes together with a fatty acid transporter gene (*Cd36*) were upregulated in livers from GPR120-deficient mice. Therefore, Overall, this study demonstrated that the lipid sensor GPR120 had a key role in sensing dietary fat and thus, in the control of energy balance in both humans and rodents.

6.3.4 Taste Buds

Matsumura et al. reported the expression of GPR120 in taste bud type II cells, as determined by double immunostaining for GPR120 and markers of type II taste cells (phospholipase-Cb2 and ⟨-gustducin) (Matsumura et al. 2009). Cartoni et al. (2010) also reported the expression of GPR120 in CV sections. Short-access test using a lick meter showed that preference for fatty acids but not for other tastes was inhibited in GPR120 KO mice (Cartoni et al. 2010).

6.3.5 Lung

Endogenous expression of GPR120 is also found in other cells and tissues. Furthermore, a GPR120 antibody that recognizes the extracellular domain of murine GPR120 has been developed. This antibody was used to detect GPR120 protein expression in lung and adipose tissues, in which GPR120 mRNA expression was already known (Miyauchi et al. 2009). Pulmonary Clara cells that expressed the Clara cell 10-kDa protein as a marker, stained positively for GPR120 with this antibody (Miyauchi et al. 2009). Further studies are needed to reveal the physiological function of GPR120 in the lung.

6.4 Genetic Contribution to Type 2 Diabetes

Recently, Taneera et al. performed a systems genomics approach to indentify genes for type 2 diabetes, and GPR120 was in the top 20 ranked list (Taneera et al. 2012). In this report, GPR120 expression in human islets was positively correlated with

