Marshall D. McCue *Editor*

Comparative Physiology of Fasting, Starvation, and Food Limitation

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Editor
Marshall D. McCue
Department of Biological Sciences
St. Mary's University
One Camino Santa Maria
San Antonio, TX 78228-8511
USA

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Preface: The Comparative Physiology of Fasting and Starvation

The physiological challenges initiated by food limitation and the risk of death by starvation were likely faced by the very first animals and show no signs of abatement within the foreseeable future. Comparative physiologists are charged with identifying and characterizing the mechanisms by which different animals persist, and even thrive, despite the often present threat of food scarcity. Over the past century only a handful of books have focused on the physiological effects of starvation and none considered this phenomenon from a broadly comparative perspective. Exploring the physiology of starvation from a comparative point of view is not simply an academic exercise. In fact, knowledge from comparative investigations routinely leads to practical applications, from the development of novel investigative techniques and the identification of new model organisms, which can lead to medical advances, to improving conditions for economically important animals, reducing damage by pest species, and to developing accurate predictions about how impending climate change will impact biological systems at various levels.

Historically, physiologists have studied fasting and starvation in their respective animal models with minimal intercourse in the literature and too little serious exchange of ideas. It is now clear that progress in understanding fasting and starvation physiology will be most rapid through the use of integrative and comparative approaches, which will require synthesis of the existing encyclopedic body of facts and data into a robust conceptual framework from which new ideas and theories will extend.

The chapters in this volume highlight the tremendous progress we have made in developing new tools and skills to study fasting and starvation. These tools range from remote sensing using global positioning systems, to DNA microarray analysis; whole-body MRI to isotope analyses of individual hairs; and analytical chemistry to population modeling. Despite the technological revolution that has occurred, future progress will continue to require input from our colleagues in the field who have detailed knowledge of the natural histories of various species. Chapters were contributed by researchers currently investigating fasting and starvation physiology using animal models that span from invertebrates to humans.

This volume summarizes the current state of the art of our field, but is also aimed at outlining the future of starvation research. To this end, in many of the chapters, the authors emphasize the need to reevaluate current paradigms and standardize methodologies in order to maximize our future efforts.

Chapter 1 introduces the ubiquitous nature of starvation and the critical role that it has had in the major mass extinction events throughout Earth's history. It also outlines the current and future ecological challenges of food limitation among humans and other animals and justifies the necessity of a comparative perspective in starvation research as we move forward.

Chapter 2 reviews the history of modern starvation research, particularly the development of the three phases of fasting that have become widely adopted by physiologists. This chapter also recapitulates the gradual transition from human-centered studies of fasting and starvation into studies of extreme fasting among new animal models including penguins, snakes, and fish.

Chapter 3 describes the interaction between starvation and population dynamics in planktonic rotifers, a group of animals that undergo extreme boom-and-bust cycles with regard to food availability. It also considers the tradeoffs arising from different reproductive strategies used by starving rotifers; some species cease egg production whereas others have apparently evolved strategies to increase reproductive output during starvation.

Chapter 4 reviews the natural differences in starvation resistance among different *Drosophila* species and describes how starvation resistance can be repeatedly observed among separate lineages using laboratory experiments. This chapter identifies life-history tradeoffs and changes in the behavioral repertoire (e.g. cannibalism) associated with evolution of starvation resistance. It concludes with a discussion of how DNA microarray analysis provides unique insight into acute starvation responses and the gradual evolution of starvation resistance.

Chapter 5 is the first comprehensive summary of physiological responses to prolonged fasting in spiders. It describes the sequential changes in body composition during starvation as well as the relationship between body mass and starvation tolerance. The chapter also introduces the idea that spiders recovering from starvation may assimilate the various nutrients differently, to preferentially refuel their depleted stores.

Chapter 6 summarizes the thermal and hormonal basis for physiological changes that take place in commercially important marine and freshwater fishes subjected to prolonged fasting. This chapter also introduces a new mathematical model based on lipostatic theory that is useful for predicting phase transitions in fasting fishes—and possibly other animals.

Chapter 7 highlights the different physiological responses to starvation measured in closely related species pairs that inhabit either terrestrial or subterranean habitats. These biological differences provide a framework for a general model to explain the convergent behavioral, morphological, and physiological adaptations used by subterranean invertebrates and vertebrates to cope with severely limited food supplies.

Chapter 8 reviews the physiological responses to prolonged starvation exhibited by distantly related species of snakes and illustrates how observed differences are related to each species' evolutionary and ecological history. It also summarizes the results of studies of wild snake populations that are under severe food limitation—one long-term study of rattlesnakes in the Ozark Mountains and several snake species that inhabit marine islands.

Chapter 9 describes the morphological cardiovascular adaptations of reptiles that feed intermittently, and explains how the form of these structures is closely integrated with the functional changes that occur as animals switch from a postabsorptive state to a postprandial state, and back again to a postabsorptive state. Special attention is given to challenges to pH homeostasis that occurs during these transitions.

Chapter 10 focuses on the physiological triggers of hypothermia that occurs in fasting birds. The author suggests that although these adaptive responses are highly variable among species they are present in some degree in every major avian family. He also explains how adaptive changes in thermal conductance, permitted by regional heterothermy, likely complement adaptive reductions in core body temperature during food limitation.

Chapter 11 describes the peculiar case of starvation that occurs among birds during their migratory flights. Apparently, migrating birds meet the dual challenges of increased energy requirements and lack of food input by efficient mobilization, delivery, and oxidation of large lipid stores previously accumulated as the result of adaptive hyperphagia and diet switches prior to migration.

Chapter 12 critically evaluates several existing hypotheses used to explain the sequential changes that occur in organ and tissue mass loss during fasting and starvation. This chapter also advances a new hypothesis that the differential rates of protein turnover (measured as carbon turnover) among tissues is adequate to explain the variability in phenotypic flexibility commonly documented among the organs of fasting birds.

Chapter 13 characterizes the physiological responses to winter food limitation among several species of small herbivorous rodents. Some of these animals are capable of dramatic reductions in their body mass set-point in the face of food limitation. The chapter also underscores the role of photoperiod in triggering hormonal and physiological changes to prepare for severe seasonal food limitation.

Chapter 14 provides a comparative review of the morphological and histological changes in various regions of the gastrointestinal tract (e.g. hypotrophy and hypoplasia) resulting from short-term and prolonged fasting among major vertebrate groups. It pays particular attention to the relationship between changes in cellular form driving changes in physiological function, as well as the tradeoffs associated with gut flexibility (e.g. reduced energy costs versus diminished functionality).

Chapter 15 explains how starvation and fasting alter the chemical composition of body lipids. Specifically, it highlights how the various physiological processes of lipid deposition, mobilization, transport, and oxidation interact to cause

characteristic changes in the fatty acid composition of starving animals (e.g. increased polyunsaturated fatty acid concentrations and overall unsaturation indices).

Chapter 16 reviews the extent to which different types of bats face starvation and characterizes their physiological responses to food limitation. The chapter discusses how starvation tolerance is highly variable among species and tends to be correlated with feeding habits (e.g. insectivory, hematophagy, or frugivory). It also describes the energetic benefits of torpor and hibernation in these unique mammals.

Chapter 17 describes the sequential physiological changes that occur in grizzly bears and black bears in response to hibernation fasting. The chapter emphasizes the peculiar abilities of these animals to preserve structure and function of skeletal and cardiac muscles and to adaptively recycle nearly 100% of their urea nitrogen during fasts that may last several months.

Chapter 18 reviews how food limitation impacts the individual physiology and the population demographics of white-tailed deer. It presents a model that describes the relationship between daily food intake and weight change. Despite behavioral and thermoregulatory adaptations starvation remains one of the greatest selective forces regulating white-tailed deer populations directly through mortality and indirectly through reduced reproductive output.

Chapter 19 summarizes the physiological responses of seals and sea lions to prolonged fasting, during which they routinely lose one-third of their body mass. It describes how these animals are capable of efficient carbon recycling and glucose production and even enter a diabetic-like state during fasting. Apparently, females of some species are able to fast for one month while they simultaneously suckle their pups.

Chapter 20 is the first review to focus exclusively on the changes in stable isotope composition that occurs in fasting and starving animals. The chapter analyzes field and laboratory studies and offers a critical evaluation of the utility of ^{15}N and ^{13}C enrichment in tissues to detect nutritional stress. It also describes how $^{13}CO_2$ breath testing can be used to characterize the transitions between fasting phases.

Chapter 21 provides historical accounts of human starvation during the Victorian Era. Hunger strikes, poor prison conditions, and regional famines provided morbid opportunities for physicians and scientists to document the progression of physiological and psychological deterioration that accompanied starvation. It documents how knowledge gained from these unintended experiments informed the following century of clinical treatment for malnutrition in humans.

Chapter 22 presents a mechanistic computational model of human metabolism and bioenergetics that is able to accurately describe the changes in body mass, body composition, and ketone and nitrogen excretion during prolonged fasting. The model is validated using actual measurements from two classic experiments of prolonged fasting in obese and lean humans.

Chapter 23 introduces a form of dietary restriction called alternate day feeding (ADF) for clinical treatment of obesity. It contrasts this treatment with traditional

caloric restriction diets and summarizes recent experiments on animals and humans that demonstrate that ADF diets preferentially reduce fat from visceral compartments. This fasting regime appears to have secondary benefits including risk reductions for cardiovascular disease risk and type-2 diabetes.

Chapter 24 synthesizes concepts presented in the previous chapters and describes how recent progress in understanding starvation physiology has exposed a new horizon of research prospects in this field. It outlines specific research questions that have been previously overlooked but will promise to complement our recent progress and concludes by identifying three major areas of starvation research that deserve more detailed investigation.

San Antonio, TX, USA Marshall D. McCue

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Chapter 1
An Introduction to Fasting, Starvation, and Food Limitation

Marshall D. McCue

1.1 Introduction

All animals are heterotrophic and must continually collect and ingest organic molecules from their respective environments. Animals rely on these organic molecules (hereafter: food) to form their tissues and as the cellular fuel to carry out the basic goals of life: growth, survival, and reproduction. When animals, for whatever reason, fail to ingest sufficient amounts of food they are considered to be fasting or starving. Some researchers use these two terms interchangeably, but most distinguish between fasting and starvation. Such distinctions can be related to degree of severity (e.g., Castellini and Rea 1992; Wang et al. 2006) or by whether the absence of feeding is under endogenous or exogenous control (sensu McCue 2007). These different definitions have advantages and disadvantages when describing particular biological situations; the following chapters will use all three semantic approaches. What these researchers do agree upon is that all animals face the potential risk of food limitation.

1.2 Ancient History of Fasting and Starvation

Food limitation is not a new phenomenon. In fact, Earth's major extinction events involve ecological factors that undoubtedly caused mass starvation events (Baker 1921; Rhodes and Thayer 1991; Table 1.1). Volcanism and asteroid activity block light required by the photosynthetic producers underlying the global food web.

M. D. McCue (✉)
Department of Biological Sciences, St. Mary's University,
One Camino Santa Maria, San Antonio, TX 78228, USA
e-mail: mmccue1@stmarytx.edu

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Table 1.1 A summary of the five major extinction events and the likely role of starvation in them

Extinction	Time (mya)	Possible factors contributing to food limitation
Ordovician–Silurian	440–450	Gamma ray burst, volcanism
Late Devonian	360–375	Volcanism, marine anoxia, global cooling
Permian–Triassic	250	Volcanism, increased temperature, UV exposure
Triassic–Jurassic	200	Volcanism, increased temperature
Cretaceous–Tertiary	65	Asteroid, volcanism

Photosynthetic organisms are also sensitive to increased electromagnetic energy (e.g., UV and gamma radiation) that likely accompanied mass extinction events (Rhodes and Thayer 1991). Anoxia can disrupt marine food webs the effects of which can upset terrestrial ecosystems. The challenges stemming from reductions in food supply can be compounded by climatic changes (e.g., ice ages) that produce temperatures that may preclude foraging and digestion. At the same time extreme temperatures can raise the maintenance energy requirements of animals (Wharton 2002; McCue 2004). Most species that have ever existed became extinct during these mass starvation events (Erwin 1998; Myers 1997); however, extant species are not immune to the omnipresent challenge of food limitation.

1.3 Fasting and Starvation in the "Wild"

Field biologists studying all types of animals can testify that death from starvation is not an uncommon event among wild animals. Despite the fact that death from starvation can be a major factor regulating animal populations (see Ullrey, this volume) ecological theorists routinely overlook its biological significance. Elaborate population models of host–parasite and predator–prey interactions and theories of interspecific versus intraspecific competition and top-down versus bottom-up regulation have been debated in the literature for nearly a century (e.g., Real and Brown 1991); unfortunately none of these explicitly consider deaths from starvation separate from other fates. This oversight is surprising since starvation is often taking place alongside these other factors.

Naturalists have documented starvation-induced mortality of wild animals for over a century (Johnston and Fleischer 1981; Marsh 1912) and some early physiologists explored starvation biology during the first quarter of the twentieth century (Benedict 1915; Lusk 1928), but controlled investigations of starvation physiology were not common until the 1960s (see Lignot and Le Maho, Chap. 2). Nevertheless, in the ensuing 50 years comparative physiologists have learned a great deal from studying fasting and starvation in various species. These studies have shown that animals employ a variety of adaptive strategies for surviving situations where food is chronically limited or acutely absent.

In the simplest terms, starvation is a situation where an organism's mass and energy inputs do not meet its mass and energy requirements. Nevertheless, this apparent simplicity should not misinform students of starvation about the variety of adaptive strategies that animals employ to survive periods of food limitation (McCue 2010). These strategies include, but are not limited to: (1) avoiding starvation by migrating to habitats where food is not limiting, (2) storing large amounts of fat in their tissues, (3) adjusting mass and energy allocation among competing sinks, (4) reducing total energy expenditure, (5) differentially mobilizing energy rich molecules, and (6) adjusting physiological set-points to tolerate disruptions in homeostasis. The following chapters provide specific examples of each of these strategies.

1.4 Viewing Starvation in the Mirror

With the exception of some filter-feeders, most animals ingest and digest food periodically in discrete events and thus experience fasting as a regular part of life. Many animals even spend the vast majority of their lives in a fasted state (Hervant, Chap. 7; McCue et al., Chap. 8) or even never eat at all (see Kirk, Chap. 3). Fasting is even a common event in the lives of humans and our first meal of the day—break-fast—marks the ending of our own nocturnal fasting period.

People often associate prolonged human fasting with weight-loss dieting (see Hall, Chap. 22; Varaday, Chap. 23) or acts of civil disobedience (see Grant, Chap. 21) without recognizing that our relationship with prolonged fasting extends back to the dawn of our species. Among apes, humans are particularly well adapted to prolonged fasting. The unpredictability of food resources throughout our evolutionary history is believed to have shaped many of the physiological mechanisms that underlie our exceptional ability to store large amounts of energy when food is available (Chakravarthy and Booth 2006). Paradoxically, the same adaptations that we once evolved to cope with starvation now underlie many modern diseases (e.g., obesity and type-2 diabetes).