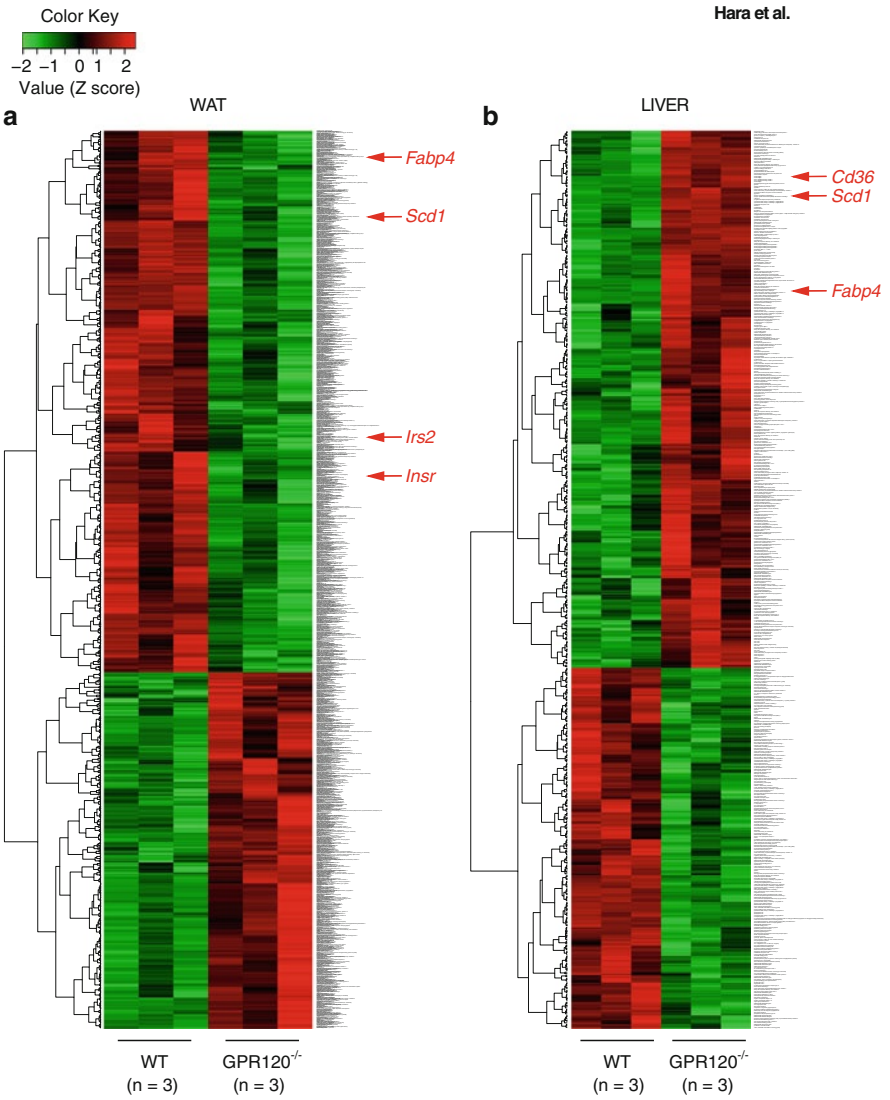

Fig. 7 Changes in gene expression and connectivity map of differentially expressed genes in 16-week-old HFD-fed GPR120-deficient mice. Heatmap comparison of (**a**) epididymal white adipose tissue (WAT) and (**b**) liver of HFD-fed WT and GPR120-deficient mice using gene expression microarray analysis. Gene changes were considered significant if $P < 0.05$ and fold-change > 1.5. The heat map was generated using z-scores across all samples

insulin secretion and insulin content, and with lower HbA1c. Although the physiological function still remains to be cleared, GPR120 expression in pancreas was detected by RT-PCR analysis with the low expression level (Gotoh et al. 2007). Second, activation of GPR120 with EPA prevented lipid-induced apoptosis, and increased cell viability. Although this is not consistent with previous reports (Nagasumi et al. 2009) that FFA1 is predominantly detected in murine pancreatic islets, these data suggest that GPR120 can protect pancreatic islets from lipotoxicity in humans.

6.5 *The Future*

Since FFARs were originally identified as the receptor for FFAs, a remarkable amount of evidence has been gathered to understand the various physiological functions of FFARs. FFARs are activated by FFAs, which are mainly derived from food and the corresponding digested or fermented products in the gastrointestinal tract. Reports using in vitro and in vivo studies indicated that the physiological functions of FFARs conclusively contribute to regulation of metabolic energy (Fig. 8). However, a number of questions remain to be answered. The relative contributions of each of these FFARs to regulation of metabolic energy in the body are currently unclear. The precise signaling mechanisms involving the activation of $[Ca^{2+}]_i$, or ERK1/2 response via G-protein dependent or G-protein independent pathways that are responsible for the reported physiological functions remain to be explored. In addition to our report that genetic analyses of GPR120 could identify a loss-of-function GPR120 variant presented in obese patients, further genetic analysis of GPR120 and other FFARs should be performed to identify gene variants associated with its protein function. Additionally, since the evidence of rare gene variants of major effect on disease risk was reported (Cirulli and Goldstein 2010), we should focus not only on common variant but also on rare variant. Moreover, because the expression, but not the function, of FFARs has been reported in several tissues, functional analysis may provide further evidence for understanding the mechanisms underlying energy metabolism associated with FFARs. Furthermore, early clinical trial evaluation has yielded beneficial results for synthetic agonists of FFA1, thereby suggesting that future research will increase the therapeutic potential of FFARs. Taken together, additional analysis of FFARs may also be important to better understand the nutrient sensing process and to develop therapeutic compounds to treat metabolic energy disorders such as obesity and type 2 diabetes.

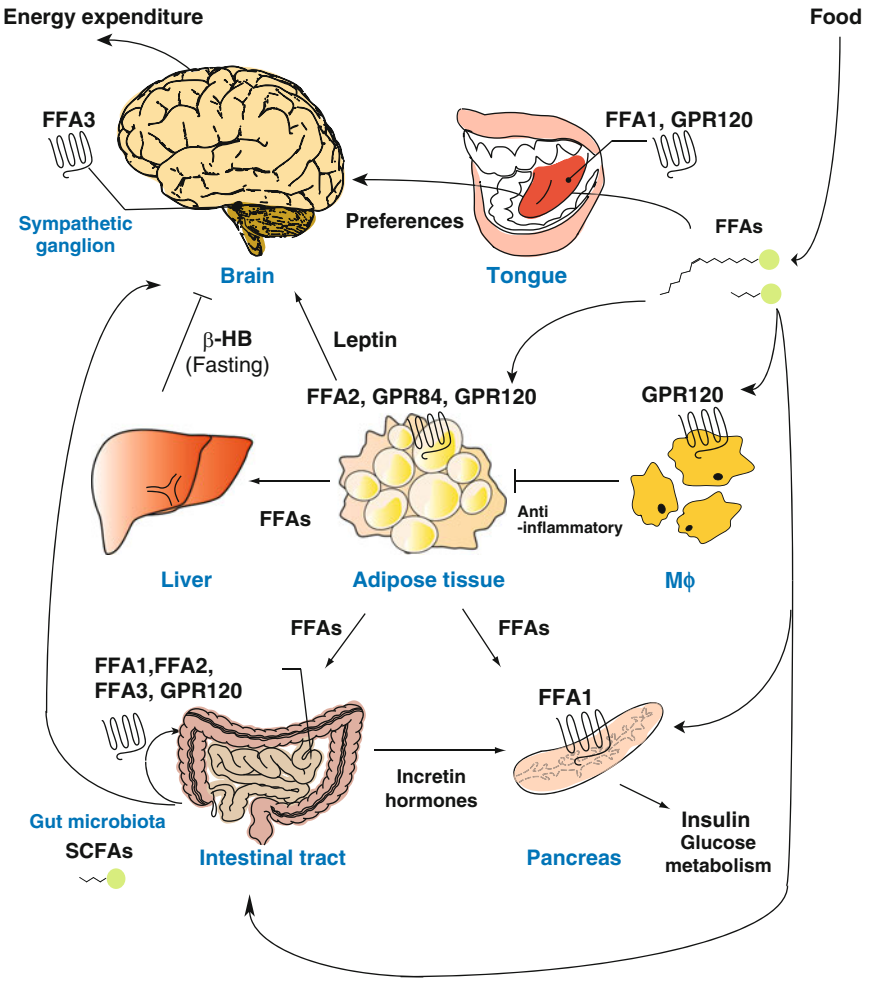

Fig. 8 Functional relationship between FFAs and FFARs. Nutritional and endogenous FFAs stimulate FFARs expressing in various tissues and thereby promote secretion of insulin and incretin hormones, regulate cell differentiations and modulate sympathetic nerve activity. FFARs play key roles in the regulation of FFAs-mediated energy homeostasis in the body

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References

Al-Lahham SH, Roelofsen H, Priebe M et al (2010) Regulation of adipokine production in human adipose tissue by propionic acid. Eur J Clin Invest 40:401–407

Alquier T, Poitout V (2009) GPR40: good cop, bad cop? Diabetes 58(5):1035–1036

Araki T, Hirayama M, Hiroi S, Kaku K (2012) GPR40-induced insulin secretion by the novel agonist TAK-875: first clinical findings in patients with type 2 diabetes. Diabetes Obes Metab 14(3):271–278

Bellahcene M, O'Dowd JF, Wargent ET et al (2012) Male mice that lack the G-protein-coupled receptor GPR41 have low energy expenditure and increased body fat content. Br J Nutr 31:1–10

Berger M, Gray JA, Roth BL (2009) The expanded biology of serotonin. Ann Rev Med 60:355

Bharate SB, Nemmani KV, Vishwakarma RA (2009) Progress in the discovery and development of small-molecule modulators of G-protein-coupled receptor 40 (GPR40/FFA1/FFAR1): an emerging target for type 2 diabetes. Expert Opin Ther Pat 19(2):237–264

Bouchard C, Pagé J, Bédard A et al (2007) G protein-coupled receptor 84, a microglia-associated protein expressed in neuroinflammatory conditions. Glia 55(8):790–800

Brantis CE, Ooms F, Bernard J (2011) Novel amino acid derivatives and their use as GPR43 receptor modulators. PCT Int. Appl. WO2011092284

Briscoe CP, Tadayyon M, Andrews JL et al (2003) The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem 278(13):11303–11311

Briscoe CP, Peat AJ, McKeown SC et al (2006) Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. Br J Pharmacol 148(5):619–628

Brown AJ, Goldsworthy SM, Barnes AA et al (2003) The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 278:11312–11319

Bullock BP, Heller RS, Habener JF (1996) Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology 137:2968–2978