Today, the amount of cultural evolution eclipses any meaningful genetic evolution (Ayala 1986), but such "progress" does not fully protect us from starvation. In his essay, "Must Men Starve?" physiologist Max Kleiber writes, "In primitive times, the killing of newly born children and weak and old people was a horrible but necessary means of keeping the tribe alive during periods of severe food shortage". Although such behavior has no place in our modern civilization, human deaths caused by starvation apparently still do, particularly in developing nations. Human populations are expected to reach their carrying capacity within the next 100 years (Harrison and Pearce 2000) and starvation will undoubtedly become more common. Kleiber reassures us with poignant optimism that at that time "None of us will be there to measure the specific dynamic effect of man's flesh as man's food…" (Kleiber 1975).

1.5 Conceptual Perspective

August Krogh wrote, "Physiology does not go forward as an ordered line of battle on a continuous front, but must be carried on, as someone has aptly said, as a guerrilla warfare against the unknown, conducted singlehanded or by small units." (Krogh 1929). Research into fasting and starvation has progressed much in this way. The lines for these "battle fronts" parallel phylogenetic relationships and the "guerrilla units" are often unified by distinct methodological approaches. This volume represents the first attempt to transcend traditional phylogenetic and methodological boundaries and consolidate our current understanding of the physiology of fasting and starvation. Rooted in the classic framework of comparative physiology, the primary goals of this effort are to better understand how animals work (sensu Schmidt-Nielsen 1972, 1994) and to identify novel comparative patterns among organisms (sensu Feder et al. 1987; Huey and Bennett 2008; Prosser 1973, 1975). Nevertheless, the chapters in this volume explore contemporary themes in organismal biology including exploring the genetic and molecular responses to starvation, the integration of multiple organ systems, and the ecological physiology of food limitation.

This volume is arranged around a phylogenetic framework that is reflective of the active areas in fasting and starvation physiology. It is apparent that some animal taxa (e.g., mammals and birds) are disproportionally represented and other groups (e.g., invertebrates and herptiles) are less well represented. In addition to identifying groups of animals in which more research in starvation physiology is needed, this volume underscores instances of phylogenetic provincialism (sensu Lutterschmidt and Hutchinson 1997) in our field. It is not surprising that researchers working with different taxa tend to use their "own" techniques, but in many cases techniques routinely used on one group of animals could be beneficially applied to studies of other groups. I hope that juxtaposing chapters with different methodologies and perspectives will facilitate a fresh exchange of ideas among comparative physiologists.

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Chapter 2
A History of Modern Research into Fasting, Starvation, and Inanition

Jean-Hervé Lignot and Yvon LeMaho

2.1 Introduction

Although detailed scientific studies on starvation physiology have been conducted since the ninteenth century, there is little uniformity in the terminology used to describe it. The term "fast" usually applies at the end of the postprandial period (basal or postabsorptive state). In this state, however, the digestive tract may still possess nutrients that can still be absorbed.

This review will briefly mention short fasting periods, underfeeding, and calorie restriction within specific nutritional conditions (low food availability, special therapeutic diets). We will also detail the physiological effects of prolonged fasting (starvation), i.e., the exclusion of all food intakes except water and thus a complete deprivation of dietary energy over extended periods. We will also focus on the history of the major scientific advances detailing starvation physiology in vertebrates (Fish, Amphibians, and "Reptiles," but mainly Birds and Mammals). Further insights into the detailed physiology of these vertebrates can be found in previously published literature (e.g., McCue 2010) and the other chapters of this book.

Depending on the species, physiology during prolonged fasting depends on different parameters such as the preferential tissues for metabolic stores, the quantity stored, their availability, and the distinct routes of mobilization. Therefore, the capability of an animal to resist prolonged fasting is determined by its

J.-H. Lignot (✉)
UMR 5119 ECOSYM Université de Montpellier II—CNRS–IFREMER,
Adaptation Ecophysiologique et Ontogénie (AOE), cc 092, Place E. Bataillon,
34095 Montpellier Cedex 05, France
e-mail: Jehan-Herve.Lignot@univ-montp2.fr

Y. LeMaho
UMR 7178 Institut Pluridisciplinaire Hubert Curien (IPHC), Département Ecologie,
Physiologie et Ethologie (DEPE), CNRS—Université de Strasbourg, 23, rue Becquerel,
67087 Strasbourg Cedex 02, France

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ability to store energy and to control its allocation during periods of food restriction. The accumulation of large energy stores to anticipate periods of food shortage is also highly valuable to survival. Furthermore, during prolonged fasting, carbohydrate, fat, and protein stores are successively depleted, therefore delaying protein catabolism for as long as possible. At the cellular level, intracellular ions (potassium, phosphorus, and magnesium) move to the extracellular space, inducing a cellular depletion of these electrolytes despite a normal serum balance. Eventually, organ dysfunction may occur, leading to a potentially fatal reduction in cardiac, renal, immune, and other functions.

The duration of prolonged fasting can therefore markedly differ between species and among individuals. Some maintain low resting energy expenditure (e.g., ectothermic and inactive species) but others can sustain physiological activities while fasting (e.g., reproduction, lactation, or migration). The key physiological differences and adaptations have now been revealed, allowing a better understanding of the diverse strategies to optimize the prefasting period, the fasting period itself, and the refeeding phase.

2.2 Early Studies

The subject of complete and partial inanition has been of interest among physiologists and clinicians for many years. Early studies investigated the physiology of fasting and starvation in humans, dogs, cats, rabbits, domestic fowls, pheasants, and pigeons. These studies mostly concerned body mass loss, urine excretion, and ketosis, i.e., the increased levels in the blood of ketone bodies formed when the liver glycogen stores are depleted (Chossat 1843; Schultz 1844; Bidder and Schmidt 1932; Falk and Scheffer 1854; Voit 1866, 1901; Schimanski 1879; Rubner 1881; Howe et al. 1912; Benedict 1915; Phillips et al. 1932; Errington 1939). The first studies on rats and mice were conducted between 1900 and 1930 (Pembrey and Spriggs 1904; Jackson 1915; Benedict et al. 1932; Benedict and Fox 1931). The first scientific studies on humans were realized between 1870 and 1890 and mostly concerned "professional fasters" (reviewed in Benedict, 1911) (see also Grant, Chap. 21). An example of this was research carried out by Luigi Luciani, professor of physiology in the University of Rome, who studied a 30-day fast undergone by Giovanni Succi, one of the professional fasters in 1889. One of his contenders, the American Henry Tanner, fasted for 40 days, while in France, Alexander Jacques fasted 50 days, drinking only an herbal mixture. Three years earlier, Steffano Merlatti had fasted for 55 days and only drank pure filtered water. Like other "hunger artists," they were regularly accused of fraud during their "careers." Nevertheless, the scientific supervision of some of these fasts allowed urine analyses and the recording of body weight. In 1911, Benedict also examined prolonged fasting (31 days) in a Maltese man, Mr. Levanzin, and recorded body temperature, arterial pulse rate, blood pressure and chemistry, urine, and respiratory parameters among other variables (Benedict 1915).

Most of the data collected from these early studies have been reviewed (Lusk 1909; Morgulis 1923; Phillips et al. 1932; Keys 1950; Grande 1964; Peret and Jacquot 1972), and indicate that carbohydrate stores provide a small but significant component of body fuel at the beginning of the fast. Thereafter, the unique sources of fuel are protein, contributing 15% of the necessary calories, and fat (Benedict 1915). Prolonged fasting can induce a dramatic body mass loss of up to 60% of the prefast weight, with an increased rate of body mass loss when starvation is prolonged. Lipid reserves can be almost completely depleted and a 30–50% decrease of protein content leads mammals and birds to death (Schimanski 1879; Phillips et al. 1932; Errington 1939). Therefore, one of the few tissues remaining preserved during prolonged fasting appears to be the central nervous system, while skin and muscle masses are reduced from one-third to half. The proportion of muscle and protein masses appears stable or slightly increased in comparison to body mass. The capacity to prolong fasting also improves when body mass and adiposity levels are high at the beginning of the fasting period (see Champagne et al., Chap. 19).

A number of early studies examined urinary nitrogen excretion during fasting (Lusk 1909; Howe et al. 1912; Benedict 1915; Goldblatt 1925; Martin and Robinson 1932; Phillips et al. 1932). While nitrogen excretion is lowered at the beginning of the fasting period before reaching a plateau, proteins only account for 7–17% of the energy expenditure and lipids appear to be the main source of energy. The excretion level also appeared to be directly linked to prior diet to fasting. With a low protein prefasting diet, the lowering of nitrogen excretion during fasting may be minimal or nonexistant. Finally, although nitrogen excretion is lowered for several days as seen in domestic fowls (Schimanski 1879) and dogs (e.g., a female fox terrier or "Oscar," the adult Scotch collie (Howe and Hawk 1911; Howe et al. 1912), a "premortal rise" preceded by a slight decrease was also demonstrated in these early studies of single or repeated fasts (Voit 1866; Schimanski 1879; Howe and Hawk 1911; Howe et al. 1912). Proteins can account for up to 50% of energy expenditure (Phillips et al. 1932; Benedict and Fox 1931) during this "premortal" rise, attributed to cell degradation, autointoxication, and secondary infection (reviewed in Morgulis 1923; Keys 1950; Peret and Jacquot 1972). However, the absence of a typical premortal rise has also been reported (Benedict and Fox 1931). Therefore, both high and low protein catabolisms were observed as the loss in body weight increased from 40 to 50% in rats and dogs (Benedict and Fox 1931; Chambers et al. 1939). Interestingly, an increased starvation tolerance was observed in the low nitrogen group, suggesting that these animals had higher body fat stores. Finally, the results presented for humans in Benedict's book "A study of prolonged fasting" (1915) and those gained from the Arrow's study on catheterized dogs (total starvation) did not show any increase in protein catabolism and led Keys et al. in "The biology of human starvation" (1950) to refute the existence of this premortal phase.

From all the data gathered, Morgulis, nevertheless, divided the progressive changes occurring in the catabolism of body tissue during a prolonged fast into four periods, which each represented a loss of approximately one-eighth of the

original body weight (Morgulis 1923). Initially, glycogen stores decrease rapidly and the ability to oxidize carbohydrate also diminishes. In the second and third phases (intermediate phases), energy is mainly derived from stored fat and the mechanism for glucose oxidation is markedly suppressed to a minimum level. Finally, the fourth phase is usually characterized by a critical exhaustion of body fat to the point where endogenous protein is needed for fuel (a "premortal" rise in ammonia excretion and thus in protein metabolism).

2.3 Therapeutic Fasting in Humans

"Therapeutic fasting" in humans using either short fasting periods or prolonged underfeeding first appeared for the treatment of diabetes and was used from 1913 until the first use of insulin in 1922 (Allen 1915a, b; Heinrich 1916; Joslin 1916). It was recommended for the treatment of convulsive disorders such as seizures and epilepsy (Guelpa and Marie 1911; Geyelin 1921; Wilder 1921). Prolonged fasting was also described as a possible treatment for obesity, as early as 1915 (Folin and Denis 1915), and the metabolism of starvation was believed to have a special significance as an indicator of the lowest maintenance requirement of the body. Later, the effects of diet composition preceding fasting as initially observed by Schimanski (1879) were studied in further detail. As seen in rats, a high fat diet induces longer survival during subsequent fasting, and present better performance during exercise than individuals having eaten isocaloric high-carbohydrate or high-protein diets (Samuels et al. 1948).

The therapeutic potential of fasting as a possible treatment for obesity as initially described by Folin and Denis (1915) was later reemphasized (Bloom 1959; Drenick et al. 1964; Duncan et al. 1965). This treatment includes total starvation, intermittent fast, and semi-starvation. However, as early as 1965, Cubberly et al. reported one death that was attributed to lactic acidosis. Spencer (1968) also reported the deaths of two patients due to heart failure while they were undergoing therapeutic starvation at 3 and 8 weeks of total starvation, respectively. During the treatment of seven grossly obese patients with long-term fasting, a young woman was reported to have died on the 7th day of refeeding following a 30 week fast (Garnett et al. 1969). Autopsy revealed fragmentation of the cardiac myofibrils. These observed deaths occurred despite patients having huge fat stores at the time of their deaths. Although prolonged therapeutic starvation was still believed to be a safe and efficient procedure at the end of the 1960s (Runcie and Thomson 1970), several side effects and complications were observed during starvation. These include breakdown in electrolyte homoeostasis (Runcie and Thomson 1970), cardiac arrhythmias (Duncan et al. 1965), and severe orthostatic hypotension, as well as severe normocytic, normochromic anemia, and gouty arthritis (Drenick et al. 1964).

Therapeutic starvation was finally stigmatized as an unsafe procedure exposing the patient to an undue risk of physical danger (but see Varaday, Chap. 23). As an

alternative to fasting, very low calorie diets (VLCDs) were developed to preserve lean body mass while maximizing weight loss as illustrated by the low carbohydrate and fat diet (Stillman and Baker 1967), the high fat and protein diet, and the "last chance diet" high in liquid protein with supplementary vitamins and minerals (Bistrian et al. l976, 1977; Linn and Stuart 1976). The last chance diet, also called the "protein-sparing, modified fast diet" (PMSF), was one of the most popular diets in the 1970s and was recently remastered by Pierre Dukan (the Dukan diet). In this "last chance diet" most of the proteins were hydrolysates made from either collagen or gelatine. Here again, the diet required close supervision after a number of human deaths were attributed to the use of liquid protein products (Center for Disease Control 1977).

Experimentally, the classic study done by Benedict in 1911 on starving humans was repeated several times by Cahill and co-workers using normal, obese, and type 2 diabetic volunteers fasting for 8–60 days (Cahill et al. 1966, 1968; Owen et al. 1967, 1969; Ruderman et al. 1976). These studies showed insulin to be the primary regulator of fuel release: glucose from the liver, amino acids from muscle, and free fatty acids from adipose tissue. They also revealed that when carbohydrate stores are exhausted and glucose levels in the blood are too low during fasting (phase 1 of fasting in man); ketogenesis is subsequently initiated to make available energy that is stored as fatty acids (phase 2 of fasting in man) (Cahill 1970; Cahill et al. 1974; Saudek and Félig 1974; Balasse 1979). In the first phase of fasting, alanine and glutamine are the key amino acids involved in gluconeogenesis. The production of β-hydroxybutyrate and acetoacetate in the liver through fatty-acid β-oxidation also markedly diminish the need for muscle proteolysis to provide gluconeogenic precursors. During the second phase of fasting, the rate of gluconeogenesis is lowered due to a decreased use of glucose by the brain. Ketone bodies and thus, indirectly, lipid reserves fuel the brain for 50–60% of its energy needs (Owen et al. 1967). No data in existing literature therefore support the existence of a rise in protein utilization in fasting humans undergoing long-term therapeutic starvation, not even in those patients who died suddenly. It was demonstrated that the causes of these deaths were not due to an inability to spare overall body protein (fasting obese patients have a lower protein utilisation than nonobese persons) (Forbes and Drenick 1979; Van Itallie and Yang 1984), but could rather be attributed to a different ability in protein conservation of the various muscles (see Bauchinger and McWilliams, Chap. 12). This is seen with the myocardial mass that is not spared as illustrated in deaths occurring during the prolonged liquid protein diet, despite the improved overall nitrogen balance in this diet. More recently, a paradoxical increase in resting energy expenditure was observed in malnourished patients near death (Rigaud et al. 2000). This increase was associated with high urinary nitrogen losses, low serum fatty acid concentrations, and a very low fat mass. This profile has never been described in humans before and is similar to that described in the king penguin and other wild animals that naturally endure long fasting periods.