Burant CF, Viswanathan P, Marcinak J et al (2012) TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet 379(9824):1403–1411

Cartoni C, Yasumatsu K, Ohkuri T et al (2010) Taste preference for fatty acids is mediated by GPR40 and GPR120. J Neurosci 30(25):8376–8382

Chandalia M, Garg A, Lutjohann D et al (2000) Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med 342:1392–1398

Chawla A, Repa JJ, Evans RM et al (2001) Nuclear receptors and lipid physiology: opening the X-files. Science 294(5548):1866–1870

Christiansen E, Urban C, Merten N et al (2008) Discovery of potent and selective agonists for the free fatty acid receptor 1 (FFA(1)/GPR40), a potential target for the treatment of type II diabetes. J Med Chem 51(22):7061–7064

Christiansen E, Due-Hansen ME, Urban C et al (2013) Discovery of a potent and selective free fatty acid receptor 1 agonist with low lipophilicity and high oral bioavailability. J Med Chem 56(3):982–992

Cirulli ET, Goldstein DB (2010) Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 11(6):415–425

Civelli O, Reinscheid RK, Zhang Y et al (2013) G protein-coupled receptor deorphanizations. Annu Rev Pharmacol Toxicol 53:127–146

Dass NB, John AK, Bassil AK et al (2007) The relationship between the effects of short-chain fatty acids on intestinal motility in vitro and GPR43 receptor activation. Neurogastroenterol Motil 19:66–74

Davis HR Jr, Mullins DE, Pines JM et al (1998) Effect of chronic central administration of glucagon-like peptide-1 (7–36) amide on food consumption and body weight in normal and obese rats. Obes Res 6:147–156

Delzenne NM, Cani PD, Daubioul C et al (2005) Impact of insulin and oligofructose on gastrointestinal peptides. Br J Nutr 93(suppl 1):S157–S161

Edfalk S, Stenberg P, Edlund H (2008) Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. Diabetes 57(9):2280–2287

Elliott RM, Morgan LM, Tredger JA et al (1993) Glucagon-like peptide-1 (7–36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol 138:159–166

Ferdaoussi M, Bergeron V, Zarrouki B et al (2012) G protein-coupled receptor (GPR)40-dependent potentiation of insulin secretion in mouse islets is mediated by protein kinase D1. Diabetologia 55(10):2682–2692

Flodgren E, Olde B, Meidute-Abaraviciene S et al (2007) GPR40 is expressed in glucagon producing cells and affects glucagon secretion. Biochem Biophys Res Commun 354(1):240–245

Fujiwara K, Maekawa F, Yada T (2005) Oleic acid interacts with GPR40 to induce Ca^{2+} signaling in rat islet beta-cells: mediation by PLC and L-type Ca^{2+} channel and link to insulin release. Am J Physiol Endocrinol Metab 289(4):E670–E677

Fukumoto S, Tatewaki M, Yamada T et al (2003) Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. Am J Physiol Regul Integr Comp Physiol 284: R1269–R1276

Fukunaga S, Setoguchi S, Hirasawa A, Tsujimoto G (2006) Monitoring ligand-mediated internalization of G protein-coupled receptor as a novel pharmacological approach. Life Sci 80(1):17–23

Galindo MM, Voigt N, Stein J et al (2012) G protein-coupled receptors in human fat taste perception. Chem Senses 37(2):123–139

Garrido DM, Corbett DF, Dwornik KA et al (2006) Synthesis and activity of small molecule GPR40 agonists. Bioorg Med Chem Lett 16(7):1840–1845

Ge H, Li X, Weiszmann J et al (2008) Activation of GPR43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology 149:4519–4526

Gosmain Y, Cheyssac C, Masson MH et al (2012) Pax6 is a key component of regulated glucagon secretion. Endocrinology 53(9):4204–4215

Gotoh C, Hong YH, Iga T et al (2007) The regulation of adipogenesis through GPR120. Biochem Biophys Res Commun 354(2):591–597

Greeley GH Jr, Jeng YJ, Gomez G et al (1989) Evidence for regulation of peptide-YY release by the proximal gut. Endocrinology 124:1438–1443

Grundy SM, Brewer HB Jr, Cleeman JI et al (2004) Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol 24:e13–e18