2.4 Integrating Data from Wild and Laboratory Models

It appeared from the different studies already mentioned that different metabolic phases can be reached depending on the species, age, and metabolic status of the individual before fasting and environmental cues such as temperature. These phases are related to modifications which occur during the catabolizing of tissues to provide sufficient energy to maintain physiological functions (Cahill 1970). In some species, rises in the rate of body mass loss and nitrogen excretion at the end of prolonged starvation are concomitant, whereas this phase is lacking in old and obese rats (Sprague–Dawley) and in humans (Cahill 1970; Goodman and Ruderman 1980; Goodman et al. 1980). Following the work carried out on humans and laboratory rats, studies performed on emperor and king penguins on-site from the mid 1970s onwards also added to our understanding of starvation physiology (Le Maho et al. 1976). Prolonged fasting in penguins has since been studied in great detail (Le Maho et al. 1976, 1981; Cherel and Le Maho 1985; Groscolas 1990; Cherel et al. 1995, 1988a, b; Robin et al. 1987, 1988).

Following the examination of changes in protein metabolism in fasting penguins, the three successive phases discovered in early studies on fasting (Schimanski 1879; Voit 1901) were further described (Le Maho et al. 1976, 1981; Goodman et al. 1980; Robin et al. 1987). The first phase (phase I) corresponds to a phase of transition between the fed state and starvation, during which the individual stops utilizing diet-derived energy. This transient phase is relatively short and lasts between several hours and several days, depending on the individual. It is characterized by a rapid decrease in daily protein losses, usually measured from nitrogen excretion. Throughout the following phase (phase II), daily protein losses remain approximately constant. This is the ketotic phase of fasting which is associated with protein sparing. The duration of this phase depends on the initial lipid mass and was shown to last for several days in rats to several months in obese geese, king penguin chicks, bears, and seals (Cherel and Le Maho 1985; Nelson 1987; Robin et al. 1987, 1988; Cherel et al. 1988a, b, c; Belkhou et al. 1991; Reilly 1991; Castellini and Rea 1992; Adams and Costa 1993; Atkinson and Ramsay 1995; Cherel and Groscolas 1999). As already pointed out by Voit (1901), the level of nitrogen excretion during phase II for any same body mass thus depends on the initial adiposity of the individual. Increased protein utilization is reflected by a rise in nitrogen excretion (Goodman et al. 1980; Le Maho et al. 1981) and characterizes the beginning of the terminal fasting phase (phase III).

Phase III is always a very brief phase, since the high daily protein losses quickly lead to the individual's death. During this phase glycemia and total plasma protein level start decreasing, and plasmatic concentration of uric acid increases while β-hydroxybutyrate values remain low and stable. Higher levels of corticosterone, the major avian glucocorticoid stimulating protein catabolism (Challet et al. 1995), are also observed during phase III (Cherel et al. 1988b; Robin et al. 1998) Therefore, wild animals that are "well-adapted" to long-term fasting achieve high levels of protein sparing during periods of fasting, with protein catabolism

contributing to only 2–10% of total energy expenditure (Nelson 1987; Robin et al. 1987, 1988; Cherel et al. 1988a, b, c; Reilly 1991; Castellini and Rea 1992; Adams and Costa 1993; Atkinson and Ramsay 1995; Cherel and Groscolas 1999), whilst these values can reach 20–40% in "non-adapted" species (Cherel et al. 1992; Lindgard et al. 1992). Furthermore, the absence of a late increase in net proteolysis in obese humans and rats (obese Zucker rats) was related to huge lipid stores which "masked" a lethal cumulative protein loss reached long before the exhaustion of fat stores (Cherel et al. 1992).

Studies conducted in the 1980 and 1990s on unexercised penguins, bears, and seals fasting spontaneously during part of their annual cycle were followed by others performed on fasting animals experiencing nutrient-demanding processes such as migration, lactation, growth, and development, for which the necessity to conserve proteins is also crucial (Costa 1991; Oftedal 1993; Piersma et al. 1993; Battley et al. 2001). An example of this is nonstop migratory birds, which have no access to supplementary water or nutrition during their multiday flight. They, therefore, have to carefully budget their body fat and protein stores to provide both fuel and life support (see Jenni-Eiermann and Jenni, Chap. 11). Some of these migratory birds can enter their third phase of fasting during the migratory season, as seen with one species of the Columbiformes order (the turtle dove) and nine other species of the Passeriformes (redstart, whinchat, whitethroat, flycatchers and warblers) (Jenni et al. 2000). The wide phenotypic flexibility of the gut and related organs can also allow migratory shorebirds and passerines to anticipate their seasonal long-distance migrations and to refuel at stopovers (Piersma et al. 1993; Klaassen and Biebach 1994; Hume and Biebach 1996; Piersma and Lindström 1997). Furthermore, high rates of fatty acid uptake by flight muscles are possible, partly due to a very high upregulation of the fatty acid translocase and fatty acid binding proteins during the migratory season (Pelsers et al. 1999; Guglielmo et al. 2002; McFarlan et al. 2009, see Price and Valencak, Chap. 15).

Concomitantly, new experimental models were also investigated, in particular the Burmese python and other snake species (Secor et al. 2000; Secor and Diamond 1995; Overgaard et al. 1999; Secor et al. 2000; Starck and Beese 2001, 2002; Secor 2003; Andrade et al. 2004; Lignot et al. 2005; Starck and Wimmer 2005; Ott and Secor 2007; Secor 2008; Cox and Secor 2008; Helmstetter et al. 2009a, b). These species with high fat reserves (although leaner than some other "well-adapted" vertebrates) can withstand long and unpredictable periods of fasting and can resume prolonged fasting by ingesting and digesting large prey items. Benedict and Fox (Benedict and Fox 1931; Benedict 1932) were the first to document the large postprandial metabolic response for the Burmese python. All the other studied species present a similar coordinated response with feeding and fasting, although to a lower degree. Cellular plasticity coupled with the up and down-regulation across the different organs and tissues of the digestive and cardiac systems is a spectacular example of the phenotypic plasticity that can occur in an organism adapted to infrequent feeding (Secor and Diamond 1997; Secor 2003, 2008; Andersen et al. 2005; McCue 2007a, b) (see also Campen and Starck, Chap. 9). This allows the snake to drastically reduce its resting energy

expenditure by up to 72% (McCue 2007a). Another selective advantage of down-regulating the digestive tract with fasting is the reduction in the cost of maintaining this energetically expensive system during long periods of fasting. This energy saving absorbs the additional cost incurred with postprandial upregulation of the quiescent gut (Secor and Diamond 1997; Secor 2003).

Other recently studied species include the marine iguana (*Amblyrhynchus cristatus*), (that is able to "shrink" by as much as 20% within 2 years of low food availability resulting from El Niño events; Wikelski and Thom 2000), aestivating anurans (Cramp and Franklin 2003; Cramp et al. 2005, 2009), and several hypogean salamanders (Hervant et al. 2001; Issartel et al. 2009, 2010). These newts live in caves or karstic and porous aquifers that are generally characterized by unpredictable and severe variations in trophic resources. They are able to tolerate up to several years without food, which may make them the best adapted vertebrates to fasting conditions (see Hervant, Chap. 7). Their general adaptive strategy, as seen with the urodel *Calotriton asper*, implies the selection of fasting adaptations similar to those observed for sit-and-wait foraging snakes. A study of this species, albeit over a short period of fasting, showed that hypogean individuals rely on a lower basal metabolic rate during fasting than their epigean counterparts, with higher energy reserves, a higher capacity for metabolic depression during food deprivation, and a higher reduction in utilization rates (Issartel et al. 2010).

The physiological effects of fasting in fish have been regularly documented for many species, especially for migrating and aestivating species as well as those that are commercially harvested (see Bar and Volkoff, Chap. 6). However, some results appeared contradictory and highly dependent on environmental conditions (Inui and Ohshima 1966; Love 1970, 1980; Dave et al. 1975; Weatherly and Gill 1987; Balasse 1979; Mendez and Weiser 1993; Navarro and Gutiérrez 1995, and for recent reviews, see Wang et al. 2006; Secor and Lignot 2010). These results, nevertheless, followed those obtained in other vertebrates. For example, the energetic costs of maintaining homeostasis during food deprivation appeared to be directly related to the animal's capacity to mobilize energy reserves such as hepatic glycogen and lipids, at least during the initial phases of fasting. As observed in examples such as carps, migratory eels, and Pacific salmon body mass loss can be drastically reduced by more than 80% (84% for male European eels after 52 months of pure fasting inducing a 13.5% decrease in body length) and up to 95% of the fat reserves can be used before catabolism of proteins starts, followed by that of carbohydrates (Pacific salmon) (French et al. 1983; Olivereau and Olivereau 1997; Shimeno et al. 1997). Prolonged fasting affects not only the digestive tract (Blier et al. 2007), but also ionic regulation, a function requiring high energy consumption (Polakof et al. 2006). There are contradictory data concerning the precise role of cortisol in regulating ion transport in fishes and the effects of cortisol during prolonged fasting remain unclear (Wendelaar Bonga 1997; Mommsen et al. 1999; Kelley et al. 2001; Peterson and Small 2004; Barcellos et al. 2010).

2.5 Refeeding after Prolonged Fasting

Over the last 20 years, refeeding after the late increase in nitrogen excretion characterizing prolonged fasting in mammals and birds has been repeatedly studied in the laboratory rat. These studies confirm that the third phase of fasting is reversible and is an essential part of the physiological adaptations to long-term food deprivation (Cherel and Le Maho 1991; Robin et al. 2008). The progressive decrease of water intake to almost zero in the third phase of fasting is progressively increased in the hyperphagic refed animals. They regain muscle mass before regaining body fat, and recover a fully functional digestive tract within less than 3 days (Cherel and Le Maho 1991; Dunel-Erb et al. 2001; Robin et al. 2008, see Lignot, Chap. 14). This hyperphagia [also documented in fish (Bélanger et al. 2002)] allows compensatory growth, and overcomes transient anorexia that may be present at the beginning of the refeeding period (Hamilton 1969). Furthermore, the extent of lipid depletion at the onset of refeeding directly impacts the body reserve restoration pattern, as illustrated by the preferential restoring of body lipids via a significant contribution from endogenous lipid production in phase III refed rats (Robin et al. 2008). Lipid use also depends on the intensity of the energy restriction (partial or total). If rats are given the ability to select their diet, refed animals following a phase II fasting period gradually decrease their preference for fatty diets (see also Overgaard and Wang, Chap. 5). In contrast, rats refed after a phase III fast increase fat intake first and then increase their protein intake (Thouzeau et al. 1995). Finally, and as previously seen in laboratory rats (Koubi et al. 1991), an "alarm signal" or "refeeding signal" has been described at the transition between phase II and phase III in fasting penguins (Robin et al. 1998; Groscolas et al. 2000). This can trigger behavioral changes such as egg abandonment and departure to refeed at sea (Robin et al. 1988; Le Maho et al. 1981; Groscolas 1990).

Since early scientific studies (see Benedict 1915), refeeding following prolonged fasting in humans has been proved to be difficult and a "refeeding syndrome" can occur, as observed with refed prisoners of war, hunger strikers, severely malnourished patients with anorexia nervosa and other critically ill patients (Schnitker 1946; Schnitker et al. 1951; Gentile et al. 2010) (see also Grant, Chap. 21). This potentially fatal syndrome is due to metabolic, intestinal and cardiorespiratory dysfunctions, as well as fluid and electrolyte imbalances (hypophosphataemia) (Crook et al. 2001; Hearing 2004) and stepwise nutritional replenishment (renourishment) is necessary before tolerating a full, unrestricted diet.

2.6 New Challenges

It is of wide biological and medical interest to elucidate the underlying mechanisms enabling metabolic adaptations occurring during prolonged fasting and at refeeding. For example, preventing loss of weight and lean body mass in critically

ill patients and avoiding the refeeding syndrome are major therapeutic objectives (Powell-Tuck 2007). Over the last 10 years, different specific studies have been carried out in laboratory rats and wild animals not only at the tissue level (e.g., intestines) but also on the major biomolecules involved (neuropeptide Y, agouti-related peptide, circulating hormones) (Habold et al. 2004, 2005, 2006, 2007; Bertile et al. 2003, 2007, 2009; Groscolas et al. 2008; Falsone et al. 2009; Spée et al. 2010; Gerson and Guglielmo 2011).

Leptin, prolactin and corticosterone, as well as proteolytic related and orexigenic genes have appeared as metabolic regulators during starvation (Bertile et al., 2003, 2007, 2009; Groscolas et al. 2008; Falsone et al. 2009; Spée et al. 2010; Gerson and Guglielmo 2011). For example, king and Adélie penguins starting their proteolytic third phase of fasting only abandon their egg when corticosterone levels are markedly high while prolactin levels have decreased (Cherel et al. 1994, 1995; Groscolas et al. 2008; Spée et al. 2010). Furthermore, phase II fasting penguins injected with corticosterone presented increased locomotor activity similar to that seen in phase III fasting birds (Spée et al. 2011). These data suggest that corticosterone and prolactin are two important hormones that certainly play a key role in the refeeding signal occurring in long-term fasting birds. It has also been suggested that corticosterone facilitates energy supply during endurance flight by regulating the composition of fuel types used during endurance flight, especially protein catabolism (Falsone et al. 2009).

Proteomic studies on phase II and phase III laboratory rat plasmatic fractions have also revealed differentially expressed levels of apolipoprotein A-IV, A-I, and E, haptoglobin, transthyretin, plasma retinol binding protein, and vitamin D binding protein (Bertile et al. 2009). It has been suggested that the marked reduction of apolipoprotein A-IV levels contributes to the refeeding signal, whilst other differentially expressed proteins during fasting were attributed to lipid metabolism and changes in insulin signaling. Finally, fatty acid transport in bird muscles markedly increases during migration (McFarlan et al. 2009) (see also Price and Valencak, Chap. 15). Nevertheless, the supplementary use of body protein catabolism during fasting still remains poorly understood. One hypothesis is that under dehydrating conditions, the catabolism of body protein could maintain water balance (Gerson and Guglielmo 2011). Migratory bats may also use convergent physiological processes during their flight (McGuire and Guglielmo 2009) (see also Ben Hamo et al., Chap. 16). For example, the cytosolic H-FABP is upregulated in the small brown bat *Myotis lucifugus* during hibernation, a physiological challenge which is highly dependent on fat stores, just like in migrating animals. (Eddy and Storey 1994; McGuire and Guglielmo 2009). Finally, fatty acid composition of the fat stores and the preferential use of n6PUFAs during exercise can modify metabolic rate and flight performance (Price and Guclielmo 2009; Guglielmo 2010; Price 2010).

Therefore, although still incomplete, a better understanding at the whole organism level of the integrated responses occurring during prolonged fasting is emerging. Close physiological comparisons between species are now possible (resting versus exercised fasting birds and mammals; fasting physiology in migratory birds and bats, preferential use of the different fatty acids, etc.). New and

exciting avenues for environmental research include the possibility of looking at severely disrupted populations and their growing food restrictions due to anthropization, climate change, reduced and more distant stop-over feeding sites, and so on. Optimizing the refeeding of fasting birds near death, unable to eat unaided and losing weight despite refeeding trials (e.g., oiled seabirds) is also a key challenge for applied research.

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Chapter 3
Starvation in Rotifers: Physiology in an Ecological Context

Kevin L. Kirk

3.1 Rotifers and Their Ecological Context

Rotifers are among the smallest and simplest animals. As a result, they have long been used as model organisms for the study of life history and population ecology. Planktonic rotifers live in a world where food abundance can change rapidly and where food limitation is common and sometimes extreme. The goal of this review is to synthesize the ecological importance of variable food levels in nature with the physiological and life history responses of rotifers to starvation in the laboratory.

There are about 2,000 described species in the phylum Rotifera, which is probably most closely related to acanthocephalan worms. There are two major classes of rotifers: Monogononta and Bdelloidea. In this review, I will focus on the monogononts, especially on the planktonic species. Most of these rotifers feed primarily on phytoplankton although some species also ingest bacteria and a few are carnivorous, eating protozoans and smaller rotifers (Gilbert and Bogdan 1984). Rotifers are eaten by crustacean zooplankton and juvenile fish.

Rotifer species vary widely in many ecologically important traits (Stelzer 2005; Thorp and Covich 2001; Wallace 2002). The planktonic species vary in body length from 80 to 2100 μm, and in dry body mass from 0.02 to 2.7 mg. Rotifers are comparatively short-lived, completing their entire life cycles in 2–16 days in the laboratory. Most are eutelic, meaning they lack cell division after egg hatch and so have a specific number of somatic cells. Rotifers are iteroparous and produce eggs individually, not in clutches. Eggs are quite large, ranging from 14 to 65% of the mother's body mass. Mature eggs are either released immediately into the water or carried by the mother until they hatch, depending on the species.

K. L. Kirk (✉)
Department of Biology, New Mexico Institute of Mining and Technology,
Socorro, NM 87801, USA
e-mail: klkirk@nmt.edu

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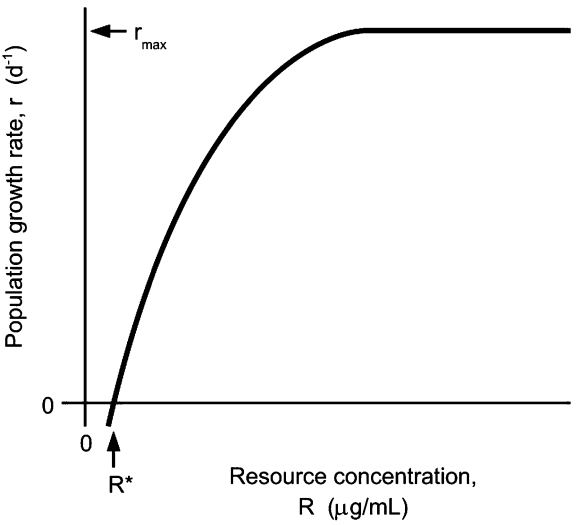

Fig. 3.1 A numerical response curve, showing how a consumer's population growth rate depends on resource concentration. R^* is the threshold resource concentration. r_{max} is the maximum population growth rate

Many species are cyclical parthenogens that can reproduce either asexually or sexually. Most offspring are produced by diploid, asexual (amictic) females making diploid eggs that hatch into amictic female clones. Occasionally, amictic females produce eggs that hatch into sexual (mictic) females. Mictic females produce haploid eggs that, if unfertilized, develop into haploid males, and if fertilized by a male's sperm, develop into diploid mictic resting eggs. One species, *Synchaeta pectinata*, is known to produce resting eggs asexually (Gilbert and Schreiber 1998). Each mictic resting egg contains a diapausing embryo that can survive in lake sediments for weeks to decades (Hairston et al. 1996). Resting eggs provide one way by which some species survive periods of low food or starvation. In lakes that freeze over in winter, mictic resting eggs remain dormant in the sediments and avoid a period of extremely low food levels. Rotifer males always starve. Males cannot feed, are much smaller than females, and have short lives spent swimming rapidly in search of mictic females (Thorp and Covich 2001; Wallace 2002).

A great deal is known about the ecology of planktonic rotifers, especially about the quantitative nature of their relationship to their food resources. The numerical response defines the relationship between resource abundance and consumer population growth rate (Fig. 3.1). Population growth rate has obvious ecological importance, as well as being a measure of evolutionary fitness that integrates information about both survival and reproduction. Two parameters describe the shape of a numerical response curve (Fig. 3.1). First, the population response to high resource levels is defined by r_{max}, the maximum population growth rate. This term defines the maximum rate of population increase when the animals are not resource limited. Second, the response to low resource levels is defined by R^*, the threshold resource abundance. R^* is the minimum resource abundance necessary

to obtain a positive population growth rate. When species compete in a constant environment, resource competition theory predicts that the species with the lowest R^* value will outcompete all other species (Grover 1997). Laboratory competition experiments with rotifers confirm this prediction (Kirk 2002; Rothhaupt 1990).

Resource competition theory predicts a tradeoff between R^* and r_{max} (Grover 1997). Species with a low R^* (a beneficial trait at low food levels) are not expected to also have a high r_{max} (a beneficial trait at high food levels). Species with the lowest R^* values are at one end of the tradeoff continuum and are called "gleaners", while species with the highest r_{max} values are at the other end of the tradeoff and are called "opportunists" (Grover 1997). Planktonic rotifers provide an excellent example of the gleaner versus opportunist tradeoff. In addition, the position of a species on the tradeoff continuum is a function of body mass; large rotifer species tend to be opportunists, while small species tend to be gleaners (Stemberger and Gilbert 1985).

Numerical response curves are obtained in the laboratory using constant food levels, but the real world is rarely constant. Fluctuations in food levels are common in lakes and ponds. A period of high wind can cause deep, nutrient-rich waters to be mixed up to shallower depths, where they stimulate phytoplankton growth, resulting in a pulse of high food for herbivorous rotifers. Food levels may then decline rapidly, as the high population growth rates of rotifers causes a population explosion of consumers that rapidly deplete the phytoplankton. Cryptomonad phytoplankton are a preferred, nutritious food for rotifers (Gilbert and Bogdan 1984; Stemberger 1981), and data from frequent sampling of cryptomonad abundance in a eutrophic pond demonstrate that food abundance can vary widely and rapidly, on timescales of a few days to a few weeks (Stewart and Wetzel 1986).

It should be noted that quantifying the abundance of phytoplankton can only give an approximate measure of the actual food abundance in nature, because the edibility and nutritional quality of phytoplankton can vary between species, between strains, and with the growth conditions of the phytoplankton (e.g., Sterner et al. 1993). Food supplementation experiments provide an alternative method for estimating food abundance in nature. In food supplementation experiments, the intensity of food limitation is used as an objective estimate of food quantity and quality. The intensity of food limitation can be expressed as Δr, the difference between the population growth rate of rotifers fed supplementary nutritious food and the population growth rate of control rotifers fed only the foods that were available in nature at the time of the experiment. In a given experiment, a high Δr means that food abundance, quality, or both are low, while a low Δr means that the existing mixture of phytoplankton in the lake is sufficient to nearly saturate the rotifer's numerical response.

Many food supplementation experiments have been conducted (Cordova et al. 2001, merriman and Kirk 2000, gonzalez and Frost 1992 see also McCue et al. Chap. 8), and from these we can arrive at some generalizations about the food environment experienced by herbivorous planktonic rotifers. First, food limitation is common. Food limitation was found in 12–100% of the experiments conducted

on five rotifer species in four lakes and ponds (reviewed in Cordova et al. 2001). Second, the frequency and intensity of food limitation varied among species. For example, in a mountain pond, *Synchaeta oblonga* was more often, and more intensely, food limited than *Keratella cochlearis* (Cordova et al. 2001). Third, the intensity of food limitation often varied greatly over time. For example, a series of 19 experiments conducted on *Keratella cochlearis* in a eutrophic pond showed that Δr ranged from near zero to near the maximum value possible (where $\Delta r = r_{max}$) over about 1 month (Merriman and Kirk 2000). Fourth, food limitation is often intense, as shown by Δr values that were close to the maximum possible in several instances (Merriman and Kirk 2000; Cordova et al. 2001). Episodes of intense food limitation can be interpreted as episodes of starvation or near starvation.

3.2 Life History Responses to Starvation

Because food levels are so variable, knowledge of R^* and r_{max} are not sufficient for understanding how rotifers cope with their food environment. It is also useful to know the starvation resistance of rotifers. Starvation resistance is determined by the animals' ability to store resources in the body during times of high resource abundance and to control their use during times of very low or zero resource abundance. In the context of resource competition theory, species with extremely high starvation resistance are called "storage specialists" (Grover 1997).

The simplest and best way to characterize an animal's starvation tolerance is to place the animal into an environment without food and observe how long it survives. Kirk (1997) compared the starvation times of nine species of planktonic rotifers, using laboratory experiments in which young adult amictic females were placed in individual volumes of filtered medium containing no food. Based on allometric relationships between body mass and metabolic rate, and between body mass and the expected size of energy reserves (e.g., Threlkeld 1979), starvation time was expected to increase with body mass (see also Overgaard and Wang, Chap. 5 and Hohtola, Chap. 10). Surprisingly, rotifer starvation time was not correlated with body mass (Kirk 1997).

When reproductive rate (fecundity) was measured during starvation, Kirk (1997) found that some rotifer species continued to reproduce when starved. In fact, some species increased their reproductive output. Other species acted like most other starving animals (McCue 2010) and reduced or stopped reproduction on transfer to zero food. Comparisons of the nine rotifer species revealed a strong inverse relationship between fecundity during starvation and starvation time. The tradeoff between fecundity and survival remained robust when fecundity during starvation was expressed as a percentage of the fecundity of fed controls, and when starvation survival was expressed relative to the survival of fed controls (Fig. 3.2). Data from two species illustrate these distinct responses. *Keratella cochlearis* stopped producing eggs within 2 days of transfer into zero food, its total fecundity when starved was only 6% of that of fed controls and it had one of the longest

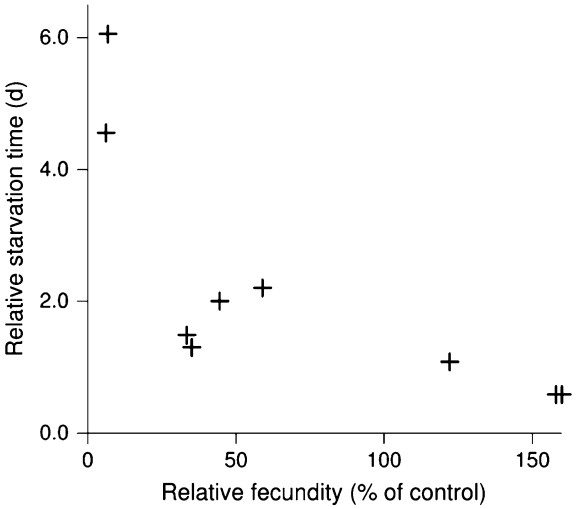

Fig. 3.2 The tradeoff between fecundity during starvation and starvation time for 10 species of planktonic rotifers. Relative fecundity is the total number of eggs produced per starving female, as a percentage of the total number of eggs produced per well-fed female, over the same time period. Relative starvation time is the time when the survival of a group of starved animals became less than 50% of the survival of a group of well-fed animals. Redrawn using data from Kirk (1997)

absolute starvation times, 4.9 days. In contrast, *Synchaeta pectinata* actually increased fecundity when starved to 160% of fed controls and it had an extremely short absolute starvation time, 0.7 days (Kirk 1997).

Weithoff (2007) conducted similar experiments on the rotifers *Cephalodella* sp. and *Elosa woralli*, and found a similar tradeoff of survival and reproduction during starvation. When placed into zero food, *Cephalodella* continued to produce eggs and had a low starvation time (about 2 days). In contrast, *Elosa* stopped reproduction at starvation onset and survived much longer (about 6 days). Yoshinaga et al. (2003) examined the starvation response of young adult *Brachionus plicatilis*. When animals were starved beginning at age 1 or 2 days, they stopped reproduction immediately and actually lived longer than fed controls.

The tradeoff between starvation survival and starvation fecundity is an example of the general life history tradeoff between survival and reproduction. Such tradeoffs are likely to be strongest under conditions of low resource availability (Stearns 1992) and may result from allocation constraints (Fig. 3.3). When adult animals are fed, the energy or materials assimilated can be allocated either to somatic maintenance (and thus to survival), to storage, or to reproduction. Under starvation conditions, resource assimilation is no longer possible and animals are faced with the problem of how to allocate their stored reserves among competing sinks (Fig. 3.3).

Most animals cease reproduction when deprived of food (McCue 2010). This response presumably evolved to increase the chances of surviving the starvation episode and then of recommencing reproduction when food becomes available. The rotifer species that are exceptions to this common pattern may have evolved in environments where episodes of starvation exceeded the animal's typical lifespan. Such an environment might favor allocation towards reproduction when food levels drop to zero, because it would be unlikely that an adult would survive a

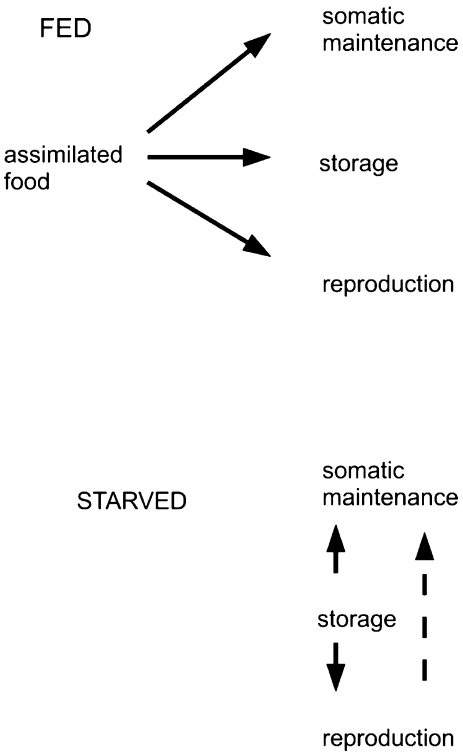

Fig. 3.3 Options for the allocation of energy and materials by adult animals that are either fed or starved. The *dotted line* indicates the reallocation from reproduction to somatic maintenance that was observed in starving *Synchaeta pectinata* by Stelzer (2001)

starvation episode. At this point, we do not know enough about the either resource fluctuations in nature or the variety of physiological and life history strategies available to rotifers to fully understand the evolutionary reasons for continued reproduction during starvation.