Hamid YH, Vissing H, Holst B et al (2005) Studies of relationships between variation of the human G protein-coupled receptor 40 gene and type 2 diabetes and insulin release. Diabet Med 22(1):74–80

Hara T, Hirasawa A, Sun Q et al (2009a) Flow cytometry-based binding assay for GPR40 (FFAR1; free fatty acid receptor 1). Mol Phamacol 75(1):85–91

Hara T, Hirasawa A, Sun Q et al (2009b) Novel selective ligands for free fatty acid receptors GPR120 and GPR40. Naunyn Schmiedebergs Arch Pharmacol 380(3):247–255

Hara T, Hirasawa A, Ichimura A et al (2011) Free fatty acid receptors FFAR1 and GPR120 as novel therapeutic targets for metabolic disorders. J Pharm Sci 100(9):3594–3601

Hara T, Hirasawa A, Ichimura A et al (2012) Physiological functions of fatty acid receptors and their therapeutic potential. Nihon Yakurigaku Zasshi 140(6):257–259

Hashimoto N, Sasaki Y, Nakama C et al (2010) Novel phenyl-isoxazol-3-ol derivative. US Patent 2010/0130559

Hirasawa A, Tsumaya K, Awaji T et al (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med 11(1):90–94

Hong YH, Nishimura Y, Hishikawa D et al (2005) Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology 146:5092–5099

Houze JB, Zhu L, Sun Y et al (2012) AMG 837: a potent, orally bioavailable GPR40 agonist. Bioorg Med Chem Lett 22(2):1267–1270

Hoveyda H, Brantis CE, Dutheuil G et al (2010) Compounds, pharmaceutical composition and methods for use in treating metabolic disorders. PCT Int. Appl. WO2010066682

Hu H, He LY, Gong Z et al (2009) A novel class of antagonists for the FFAs receptor GPR40. Biochem Biophys Res Commun 390(3):557–563

Hudson BD, Christiansen E, Tikhonova IG et al (2012) Chemically engineering ligand selectivity at the free fatty acid receptor 2 based on pharmacological variation between species orthologs. FASEB J 26(12):4951–4965

Humphries PS, Benbow JW, Bonin PD et al (2009) Synthesis and SAR of 1,2,3,4-tetrahydroisoquinolin-1-ones as novel G-protein-coupled receptor 40 (GPR40) antagonists. Bioorg Med Chem Lett 19(9):2400–2403

Ichimura A, Hirasawa A, Hara T et al (2009) Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. Prostaglandins Other Lipid Mediat 89(3–4):82–88

Ichimura A, Hirasawa A, Poulain-Godefroy O et al (2012) Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature 483(7389):350–354

Inoue D, Kimura I, Wakabayashi M et al (2012) Short-chain fatty acid receptor GPR41-mediated activation of sympathetic neurons involves synapsin 2b phosphorylation. FEBS Lett 586:1547–1554

Itoh Y, Kawamata Y, Harada M et al (2003) Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature 422(6928):173–176

Kaku K, Araki T, Yoshinaka R et al (2013) Randomized, double-blind, dose-ranging study of 1089 TAK-875, a novel GPR40 agonist, in Japanese patients with inadequately controlled type 2 diabetes. Diabetes Care 36(2):245–250

Karaki S, Mitsui R, Hayashi H et al (2006) Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. Cell Tissue Res 324:353–360

Karaki S, Tazoe H, Hayashi H et al (2008) Expression of the short-chain fatty acid receptor, GPR43, in the human colon. J Mol Histol 39:135–142

Katsuma S, Hatae N, Yano T et al (2005) Free fatty acids inhibit serum deprivation-induced apoptosis through GPR120 in a murine enteroendocrine cell line STC-1. J Biol Chem 280(20):19507–19515

Kebede M, Alquier T, Latour MG et al (2008) The fatty acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. Diabetes 57(9):2432–2437

Kebede M, Ferdaoussi M, Mancini A et al (2012) Glucose activates free fatty acid receptor 1 gene transcription via phosphatidylinositol-3-kinase-dependent O-GlcNAcylation of pancreas-duodenum homeobox-1. Proc Natl Acad Sci USA 109(7):2376–2381