In the starvation experiments described above, rotifers were provided with high food levels prior to starvation onset. This was done to ensure that all species began the experiments at the same physiological state. In the natural environment, however, it is more likely that periods of starvation would be preceded by periods of low food levels. When *Brachionus calyciflorus* and *Synchaeta pectinata* were fed low food prior to starvation onset, they were less resistant to starvation. This effect was especially pronounced in *Synchaeta*: when acclimated to a low food level its starvation time declined to almost half that of animals acclimated to a high food level (Kirk 1997). Animals that were food-limited prior to starvation may have had a smaller reserve size than did animals fed maximally prior to starvation. Thus, these rotifers do not use low food levels in the present as a signal to increase their allocation to stored reserves in "anticipation" of a possible starvation episode in the future.

Different life stages have different options with regard to how they allocate materials within their body, and so may have different responses to starvation. The starvation times of juvenile *Brachionus calyciflorus* (Kirk 1997) and juvenile

Brachionus plicatilis (Yoshinaga et al. 2003) were greater than the starvation times of young adults. This suggests that rotifer eggs are provided with large amounts of reserves by the mother, more than are required to simply complete embryonic development. Moreover, starvation tolerance is further extended by the fact that juveniles need not allocate energy to reproduction. The high starvation tolerance of juvenile rotifers may have implications for those species that continue to reproduce when starved. In those species, a juvenile from a newly produced egg may have a greater chance of surviving a starvation episode than does its adult mother. This possibility underscores the fitness advantage of starving mothers to focus her resources toward reproduction.

Relatively little is known about the starvation response of bdelloid rotifers, but it appears that they have unusually high starvation resistance. Ricci and Perletti (2006) found that the median starvation time of the bdelloid *Macrotrachela quadricornifera* was about 40 days. The long starvation time of this bdelloid may be related to the high stress resistance of bdelloids in general (Gladyshev and Meselson 2008).

3.3 Life History Responses to Low Food

The response to zero food is likely to be related to the response to low food. Many types of animals, from rotifers to rats, live longer when fed submaximal food levels (see Varaday Chap. 23). The longevity response to dietary restriction may, in part, have its origins in a change in physiological allocation patterns at different food levels (Fig. 3.3). When food level declines, animals may shift their allocation away from reproduction and towards somatic maintenance, thus prolonging survival and making reproduction possible in a future, more favorable environment (Kirkwood and Shanley 2005). The anti-aging effect of dietary restriction may have evolved as an adaptation to life in variable environments, in particular to environments with frequent episodes of starvation (Holliday 1989; Masoro and Austad 1996).

In a comparative analysis of 10 rotifer species, Kirk (2001) found that most, but not all, species of rotifers increased their lifespan and slowed their rate of aging when grown in low food concentrations. There was a large amount of interspecific variation, ranging from a 130% increase in lifespan under dietary restriction (*Asplanchna girodi*) to a 29% decrease in mean life span (*Brachionus plicatilis*). Most significantly, there was a connection between the response of a species to low food and its response to starvation. Those species that continued to reproduce during starvation did not increase longevity under dietary restriction. The two extremes of this pattern are shown by comparing *Brachionus calyciflorus* and *Synchaeta pectinata*. *Brachionus* increased its lifespan to 136% of well-fed controls when under dietary restriction and its fecundity when starved was only 45% of well-fed animals. In contrast, *Synchaeta* decreased its life span to 48% of well-fed controls when under dietary restriction and its fecundity when starved was 160% of well-fed animals (Kirk 1997, 2001).

Weithoff's (2007) experiments on *Cephalodella* and *Elosa* confirm the connection between the response to low food and the response to starvation. *Cephalodella*, which continues to reproduce when starved, showed no increase in life span in response to dietary restriction. In contrast, *Elosa*, which stops reproduction when starved, increased its life span by about 50% in response to dietary restriction (Weithoff 2007).

3.4 Physiological Responses to Starvation

At the organismal level, starvation resistance depends on three physiological parameters: the size of the body's reserves that were built up during periods of high food, the rate of use of the reserves during starvation, and the pattern of allocation of the reserves to various physiological functions.

Metabolic rate is a good measure of how quickly reserves are used during starvation. It is common for many macroscopic animals, including humans, to reduce their whole-body metabolic rates during starvation (McCue 2010). Kirk et al. (1999) measured how the metabolic rates of four planktonic rotifer species changed during starvation. There was considerable variation between species. Some rotifers reduced metabolic rates when starved, and some did the opposite. After a time interval equal to the median starvation time, the metabolic rates of surviving animals varied from 148% (*Asplanchna priodonta*) to 42% (*Brachionus calyciflorus*) of the initial, well-fed metabolic rate.

To determine the size of usable material reserves, Kirk et al. (1999) placed well-fed rotifers into zero food conditions. At various time intervals, groups of rotifers were sampled, dried, and weighed. This procedure was continued until most of the rotifers had died of starvation. The minimum body mass was defined as the body mass just prior to death by starvation. The difference between the initial body mass and the minimum body mass was used as an index of the quantity of stored reserves. Reserve size varied from 42 to 71% of body mass.

Interspecific comparisons revealed some interesting relationships between physiology and survival during starvation (Kirk 1997; Kirk et al. 1999). As expected, starvation time was positively correlated with reserve size: species with larger reserves were able to survive for longer periods at zero food. However, there was no interspecific correlation between starvation time and either metabolic rate, or changes in metabolic rate, during starvation. Thus, both reserve size and fecundity during starvation were more important for starvation survival than was the rate of energy expenditure.

Stelzer (2001) conducted detailed investigations into the responses of *Synchaeta pectinata* to low food and starvation conditions. He was particularly interested in how the animal changed its pattern of allocation in different environments. Because *Synchaeta* has transparent body walls, Stelzer was able to quantify allocation patterns in living animals by measuring the volume of the ovary (the primary storage site) and the volume of each egg as it was produced. As an egg is made, the

volume of the oocyte grows as cytoplasm moves into the egg from the ovary vitellarium (yolk gland). By quantifying this volumetric change, Stelzer (2001) was able to calculate an index of allocation towards reproduction within a given egg-laying interval. This index, called the reproductive effort, was defined as the proportion of total ovary volume before egg deposition that was used for the production of the egg.

Life history theory predicts that the optimum reproductive effort should often increase as food levels decrease (Stearns 1992). This makes sense, because low food levels are likely to reduce the survival of adults and so reduce their chances of reproducing in the future. Under low food conditions, it is advantageous to reproduce now, i.e., to increase the current reproductive effort. Stelzer (2001) results support the theoretical prediction; as food level declined from high (about half that needed for r_{max}) to low ($<R^*$), the reproductive effort of *Synchaeta* increased from 0.36 to 0.92. In other words, at very low food levels rotifer mothers allocated 92% of their ovary volume towards the production of one last egg (Stelzer 2001).

When *Synchaeta* adults were starved, comparisons between individuals indicated that the size of the ovary was positively correlated with starvation time (Stelzer 2001). This implies that the ovary contents can be used not only for reproduction, but also for somatic maintenance to enhance starvation survival (see also McCue et al. Chap. 8). Starved *Synchaeta* were even observed to resorb material from developing eggs back into the ovary (Stelzer 2001). In other words, *Synchaeta* can reallocate resources previously alloted to reproduction, and use them to enhance starvation survival (see Fig. 3.3).

3.5 Optimal Allocation Theory

The success of an animal in an environment with variable food levels depends on how it allocates resources within its body. Different environments, with different temporal patterns of food levels, may select for different types of allocation strategies. Evolutionary ecologists have attempted to understand the relationship between allocation patterns and fitness (e.g., Fischer et al. 2009). Shertzer and Ellner (2002) formulated a dynamic energy budget model for rotifers, in which allocation decisions are determined by the current state of an animal, for example, body mass, storage mass, and life stage. The model was parameterized using the characteristics of *Brachionus calyciflorus*. It provided predictions of the optimal allocation towards stored reserves in a variety of simulated environments, having different probabilities of the occurrence of starvation episodes. Shertzer and Ellner (2002) predicted that environments with a higher probability of starvation episodes will select for allocation strategies that result in smaller adult body mass, increased reserve size (as a fraction of total body mass), and longer starvation times. The model was also used to predict starvation times for *Brachionus calyciflorus*, and the predictions closely matched data from Kirk (1997).

3.6 Future Research

Rotifers have given us insight into the variety of responses that animals can have to both starvation and very low food levels. There are, however, areas where we know relatively little about rotifers. We need to know more about the biochemical composition of the body reserves that are used by rotifers during starvation. This knowledge could be used to formulate methods to determine the physiological state of animals collected in the wild (Stelzer 2001; Tessier and Goulden 1982). Also, we need additional ways to quantify the allocation of resources in the bodies of living rotifers. This data would enable us to observe more subtle changes in allocation during various levels of food limitation and starvation. Finally, we need more information about the intensity of food limitation and the frequency of starvation experienced by rotifers in their natural habitats. This would help us formulate models with which to understand the evolutionary explanation for the dramatic diversity of the response of rotifers to low food levels and starvation.

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Chapter 4
Drosophila as a Model for Starvation: Evolution, Physiology, and Genetics

Allen G. Gibbs and Lauren A. Reynolds

4.1 Introduction

Drosophila melanogaster is one of the primary genetic models for understanding how nutritional limitation affects cellular physiology, because many of the molecular and cellular signaling pathways are shared among invertebrates and vertebrates. To a lesser extent, it is a model for organismal responses, although differences in endocrine systems sometimes make the link to vertebrates one of analogy rather than homology. *Drosophila* is also an excellent model for the evolution of starvation responses. The evolutionary history of the genus has been well studied, and *D. melanogaster's* short generation time and ease of maintenance have allowed experimental evolution studies on starvation resistance. We review here studies of starvation in *Drosophila* at multiple levels of organization, from species to molecules. A great advantage of *Drosophila* is the ability to traverse these levels relatively easily, and information across all levels is now being integrated in many labs around the world.

It is important to recognize at the outset that *D. melanogaster* is only a *model* for other species, including other *Drosophila* species. We were charged with reviewing the physiology of starvation specifically in *Drosophila*, and so we do not refer to the large and interesting body of related work done with *Manduca, Locusta, Bombyx*, and a wide variety of other insects. The literature on *Drosophila* alone is extensive—our recent Web of Science search for "drosophila and feeding" returned nearly 2000 citations. This review will therefore necessarily skim the surface and omit a great deal of interesting information about starvation in *Drosophila*.

A. G. Gibbs (✉) · L. A. Reynolds
School of Life Sciences, University of Nevada, Las Vegas, NV 89154-4004, USA
e-mail: allen.gibbs@unlv.edu

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4.2 Starvation Resistance in Natural Populations

The role of starvation stress in the ecology of *Drosophila* species is very poorly understood; in fact, the ecology of *Drosophila* in general is poorly understood. It is clear, however, that *Drosophila* species vary greatly in their ability to survive starvation stress. van Herrewege and David (1997) found that *Drosophila* species differed up to 5-fold in their survival in humid air. Starvation resistance was highly temperature dependent, with flies surviving approximately twice as long at 17°C as at 25°C. Species from temperate regions tended to survive longer than tropical species. The temperate species studied also tended to be larger, which may have contributed to longer survival times (Fig. 4.1). On the other hand, flies from temperate populations of two species were larger than tropical congeners, but size had little effect on starvation resistance.

Many *Drosophila* species have broad geographic ranges, allowing intraspecific studies of local adaptation in starvation resistance. The Indian subcontinent has been particularly well studied. Northern populations of several species have lower starvation resistance compared to southern, subtropical populations (Parkash et al. 1994; Parkash and Munjal 2000; Sisodia and Singh 2010). Starvation resistance also increases with latitude in Australian populations of *D. birchii* (Griffiths et al. 2005).

In eastern North America, an opposing latitudinal cline occurs. Populations of *D. melanogaster* in the north are more starvation resistant than southern populations (Schmidt et al. 2005; Schmidt and Paaby 2008). Robinson et al. (2000) also found no correlation between latitude and starvation resistance in *D. melanogaster* from South America. In Australia, differences in starvation resistance between populations of *D. melanogaster* were found, but these were not correlated with environmental conditions (Hoffmann et al. 2001, 2005; Hoffmann and Weeks 2007), whereas Philippine *Drosophila* species varied within, but not among, populations (van der Linde and Sevenster 2006).

The explanation(s) for differing geographic patterns in starvation resistance are not clear. Parkash and Munjal (2000) argue that tropical populations are more susceptible to starvation because of higher metabolic rates related to high habitat temperatures. In North America, northern populations of *D. melanogaster* are more likely to undergo reproductive diapause under simulated winter conditions (Schmidt et al. 2005). Schmidt and Paaby (2008) concluded that females able to use reproductive diapause to overwinter are more resistant to stress in general, including starvation. Australian populations also differ in reproductive patterns in the winter (Mitrovski and Hoffmann 2001; Hoffmann et al. 2003), suggesting a potential link between reproduction and stress resistance.

It should also be noted that the latitudinal ranges for these studies differ. For example, the northernmost Indian populations studied were from similar latitudes to the southernmost North American populations. Differing types of selection at the extreme latitudes could result in higher starvation resistance in both regions. For example, global scale atmospheric circulation patterns (Hadley cells) create

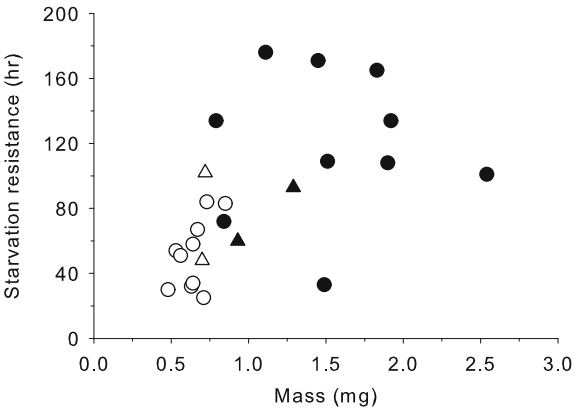

Fig. 4.1 Starvation resistance of 22 species of *Drosophila.* Male flies were assayed at 25°C. *Open circles*, tropical species; *filled circles*, temperate species. *Triangles* indicate tropical and temperate populations of *D. melanogaster* and *D. simulans*. Data modified from van Herrewege and David (1997)

generally lower humidity approximately 30° north and south of the equator. Natural selection for surviving desiccation could tradeoff against starvation resistance (Parkash et al. 1994; Parkash and Munjal 2000; Parkash et al. 2012).

An alternative to comparative studies of starvation resistance is to study its evolution in the laboratory. *Drosophila melanogaster* is a widely used experimental model for the evolution of stress resistance (Garland and Rose 2009). The use of replicated populations (and unselected control populations) under controlled conditions allows correlations and tradeoffs between traits to be assessed and tested in a rigorous manner, although laboratory environments are not necessarily as simple as they appear (Gibbs and Gefen 2009). Starvation resistance evolves rapidly when populations are subjected to strong selection each generation (Rose et al. 1992). Selection on a poor diet (lemons) also results in increased starvation resistance (Harshman et al. 1999). Most studies have involved selection for adult starvation resistance, but at least one study on larval selection has been performed (Kolss et al. 2009).

4.3 Physiological Mechanisms of Starvation Resistance

At the organismal level, there are three mechanisms by which starvation resistance can be increased, as illustrated in Fig. 4.2. Animals can store more energy (lipids, carbohydrates, protein), they can consume it at a slower rate, or they can tolerate loss of a greater fraction of their initial energy supply. These mechanisms are not mutually exclusive. A fourth, behavioral strategy is cannibalism. When flies are starved in groups, in principle the longest survivors can consume those that have already died. This behavior is not seen in wildtype flies (Huey et al. 2004), but could evolve in starvation-selected populations.

Starvation resistance is positively correlated with lipid content among different *Drosophila* species (van Herrewege and David 1997; Bharathi et al. 2003). In fact,

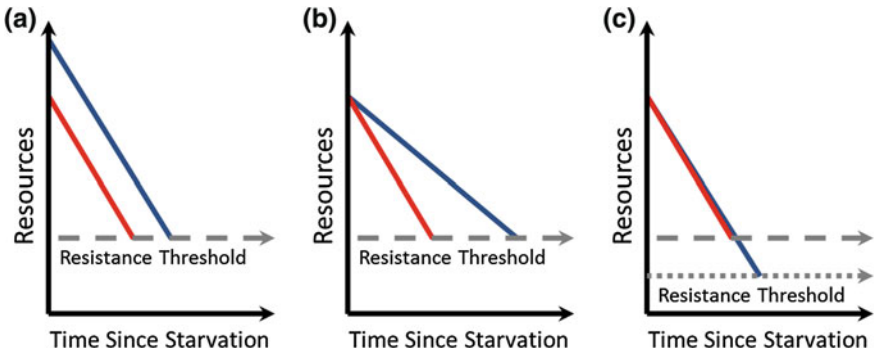

Fig. 4.2 Potential organismal mechanisms to increase starvation resistance. **a** Increased energy storage. **b** Reduced energy consumption. **c** Lower energetic threshold for mortality

the differences between tropical and temperate species seen in Fig. 4.1 are largely due to higher relative lipid content. Similar correlations between lipid content and starvation resistance occur within species (Parkash et al. 2005; Ballard et al. 2008; Sisodia and Singh 2010), although Jumbo-Lucioni et al. (2010) found that these traits were not genetically correlated in a set of 40 inbred lines. Greatly increased lipid storage is a consistent finding in starvation selection experiments (Chippindale et al. 1996; Djawdan et al. 1997; Harshman et al. 1999; Schwasinger-Schmidt et al. 2012). Lipid contents are generally much higher than in natural populations, suggesting that lipid storage has an evolutionary cost. Carbohydrates have received far less attention than lipids as energy stores, but also increase under starvation selection (Djawdan et al. 1997). Thus, energy storage, particularly in the form of lipids, is a consistent marker for starvation resistance.

The relationship between metabolic rates and starvation resistance is murkier. Surprisingly, no systematic comparative studies of metabolic rates in natural populations of *Drosophila* appear to have been done, at least not in the context of starvation stress. Metabolic rates differ substantially among species (Gibbs et al. 2003; Marron et al. 2003). Some of this variation may be related to water conservation, as desert (cactophilic) *Drosophila* have lower metabolic rates than other species after correction for body size and phylogenetic relationships (Gibbs et al. 2003). Tolerance of low energy content has not been studied (Rion and Kawecki 2007).

In starvation selection experiments, the evidence for evolution of reduced metabolism is mixed. Starvation-selected flies often have lower mass-specific metabolic rates than controls (Djawdan et al. 1997; Harshman et al. 1999). However, they are also larger because of their greater energy stores; when this is taken into consideration metabolic differences may disappear (Djawdan et al. 1997). Baldal et al. (2006) found that starvation-selected females actually tended to have higher metabolic rates than controls in the absence of food. No differences were seen when food was present, but metabolic rates are consistently lower when flies are starved than when they are fed (Djawdan et al. 1997; Baldal et al. 2006).

Harshman and Schmid (1998) also found no relationship between metabolic rates and starvation resistance. More recently, Schwasinger-Schmidt et al. (2012) found some support for the idea that starvation-selected flies are less active, and therefore should have lower metabolic rates (see also Hervant, Chap. 7). In summary, lower metabolic rates may contribute to increased starvation resistance in *Drosophila*, but their contribution is inconsistent and is certainly less significant than differences in energy storage.

4.4 Starvation and Life History Traits

A fundamental tradeoff in life history evolution exists between allocation of resources to survival and reproduction (see also Kirk, Chap. 3). This tradeoff can be alleviated by acquiring more resources (de Jong 1993), as exemplified by lipid accumulation in starvation-selected populations of *Drosophila*. Resource acquisition may have its own costs, however. Starvation-selected flies take longer to develop (Chippindale et al. 1996; Harshman et al. 1999) and have lower fecundity than controls (Wayne et al. 2006; Kolss et al. 2009). This is despite their larger body size and higher lipid content, factors that are generally correlated with higher fecundity in insects.

This conundrum may be explained by the complex life cycle of *Drosophila*. Holometabolous insects have striking differences in life history from vertebrates. In the case of *D. melanogaster*, eggs hatch into a larva that is essentially a feeding and growth machine. Over 3 days, the larva increases in mass by approximately 200-fold (Church and Robertson 1966). Soon thereafter it enters a 15–24 h wandering phase, during which it ceases feeding, leaves the media, and searches for a pupation site. The larva selects a spot, secretes a glue protein that adheres the animal to the substrate, and undergoes metamorphosis. Approximately 4 days later, an adult fly emerges from the pupal case. The adult feeds and allocates resources between somatic maintenance and reproduction. Thus, the life history of *Drosophila* can be broadly separated into 3 nutritional states: a feeding and growth stage, a non-feeding period lasting from late larval through early adult development, and a feeding but non-growing adult stage.

Drosophila pupae consume less than half of their stored lipids during metamorphosis, so flies eclose to adulthood with an energetic reserve (Merkey et al. 2011). Starvation-selected adults eclose with greater lipid stores than unselected controls, so that differences in energy storage occur before adulthood as well as in the young adult (Chippindale et al. 1996). This may be achieved by higher larval feeding rates to grow faster, extending the larval feeding period, reduced energy expenditure during metamorphosis, or some combination of these. Pre-adult stages of starvation-selected lines have not been well characterized, but selected lines do have longer egg-to-adult development times, suggesting a longer feeding period (Chippindale et al. 1996). Within these populations, individuals with longer development times also survived starvation longer.

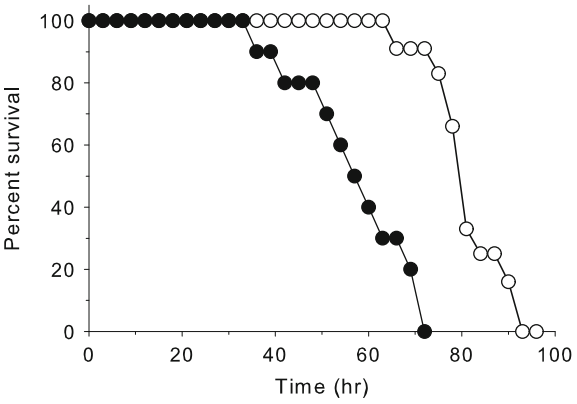

Fig. 4.3 Inhibition of programmed fat cell death increases starvation resistance in *D. melanogaster*. *Filled symbols*, control flies; *open symbols*, flies in which fat cell death was inhibited by expression of *diap* (*Drosophila* inhibitor of apoptosis) in the larval fat body. Data modified from Aguila et al. (2007)

Larvae store energy in the larval fat body. The fat body is unique to insects and serves many functions in addition to energy storage, including but not limited to immune responses, detoxification, and endocrine secretion (Hoshizaki 2005). In comparison to other larval tissues, larval fat body is unusual in that its cells remain intact during metamorphosis and are present in the young adult (Nelliot et al. 2006). Most larval tissues undergo programmed cell death in the pupa, with their contents being used to support proliferation of the imaginal disk cells that will form the adult tissues. Larval fat cells escape this fate, then undergo programmed cell death in the first 48 h of adult life (Aguila et al. 2007). Nutrients released at this time are used to support adult tissues and reproduction (Min et al. 2006; O'Brien et al. 2008).

Recent evidence suggests that the larval fat body has an important role in starvation resistance in young adult flies. Aguila et al. (2007) observed that newly eclosed female adults survived starvation stress over twice as long as 3–10-day-old females. The authors then used a genetic manipulation to delay death of the larval fat cells by approximately 2 days. These females survived starvation ~24 h longer than unmanipulated flies (Fig. 4.3). These flies also had lower fecundity, suggesting that larval resources are also important for reproduction (Aguila, Hoshizaki, and Gibbs, unpublished observations).

Together, these findings suggest that starvation selection affects the physiology of the larval fat body. Increased lipid storage during the larval stage is certainly consistent with this idea. Because all cell division in this tissue occurs embryonically (Hoshizaki 2005), this probably reflects more lipid per cell rather than more fat cells. Starvation-selected females also have lower early adult fecundity than controls, despite having more ovarioles (Wayne et al. 2006). Preliminary evidence suggests that fat cell death is delayed in starvation-selected populations (Reynolds and Gibbs, unpublished data), which would cause lower fecundity. The onset of the wandering stage and developmental events in the fat body are regulated by the steroid hormone, 20-hydroxyecdysone (20E; Riddiford and Truman 1993; Rusten et al. 2004; Hoshizaki 2005; Bond et al. 2011). The hormonal basis for fat body changes in all stages of starvation-selected flies is unknown, but 20E signaling is likely to be involved.

4.5 Metabolic Responses to Starvation Stress

Drosophila melanogaster is a widely studied model for starvation responses, but the vast majority of studies have used the third and last larval instar. In adults, food deprivation causes increased activity (Connolly 1966; Knoppien et al. 2000; Farhadian et al. 2012). Increased energy consumption would appear counterintuitive, but in nature waiting for the next rotting banana to appear makes no sense (see also McCue et al., Chap. 8). Laboratory-selected flies do not have the option of finding a new food source, so they reduce their activity when food is absent (Williams et al. 2004). When food is returned, flies increase their feeding rate and allow more food to accumulate in their crop relative to unstarved controls (Farhadian et al. 2012).

The primary fuel consumed during starvation stress is lipid (Marron et al. 2003), by mechanisms closely resembling, and sometimes homologous to, mammalian regulation of lipolysis (Arrese and Soulages 2010). Neurosecretory cells in the ring gland secrete adipokinetic hormone (AKH), which activates lipolysis via G protein-mediated phosphorylation of one of the primary proteins associated with lipid droplets in the fat body, lipid storage droplet protein-1 (LSD1), a member of the perilipin protein family. As starvation progresses, transcription of *brummer* (*bmm*) is activated (Groenke et al. 2007). Brummer is the *Drosophila* homolog of adipose triglyceride lipase (Groenke et al. 2005). Lipids are transported in the hemolymph bound to lipophorins, probably in the form of diacylglycerides, rather than triacylglycerides (Canavoso et al. 2001). Oenocytes, specialized cells attached to the inner surface of the animal, take up some of these lipids and store them in a manner analogous to mammalian hepatocytes (Gutierrez et al. 2007). Most lipids, however, presumably are absorbed and metabolized by cells throughout the body.

In addition to AKH signaling, the insulin signaling pathway regulates nutrient uptake, storage, and metabolism. This pathway is well conserved between flies and mammals, making *Drosophila* an excellent model for mammals (Fig. 4.4). *Drosophila melanogaster* has 7 insulin-like peptides (dILPs) that are homologous to the insulin family in vertebrates, as well as a homologous insulin receptor. The dILPs are expressed at different times by different tissues, but there are some overlapping functions. The most important in terms of nutritional status are dILPs expressed by 7 neurosecretory cells (NSCs) in the brain. Ablation of these cells in larvae or adults results in elevated hemolymph trehalose and excess lipid accumulation, analogous to the condition in diabetic mammals (Belgacem and Martin 2006). However, release of dILPs is not dependent on lipid or carbohydrate levels; instead it depends on an amino acid sensing mechanism in the fat body (Geminard et al. 2009).

Drosophila have only one insulin receptor (InR), which can bind all 7 dILPs. Binding activates an intracellular signaling pathway strongly resembling, but less redundant than, mammalian insulin signaling (Teleman 2010). Events include activation of PI3 kinase (PI3 K), followed by the protein kinase Akt. Akt then

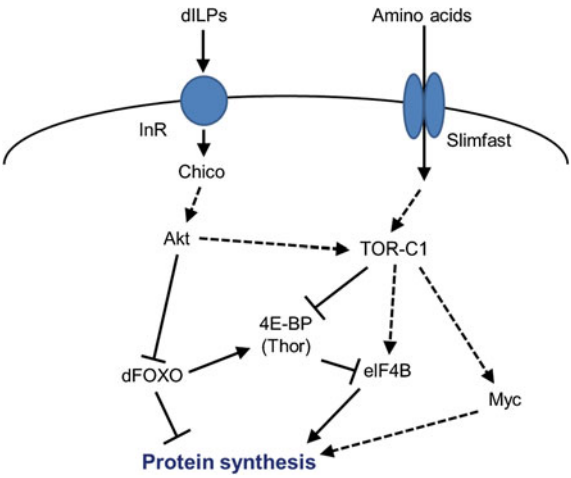

Fig. 4.4 Insulin/TOR signaling in *Drosophila*. Only members of these pathways mentioned in the text are shown. *Arrows* indicate activation of the downstream component; *blocked lines* indicate inhibition. *Dashed lines* indicate an indirect effect mediated by one or more intermediate steps. A more complete diagram can be found in Teleman (2010)

phosphorylates a variety of proteins, including dFOXO, the single *Drosophila* member of the FOXO family of transcription factors. dFOXO regulates transcription of numerous targets (Teleman et al. 2008), including 4E-binding protein (4E-BP, or Thor, a general inhibitor of translation). Phosphorylation of dFOXO decreases *Thor* expression, allowing greater protein synthesis.

Akt also indirectly regulates TOR (Target of Rapamycin), a central regulator of cellular metabolism. The TOR-C1 form of TOR increases ribosomal synthesis, inhibits translational repression by phosphorylating *Thor*, and stimulates amino acid uptake via the amino acid transporter, Slimfast. There is extensive crosstalk and feedback among various branches of the insulin signaling pathway. Accumulation of amino acids activates TOR, thereby activating amino acid transport. dFOXO regulates the expression of *myc*, a target of TOR that stimulates ribosome synthesis (Teleman et al. 2008). dFOXO and TOR pathways also intersect via their opposing effects on the expression and activity of 4E-BP.

The alphabet-soup description above includes only a few components of the insulin/TOR signaling pathway, but it provides a framework for understanding how starvation affects signaling. During starvation in *Drosophila*, secretion of dILPs by the neurosecretory cells decreases. Food-seeking behavior increases, mediated by neural S6 kinase, a downstream target of insulin signaling. AKH secretion also stimulates activity (Lee and Park 2004; Isabel et al. 2005). Phosphatidylinositol-(3,4,5)-triphosphate levels decline, Akt becomes dephosphorylated, and dFOXO is recruited to the nucleus. *Thor* expression increases, and existing Thor protein becomes dephosphorylated and can inhibit elongation initiation factor eIF4B, thereby inhibiting protein synthesis. dFOXO and TOR inputs inhibit *myc* transcription, thereby inhibiting ribosome biogenesis. The overall result is a general reduction in energy-intensive biosynthetic activities. In addition TOR-mediated autophagy of fat cell contents commences, generating nutrients that

can be used to support metabolism in the rest of the body (Scott et al. 2004; McPhee and Baehrecke 2009).