Keenan MJ, Zhou J, McCutcheon KL et al (2006) Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. Obesity (Silver Spring) 14:1523–1534

Kim DY, Camilleri M (2000) Serotonin: a mediator of the brain–gut connection. Am J Gastroenterol 95:2698–2709

Kim BJ, Carlson OD, Jang HJ et al (2005) Peptide YY is secreted after oral glucose administration in a gender-specific manner. J Clin Endocrinol Metab 90:6665–6671

Kimura I, Inoue D, Maeda T et al (2011) Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proc Natl Acad Sci USA 108:8030–8035

Kotarsky K, Nilsson NE, Olde B, Owman C (2003) Progress in methodology. Improved reporter gene assays used to identify ligands acting on orphan seven-transmembrane receptors. Pharmacol Toxicol 93(6):249–258

Lagerstrom MC, Schioth HB (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat Rev Drug Discov 7(4):339–357

Lan H, Hoos LM, Liu L et al (2008) Lack of FFAR1/GPR40 does not protect mice from high-fat diet-induced metabolic disease. Diabetes 57(11):2999–3006
Latour MG, Alquier T, Oseid E et al (2007) GPR40 is necessary but not sufficient for fatty acid stimulation of insulin secretion in vivo. Diabetes 56(4):1087–1094
Le Poul E, Loison C, Struyf S et al (2003) Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem 278:25481–25489
Lee T, Schwandner R, Swaminath G et al (2008) Identification and functional characterization of allosteric agonists for the G protein-coupled receptor FFA2. Mol Pharmacol 74:1599–1609
Leonard JN, Chu ZL, Bruce MA et al (2006) GPR41 and modulators thereof for the treatment of insulin-related disorders. PCT Int. Appl. WO2006052566
Liou AP, Lu X, Sei Y et al (2011) The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. Gastroenterology 140(3):903–912
Longuet C, Broca C, Costes S et al (2005) Extracellularly regulated kinases 1/2 (p44/42 mitogen-activated protein kinases) phosphorylate synapsin I and regulate insulin secretion in the MIN6 beta-cell line and islets of Langerhans. Endocrinology 146:643–654
Lu X, Zhao X, Feng J et al (2012) Postprandial inhibition of gastric Ghrelin secretion by long-chain fatty acid through GPR120 in isolated gastric Ghrelin cells and mice. Am J Physiol Gastrointest Liver Physiol 303(3):G367–G376
Luo J, Swaminath G, Brown SP et al (2012) A potent class of GPR40 full agonists engages the enteroinsular axis to promote glucose control in rodents. PLoS One 7(10):e46300
Ma D, Zhang M, Larsen CP (2010) DHA promotes the neuronal differentiation of rat neural stem cells transfected with GPR40 gene. Brain Res 1330:1–8
Maslowski KM, Vieira AT, Ng A et al (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461:1282–1286
Matsumura S, Mizushige T, Yoneda T et al (2007) GPR expression in the rat taste bud relating to fatty acid sensing. Biomed Res 28(1):49–55
Matsumura S, Eguchi A, Mizushige T et al (2009) Colocalization of GPR120 with phospholipase-Cbeta2 and alpha-gustducin in the taste bud cells in mice. Neurosci Lett 450(2):186–190
Miyauchi S, Hirasawa A, Iga T et al (2009) Distribution and regulation of protein expression of the free fatty acid receptor GPR120. Naunyn Schmiedebergs Arch Pharmacol 379(4):427–434
Nagasaki H, Kondo T, Fuchigami M et al (2012) Inflammatory changes in adipose tissue enhance expression of GPR84, a medium-chain fatty acid receptor: TNFα enhances GPR84 expression in adipocytes. FEBS Lett 586(4):368–372
Nagasumi K, Esaki R, Iwachidow K et al (2009) Overexpression of GPR40 in pancreatic beta-cells augments glucose-stimulated insulin secretion and improves glucose tolerance in normal and diabetic mice. Diabetes 58(5):1067–1076
Naik H, Vakilynejad M, Wu J et al (2012) Safety, tolerability, pharmacokinetics, and pharmacodynamic properties of the GPR40 agonist TAK-875: results from a double-blind, placebo-controlled single oral dose rising study in healthy volunteers. J Clin Phamacol 52(7):1007–1016
Nilsson NE, Kotarsky K, Owman C et al (2003) Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. Biochem Biophys Res Commun 303:1047–1052
Ogawa T, Hirose H, Miyashita K et al (2005) GPR40 gene Arg211His polymorphism may contribute to the variation of insulin secretory capacity in Japanese men. Metabolism 54(3):296–299
Oh DY, Talukdar S, Bae EJ et al (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 142(5):687–698
Owira PM, Winter TA (2008) Colonic energy salvage in chronic pancreatic exocrine insufficiently. J Parenter Enteral Nutr 32:63–71
Parker HE, Habib AM, Rogers GJ et al (2009) Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. Diabetologia 52(2):289–298