This general pattern is likely to differ in a tissue-specific manner. It can also vary depending upon developmental stage. The pupa does not feed, yet needs to devote a significant fraction of metabolism to building adult tissues. Beginning in the wandering stage of the third instar, 20E signaling induces the larval fat body to express *dILP6* (Slaidina et al. 2009) and activates lipid catabolism (Wang et al. 2010). Inhibition of *dILP6* transcription in the fat body results in smaller adults, but these have high triglyceride levels and are more starvation resistant than control flies. Additional experiments revealed that *dILP6* expression is regulated by dFOXO, providing a further example of the intersection between these pathways. In another example of signaling crosstalk, recent work suggests that dFOXO regulates expression of *dDOR*, a coactivator of the ecdysone receptor (Francis et al. 2010).

Mammalian researchers will note that we have barely mentioned sugar homeostasis in our discussion of insulin signaling (see Champagne et al., Chap. 19). To some extent this is due to the focus on the *Drosophila* larva, a very rapidly growing stage that requires high levels of amino acids to support biosynthesis. In fact, a common control treatment for 'starvation' (lack of amino acids) is a diet containing sucrose to allow animals to continue to manufacture ATP.

In *Drosophila*, the primary signal for insulin secretion is the presence of amino acids, not carbohydrates. The primary site for sensing overall nutritional status is the fat body (Colombani et al. 2003). One or more factors secreted by the fat body stimulates dILP secretion by the NSCs when amino acids are abundant (Geminard et al. 2009). When amino acid levels are low or the Slimfast amino acid transporter is inactivated, dDILP secretion is reduced. Thus, the NSCs and fat body are in reciprocal communication with each other. The identity of the signal released by the fat body is unknown, but the fat body is known to produce numerous growth factors (Britton and Edgar 1998; Kawamura et al. 1999).

Under prolonged starvation, an additional energy source available to female flies is reabsorbed eggs (Wilson 1985; McCall 2004). Oogenesis is initiated from germline stem cells situated at the anterior tip of each ovariole, the germarium. An egg chamber or follicle forms, comprising the oocyte and nurse cells enclosed in a layer of follicle cells (Wu et al. 2008). In well-fed laboratory strains of *D. melanogaster*, new egg chambers are formed continuously over most of an adult female's life span. Reabsorption during starvation is initiated by apoptosis of the nurse cells (Terashima and Bownes 2005, 2006), and there is increased cell death in the germarium (Drummond-Barbosa and Spradling 2001; Pritchett et al. 2009). One might predict that starvation-selected flies would contain fewer ovarioles than control flies, but this is not the case (Wayne et al. 2006). Reduced fecundity in these populations may instead be caused by lower activity of the germline stem cells or increased egg reabsorption, but this has not been investigated.

4.6 Genomics of Starvation Resistance

As the first multicellular eukaryote with a sequenced genome, *D. melanogaster* has been the subject of numerous genomic analyses, including several related to starvation stress. Harbison et al. (2004) identified nearly 400 genes associated with starvation resistance, many of them associated with cell fate determination. The authors suggest that these genes may affect resource allocation during development, setting the conditions for survival later. This pattern is consistent with selection experiments in which larval resource acquisition is a major determinant of adult starvation resistance (Chippindale et al. 1996). Analyses of quantitative trait loci (QTLs) have identified several genomic regions associated with differences in starvation resistance and energy storage (Vieira et al. 2000; Harbison et al. 2005; Wang et al. 2005).

Microarray experiments have shown that up to 25% of the transcriptome can be affected by starvation (Harbison et al. 2005). The first such transcriptome analysis was performed by Zinke et al. (2002). The focus of this study was sugar-related gene expression, so larvae fed sugar were compared with starved larvae and those fed with sugar and protein. Several genes associated with lipid catabolism were upregulated specifically in starved larvae, whereas lipid synthetic genes were upregulated in larvae fed only sugar. These results are consistent with the idea that starved larvae rely on endogenous lipid to survive, while sugar-fed larvae use this resource to make ATP, with any excess going to lipid synthesis. Surprisingly, Harbison et al. (2005) found that genes for biosynthetic proteins tended to increase in expression in starved flies. Transcriptional networks affecting energy storage and metabolism have also been identified (Jumbo-Lucioni et al. 2010). Transcripts correlated with lipid content included several that have human homologs and have been associated with obesity in mice.

The studies above assayed whole-body gene transcription, but different tissues will respond differently to starvation (e.g. fat body and oenocytes). Immune function genes are downregulated in several tissues (Farhadian et al. 2012). In ovaries, changes in expression of multiple members of the insulin/TOR signaling are consistent with an inhibition of protein synthesis and cell growth (Terashima and Bownes 2005). Decreased expression of ovary-specific genes, such as yolk proteins, can also be detected in whole-animal experiments (Bauer et al. 2006). Starvation selection also affects gene expression. Sorensen et al. (2007) found that over 200 genes were constitutively downregulated in starvation-selected lines, including many involved in transcription and glycolysis, suggesting that overall metabolism may be lower. Interestingly, the specific genes identified differed from those differentially expressed during starvation stress (Harbison et al. 2005). Thus, acute and evolutionary responses to starvation appear to rely on different mechanisms.

Genomic studies of starvation in natural populations of *Drosophila* have also been performed. In both North America and Australia, latitudinal clines in allele

frequencies of the insulin receptor have been observed in *D. melanogaster* (Paaby et al. 2010). In North America, this cline parallels a cline in starvation resistance (Schmidt et al. 2005; Schmidt and Paaby 2008). No latitudinal clines were detected, however, for the InR substrate, Chico. This finding is consistent with genomic comparisons among *Drosophila* species, which show that evolution of downstream members of the insulin signaling pathway tends to be more constrained than that of upstream proteins (Alvarez-Ponce et al. 2009, 2012).

4.7 Summary

More is known about starvation responses in *Drosophila* than in any other insect, perhaps any other animal. The genetic resources available for *D. melanogaster* have made it a widely used model to study regulation of energy storage and mobilization. For example, many aspects of TOR signaling were initially identified in *Drosophila*, then studied in mammalian systems (Martin and Hall 2005). Genetic advantages notwithstanding, fruitflies are too small for convenient study of some aspects of starvation. For this reason, hemolymph transport of lipids is far better understood in larger insects such as *Manduca* (Arrese et al. 2001). Presumably *Drosophila* also convert triacylglycerides to diacylglycerides before releasing them into the hemolymph, but this has not been well studied. Life history differences among species will also affect how insects respond to starvation. Adult *Bombyx* moths do not feed, so starvation-induced reabsorption of eggs does not make sense and presumably does not occur. *Drosophila* is an excellent *model*, but comparative studies of insect starvation are still needed.

Comparative studies within the genus *Drosophila* should be very informative. *Drosophila* use a wide variety of host plants in nature, differing greatly in their spatial and temporal availability, as well as nutritional content (Markow and O'Grady 2008). Starvation resistance varies widely across the genus. Within species, local populations exhibit variation that in many cases suggests local adaptation to environmental conditions. At the time of this writing, genome sequences are available for 19 species of *Drosophila*, from many different nutritional habitats. A century of genetic research on *D. melanogaster*, intensive study of evolution in the genus *Drosophila*, and rapidly expanding genomic resources for *D. melanogaster* and its relatives provide many opportunities to deepen our understanding of starvation biology in insects and other animals.

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Chapter 5
Metabolic Transitions During Feast and Famine in Spiders

Johannes Overgaard and Tobias Wang

5.1 Introduction

Spiders occur in virtually all terrestrial ecosystems and have adapted to varying food availability by means of diverse foraging strategies and appropriate physiological responses. The versatile feeding ecology of spiders has been reviewed by Turnbull (1973), Nentwig (1987), Wise (1995), Foelix (1996) and Wilder (2011), and we will merely mention some generalizations attributable to the feeding ecology of most spiders. The majority of spiders use either a web-building or a 'sit-and-wait strategy' for prey capture. Both strategies may involve prolonged food deprivation due to the stochastic encounter between prey and predator (Wise 1995; Wilder 2011). Prolonged food deprivation may also occur during overwintering, particularly for temperate species (Anderson 1974), although several species do actively forage and digest meals at low temperature (Aitchison 1984; Foelix 1996; Korenko and Pekár 2010; Wise 1995).

There is some indirect evidence that spiders are restricted by prey abundance in their natural environment. Field studies using artificial food supplementation documented increases in growth rate, population density, and reproductive output (Wise 1995; Wilder 2011). These findings suggest that spiders are often food limited in their natural environment to an extent that lowers optimal population growth rates. In this respect it has been suggested that the sit-and-wait strategy employed by many spiders is particularly adaptive when prey is scarce and occurs at irregular intervals (Wise 1995) (see also Hervant, Chap. 7; McCue et al., Chap. 8).

Spiders are almost exclusively carnivorous predators (Foelix 1996; Wise 1995), although there are a few examples of nectar and pollen feeding (Smith and Mommsen

J. Overgaard (✉) · T. Wang
Zoophysiology, Institute of Bioscience, Aarhus University,
Aarhus C, 8000 Aarhus, Denmark
e-mail: Johannes.overgaard@biology.au.dk

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1984; Wu et al. 2011), and scavenging on dead food items has also been documented (Sandidge 2003; Vetter 2011). Predatory spiders are most often polyphagous, feeding on many different prey types and spiders are renowned for being able to subdue large prey that may even be bigger than the spider itself; there are even some (rare) examples of spiders feeding on small vertebrates (McCormick and Polis 1982). Nevertheless, the vast majority of spider prey is insects and other arthropods of similar or smaller size than the spider itself (Foelix 1996; Nentwig 1987; Wilder 2011; Wise 1995). Here we review the existing knowledge of feeding and fasting responses of arachnids and discuss how their physiological adaptations allow them to thrive in environments with spatial and temporal variation in food availability.

5.2 Starvation Tolerance

Historically, it has been proposed that spiders are particularly well adapted for long-term starvation because their standard metabolic rate was estimated at approximately only 50% of other arthropods (Anderson 1970). More recent studies, however, indicate that the reports of low metabolism of spiders appear to be an artifact because studies were performed on starved spiders (Lighton and Fielden 1995). It was also suggested that some families of 'primitive' spiders should be endowed extraordinary low energy requirements, thus rendering them particularly starvation tolerant (Carrel and Heathcote 1976). This idea was not supported by a subsequent and more comprehensive study of arachnid metabolism, which failed to find such phylogenetic patterns of lowered metabolism (Greenstone and Bennett 1980). It seems, therefore, that spiders in general do not have extraordinary low costs of living. Even so, a plethora of non-scholarly sources report that large spiders (tarantulas) can survive starvation for more than a year; in our lab, we have kept mature female tarantulas (*Acanthoscurria geniculata*) at 28°C without food for more than 220 days without mortality or signs of distress (Knudsen 2011). These anecdotal observations are corroborated by a few controlled experimental studies. Anderson (Anderson 1974), for example, reported average survival of 208 and 275 days without food at 20°C for the wolf spider *Lycosa lenta* and the cribellate web builder *Filistata hibernalis*, respectively. Tanaka (Tanaka and Itô 1982) measured starvation tolerance of the wolf spider *Pardosa astrigera* at 25°C to be ~29 and 54 days for males and females, respectively, and Jensen et al. (Jensen et al. 2010) measured starvation tolerances of 35, 40, or 48 days for *Pardosa prativaga* at 25°C depending on the spiders nutritional status at the onset of starvation.

Although, data are only available for a few species, Fig. 5.1a shows that starvation tolerance increases with body mass with a power of 0.388 (see also Hohtola, Chap. 10). However, given the limited data set it is highly unlikely that this value represents a generalized pattern. Assuming that starvation tolerance is determined by the amount of energy reserves available at the onset of starvation and the rate of utilization during food deprivation (i.e., metabolism), it would be expected that starvation tolerance scales with a factor ~0.66, as a reflection of the generalized

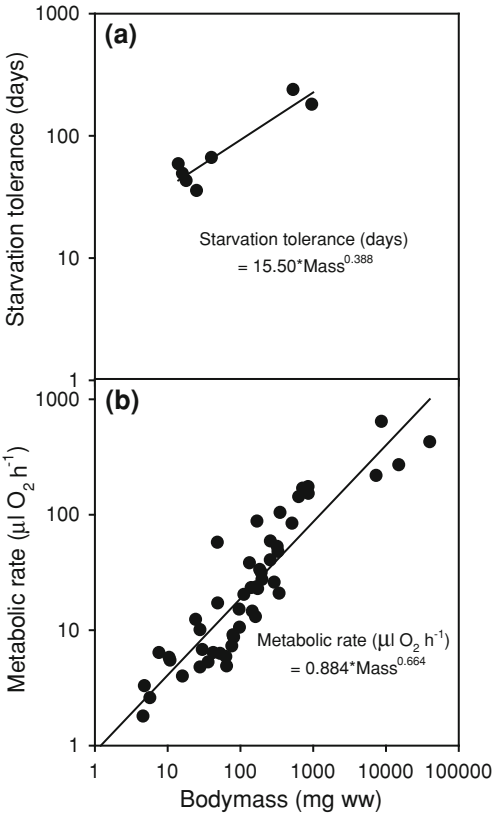

Fig. 5.1 **a** Relationship between starvation tolerance and body mass. Data are normalized to a temperature of 22°C using a $Q_{10} = 2$ and for each species/sex/feeding group the average time to death is plotted against the initial body mass. **b** Relationship between body mass and metabolic rate. Data are normalized to a temperature of 22°C using a $Q_{10} = 2$. Data are compiled from (Anderson 1974; Greenstone and Bennett 1980; Tanaka and Itô 1982; Jensen et al. 2010; Knudsen 2011; Nespolo et al. 2011)

relationship between body mass and metabolic rate of spiders (Fig. 5.1b). This scaling exponent of standard metabolism is within the normal range found for living organisms (Schmidt-Nielsen 1984) as is the proportional relationship of total lipid reserves in spiders (~1.01) (Lease and Wolf 2011). It follows that starvation tolerance among spiders, collectively, should follow the metabolic rate and body size relationship (see Fig. 5.1b). One consequence of this scaling relationship is that a large tarantula of 40 g should survive 16 times longer without food than a 10 mg species (Greenstone and Bennett 1980). Another consequence is that spiderlings will be more sensitive to variable prey abundance as is also indicated by field studies (Wise 1995). It should be noted, however, that metabolism varies considerably between species of the same mass (Fig. 5.1b) and other factors such as sex, age, and seasonal differences in energy storage does also influence starvation tolerance (Jensen et al. 2010; Lease and Wolf 2011). Although only a few studies have directly estimated starvation tolerance they suggest that large differences are to be expected due to body size, interspecific differences, and obviously also due to temperature which will potentially increase starvation tolerance several fold by its influence on the total energy requirement (Anderson 1970, 1974; Ford 1977; McCue 2004; Seymour and Vinegar 1973; Shillington 2005).