Robertson MD, Bickerton AS, Dennis AL et al (2005) Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 82:559–567

Salehi A, Flodgren E, Nilsson NE et al (2005) Free fatty acid receptor 1 (FFA(1)R/GPR40) and its involvement in fatty-acid-stimulated insulin secretion. Cell Tissue Res 322(2):207–215

Samuel BS, Shaito A, Motoike T et al (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci USA 105:16767–16772

Saniere LRM, Pizzonero MR, Triballeau N et al (2012) Preparation of azetidine derivatives as GPR43 antagonists useful in the treatment of metabolic and inflammatory diseases. PCT Int. Appl. WO2012098033

Sawzdargo M, George SR, Nguyen T et al (1997) A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. Biochem Biophys Res Commun 239:543–547

Schmidt J, Smith NJ, Christiansen E et al (2011) Selective orthosteric free fatty acid receptor 2 (FFA2) agonists: identification of the structural and chemical requirements for selective activation of FFA2 versus FFA3. J Biol Chem 286(12):10628–10640

Selway J, Rigatti R, Storey N et al (2012) Evidence that Ca within the microdomain of the L-type voltage gated Ca channel activates ERK in MIN6 cells in response to glucagon-like peptide-1. PLoS One 7(3):e33004

Shah BP, Liu P, Yu T et al (2012) TRPM5 is critical for linoleic acid-induced CCK secretion from the enteroendocrine cell line, STC-1. Am J Physiol Cell Physiol 302(1):C210–C219

Shimpukade B, Hudson BD, Hovgaard CK et al (2012) Discovery of a potent and selective GPR120 agonist. J Med Chem 55(9):4511–4515

Sidhu SS, Thompson DG, Warhurst G et al (2000) Fatty acid-induced cholecystokinin secretion and changes in intracellular Ca^{2+} in two enteroendocrine cell lines, STC-1 and GLUTag. J Physiol 528(Pt 1):165–176

Sleeth ML, Thompson EL, Ford HE et al (2010) Free fatty acid receptor 2 and nutrient sensing: a proposed role for fiber, fermentable carbohydrates and short-chain fatty acids in appetite regulation. Nutr Res Rev 23:135–145

Steneberg P, Rubins N, Bartoov-Shifman R et al (2005) The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis and impaired glucose homeostasis in mouse. Cell Metab 1(4):245–258

Sum CS, Tikhonova IG, Neumann S et al (2007) Identification of residues important for agonist recognition and activation in GPR40. J Biol Chem 282(40):29248–29255

Sum CS, Tikhonova IG, Costanzi S, Gershengorn MC (2009) Two arginine-glutamate ionic locks near the extracellular surface of FFAR1 gate receptor activation. J Biol Chem 284(6):3529–3536

Sun Q, Hirasawa A, Hara T et al (2010) Structure-activity relationships of GPR120 agonists based on a docking simulation. Mol Pharmacol 78(5):804–810

Suzuki T, Igari S, Hirasawa A et al (2008) Identification of G protein-coupled receptor 120-selective agonists derived from PPARgamma agonists. J Med Chem 51(23):7640–7644

Takeuchi M, Hirasawa A, Hara T et al (2013) FFA1-selective agonistic activity based on docking simulation using FFA1 and GPR120 homology models. Br J Pharmacol 168(7):1570–1583

Tanaka T, Katsuma S, Adachi T et al (2008) Free fatty acids induce cholecystokinin secretion through GPR120. Naunyn Schmiedebergs Arch Pharmacol 377(4–6):523–527

Taneera J, Lang S, Sharma A et al (2012) A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab 16(1):122–134

Tazoe H, Otomo Y, Karaki S et al (2009) Expression of short-chain fatty acid receptor GPR41 in the human colon. Biomed Res 30:149–156

Tikhonova IG, Sum CS, Neumann S et al (2007) Bidirectional, iterative approach to the structural delineation of the functional "chemoprint" in GPR40 for agonist recognition. J Med Chem 50(13):2981–2989

Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783

Tolhurst G, Heffron H, Lam YS et al (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61:364–371

Tomita T, Masuzaki H, Iwakura H et al (2006) Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumors in humans: evidence for GPR40 expression in pancreatic beta cells and implications for insulin secretion. Diabetologia 49(5):962–968

Tsujihata Y, Ito R, Suzuki M et al (2011) TAK-875, an orally available G protein-coupled receptor 40/free fatty acid receptor 1 agonist, enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia in type 2 diabetic rats. J Pharmacol Exp Ther 339(1):228–237

Turton MD, O'Shea D, Gunn I et al (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72

Ulven T (2012) Short-chain free fatty acid receptors FFA2/GPR43 and FFA3/GPR41 as new potential therapeutic targets. Front Endocrinol (Lausanne) 3:111

Venkataraman C, Kuo F (2005) The G-protein coupled receptor, GPR84 regulates IL-4 production by T lymphocytes in response to CD3 crosslinking. Immunol Lett 101(2):144–153

Vettor R, Granzotto M, De Stefani D et al (2008) Loss-of-function mutation of the GPR40 gene associates with abnormal stimulated insulin secretion by acting on intracellular calcium mobilization. J Clin Endocrinol Metab 93(9):3541–3550

Wang J, Wu X, Simonavicius N et al (2006) Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. J Biol Chem 281(45):34457–34464

Wild S, Roglic G, Green A et al (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27(5):1047–1053

Wittenberger T, Schaller HC, Hellebrand S (2001) An expressed sequence tag (EST) data mining strategy succeeding in the discovery of new G-protein coupled receptors. J Mol Biol 307(3):799–813

Xiong Y, Miyamoto N, Shibata K et al (2004) Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci USA 101:1045–1050

Yamashita T (2008) A putative link of PUFA, GPR40 and adult-born hippocampal neurons for memory. Prog Neurobiol 84(2):105–115

Yashiro H, Tsujihata Y, Takeuchi K et al (2012) The effects of TAK-875, a selective G protein-coupled receptor 40/free fatty acid 1 agonist, on insulin and glucagon secretion in isolated rat and human islets. J Pharmacol Exp Ther 340(2):483–489

Yonezawa T, Haga S, Kobayashi Y et al (2008) Unsaturated fatty acids promote proliferation via ERK1/2 and Akt pathway in bovine mammary epithelial cells. Biochem Biophys Res Commun 367(4):729–735

Yousefi S, Cooper PR, Potter SL et al (2001) Cloning and expression analysis of a novel G-protein-coupled receptor selectively expressed on granulocytes. J Leukoc Biol 69(6):1045–1052

Zaibi MS, Stocker CJ, O'Dowd J et al (2010) Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 584:2381–2386

Zhang X, Yan G, Li Y et al (2010) DC260126, a small-molecule antagonist of GPR40, improves insulin tolerance but not glucose tolerance in obese Zucker rats. Biomed Pharmacother 64(9):647–651

Zhao YF, Pei J, Chen C (2008) Activation of ATP-sensitive potassium channels in rat pancreatic beta-cells by linoleic acid through both intracellular metabolites and membrane receptor signaling pathway. J Endocrinol 198(3):533–540

Zhou C, Tang C, Chang E et al (2010) Discovery of 5-aryloxy-2,4-thiazolidinediones as potent GPR40 agonists. Bioorg Med Chem Lett 20(3):1298–1301

Zhu JX, Wu XY, Owyang C et al (2001) Intestinal serotonin acts as a paracrine substance to mediate vagal signal transmission evoked by luminal factors in the rat. J Physiol 530:431–442