5.3 The Respiratory and Metabolic Response to Fasting

Spiders respond to long-term fasting by reducing their metabolic rate and several studies have reported that fasting metabolism decreases to 30–80% of the post-digestive value (Fig. 5.2a). The underlying processes involved in this metabolic depression are not well described and may not even represent a true example of metabolic depression (Guppy and Withers 1999). Thus, one important component could be the replacement of dry mass with water (discussed below), since most of the 'metabolic depression' disappears when metabolic rate is expressed relative to dry rather than wet mass (Knudsen 2011). Moreover metabolic rate typically remains elevated for several days after feeding (Knudsen 2011) and some of the apparent 'starvation-induced hypometabolism' during food deprivation may simply represent the termination of the prolonged specific dynamic action (SDA) response (discussed below).

Long-term starvation has been associated with a transition to lipid catabolism as attested by reduction in respiratory exchange ratio (RER) (Tanaka et al. 1985). However, small changes in RER are often difficult to measure, and few other studies have confirmed that such reductions are indicative of a general response during food deprivation (Fig. 5.2b) (Collatz and Mommsen 1975; Jensen et al. 2010; Knudsen 2011). Instead, it seems that RER is slightly higher during digestion (Jensen et al. 2010), and it is possible that changes in RER reflect a transition in energy catabolism between feeding and fasting rather than a progression of fasting per se.

5.4 Changes in Body Mass and Body Composition

As a consequence of the continued catabolism of energy stores during food deprivation spiders lose body mass when fasting. The body mass reductions in fasted spiders are generally smaller (0–20%) (Anderson 1974; Knudsen 2011; Stewart and Martin 1970; Tanaka and Itô 1982) than the mass loss of starving vertebrates (e.g., 50% of initial body mass) (McCue 2010) (see also Champagne et al., Chap. 19; Lignot and Le Maho, Chap. 2). It is possible that the smaller flexibility in mass relates to the relative high mass of the exoskeleton, which does not change mass during starvation (see Kirk, Chap. 3).

The RER values of food-deprived spiders are generally below 0.8 (Fig. 5.2b) and fasting spiders are assumed to rely primarily on their lipid reserves implying that the size of the lipid reserve is an important determinant of starvation tolerance. Studying *P. prativaga* in different nutritional status, Jensen et al. (Jensen et al. 2010) found that spiders with high lipid content survived food deprivation significantly longer than conspecifics with high protein content. This difference was found despite a lower total body mass of the lipid-rich spiders, indicating that starvation tolerance may correlate better with total lipid content than body mass.

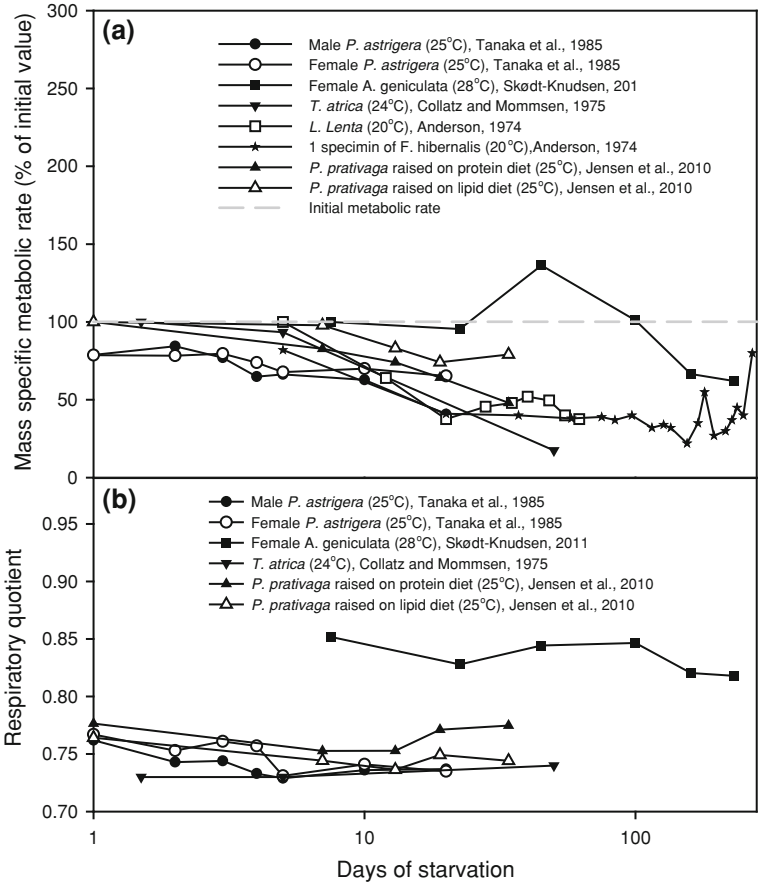

Fig. 5.2 **a** Normalized mass-specific metabolic rate of spiders plotted against duration of starvation period. **b** Estimates of respiratory exchange ratio (RER) during starvation. Data are compiled from species that differ in size, ecology, and experiments are run at different temperatures so that duration of food deprivation is not directly comparable in terms of the starvation stress imposed

Preferential use of lipids is also confirmed by two studies investigating the body composition of spiders before and after fasting. Collatz and Mommsen (1975) found that 47% of the lipid stores were oxidized during 50 days of fasting in *Tengenaria atrica*, while only 9% of the protein mass had disappeared (Fig. 5.3a). Carbohydrate stores were depleted even more (61%), but contributed little to the overall catabolism because carbohydrates only constitute a small fraction of the total energy reserve. In a comparison of recently fed *A. geniculata* with spiders that had been starved for 220 days, Knudsen (2011) showed that almost two-thirds of the dry mass loss could be ascribed to reduced lipid stores, while protein loss accounted for the other third. Given the higher energy density of lipids this finding also suggests that lipids are the preferred energy source during starvation

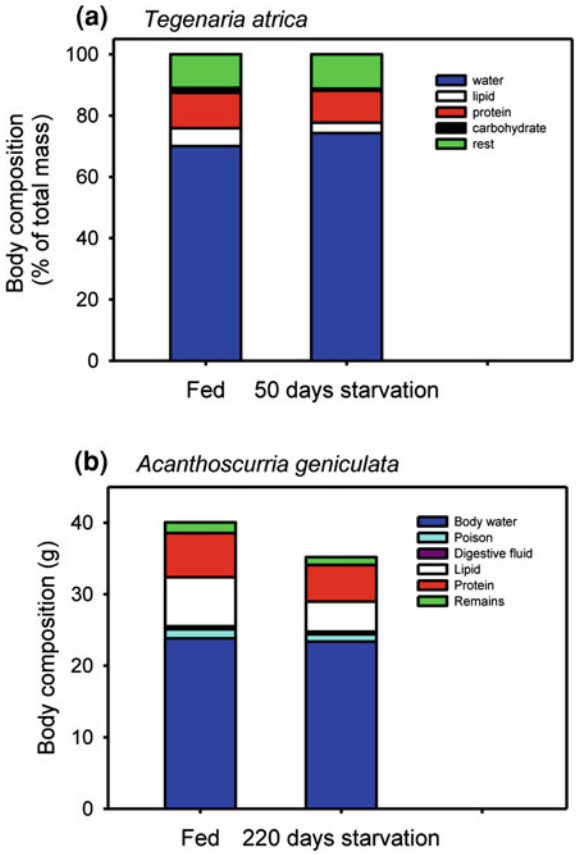

Fig. 5.3 **a** Fractional body composition of *T. atrica* before and after 50 days of starvation. Data are from (Collatz and Mommsen 1975). **b** Average mass of body constituents measured before and after an average of 220 days of starvation in *A. Geniculata*. Data are adapted from (Knudsen 2011)

(Fig. 5.3b). The importance of lipids during food deprivation is also suggested by the seasonal change in preference toward a more lipid-rich prey during the autumn when spiders prepare for winter. In fact, spiders in the fall have a greater preference for lipid-rich prey than those caught in spring immediately before their reproductive season (Bressendorff and Toft 2011; Collatz and Mommsen 1975).

Given the limited amount of data, it remains unknown whether spiders exhibit the typical three phases of starvation (reviewed by McCue 2010 and Wang et al. 2006) where there is a progressive switch from carbohydrate (phase I) to lipid (phase II) to protein catabolism (phase III). Nevertheless, Collatz and Mommsen (1975) demonstrated that carbohydrate and lipid stores are preferentially oxidized during the initial phases of food deprivation. Moreover, metabolic rate data from one individual approaching the lethal limit of starvation (Anderson 1974) increased activity (escape response), which is also often documented in animals during phase III (Wang et al. 2006). This observation does not, however, directly indicate which energetic fuel was used during the final phases of starvation. It is,

for example, likely that the increased activity is simply related to a shift toward a more active feeding strategy induced by low energy reserves.

Studies by Collatz and Mommsen (1975) and Knudsen (2011) have reported increased proportion of water while dry mass decreased during food deprivation (Fig. 5.3a). In the case of *A. geniculata,* this resulted in an increase in water content from 1.6 to 2.3 g water per g dry mass over a 200 day fasting period. Thus, total water content seems to be maintained during fasting in spiders. This is in accordance with Stewart and Martin (1970) reporting that the bodies of starved spiders had increased relative water content. Effective replacement of organic mass with water during prolonged starvation is also seen in a number of invertebrates and vertebrates (reviewed in McCue 2010), but the underlying cause for this response is not well understood for spiders. An overall increase in water content could be linked to the low water content of adipose tissue which is catabolized (~10% water). Loss of lipid stores would therefore increase the relative water content in the starved and lean animals. It is also possible that the increased water content is merely a response to fill the otherwise "empty" volume of the fasted spiders' exoskeleton. Thus, spiders actively drink to replace fluids/volume that is lost during fasting, egg-laying, or bleeding (Stewart and Martin 1970). This latter suggestion may be particularly important for spiders, where the water/hemolymph content must be defended to ensure adequate hydrostatic pressure for locomotion (Stewart and Martin 1970). Thus, spiders lack extensor muscles in both the femur-patella joint and the tibia-metatarsal joint and leg extension is therefore dependent on hydraulics driven by the generation of adequate hemolymph pressure (Foelix 1996).

5.5 Summary of Starvation

Starvation tolerance of spiders depends on a range of factors that include body size, lipid storage, and the ability to reduce metabolism during starvation. Environmental temperature will also have a tremendous impact on the starvation tolerance of many spiders, particularly those that survive winters without feeding, but few studies have characterized such effects. Combined with the ability of starving spiders to continue reproductive behavior, albeit with smaller and fewer eggs (Nakamura 1987) their exceptional starvation tolerance enables spiders to persevere in environments with scarce and/or strongly fluctuating food availability. A particular interesting aspect of the starvation physiology of spiders that is currently poorly understood is how starvation affects behavior and feeding strategy of spiders. Do spiders, for example, seek cooler environments when food deprived and/or do they enhance their active feeding efforts by relocating or by actively seeking prey? Spiders, particularly those that use webs, are especially well suited for such studies as these small arthropods are relatively easy to (re)locate under field-like conditions where prey density can be controlled.

5.6 Responses to Feeding

Spiders use venom to kill their prey and during the subsequent feeding they employ extraoral digestion, where they regurgitate digestive fluids over or into the prey. The resulting digesta is ingested by the specialized sucking stomach, a behavior that is repeated sequentially until feeding stops. Detailed anatomical, structural, and functional descriptions of the digestive apparatus of spiders and other arthropods using extraoral digestion are found in Cohen (1995), Collatz (1987) and Foelix (1996). Here we will only emphasize that extraoral digestion entails some potential adaptive benefits since this mode of feeding permits spiders to reduce handling time and increase the relative quality of the digested food. Animals feeding by means of extraoral digestion only ingest the energetically favorable parts of the prey and need not expend additional resources to process toxic matter or the cuticula of arthropod prey. Extraoral digestion also allows the predator to expand its repertoire of prey size and shape to include items that cannot be swallowed (Cohen 1995). Extraoral digestion, however, also poses some challenges since the absolute and relative assimilation of macronutrients is based on preabsorptive processes. Any energetic savings from atrophy of the digestive apparatus must therefore be balanced by the need to rapidly restore digestive functions because the available energy of the prey remains outside the spider until it is degraded. The potential time constraint of feeding also entails that the relative absorption of macronutrients depends on the composition and efficiency of the hydrolytic enzymes released by the predator.

5.7 The Digestive System and Digestive Enzymes of Spiders

The digestive system of spiders consists of a small mouth-opening covered by bristles that act as a sieve to exclude larger food particles. The initial internal structures, the pharynx and esophagus, are both lined with a thin cuticular layer and eventually lead up to a muscular sucking stomach that generates the pumping action during feeding. Unidirectional flow of nutrients from the prey to the midgut is ensured by valves situated at the entrance and exit of the stomach. The midgut is a large, extensible structure within both the cephalothorax and the abdomen (Fig. 5.4). This structure is characterized by extensive branching (diverticula) that may extend into the legs in the cephalothorax and the branching of the midgut is even more elaborate in the abdomen. The midgut terminates at the stercoral pocket where feces are stored until defecation (Collatz 1987; Foelix 1996). The midgut epithelium contains both secretory and absorptive cells involved in digestive processes, and the midgut also serves as a storage site for glycogen and lipids. Because of the multiple functions that are homologous to the insect fat body, the midgut is sometimes referred to as the liver, pancreas, or hepatopancreas (Collatz 1987; Laino et al. 2009, 2011).

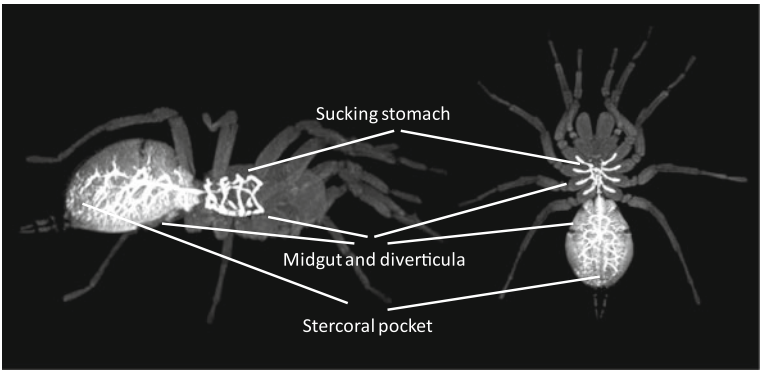

Fig. 5.4 Lateral and coronal view of the gastrointestinal tract of the tarantula (*Acanthoscurria geniculata*) visualized using MRI. The midgut is visually enhanced following ingestion of MRI contrast agent. The midgut branches in both the front (prosoma or cephalothorax) and anterior (opistosoma or abdomen) and in the abdomen these branches become more diffuse and subdivide into lobules. Pictures are modified from (Lauridsen et al. 2011). See (Foelix 1996) for more detailed anatomical descriptions of the spider's digestive apparatus

Once the prey is subdued and feeding commences, the digestive enzymes are released from the secretory cells of the midgut (Foelix 1996). The digestive juice regurgitated by the spider has high protein content and is densely packed with digestive enzymes including proteases, amylases, esterases, lipases, nucleases, and chitinase (Collatz 1987; Mommsen 1978a, b, c). The high enzymatic capacities found in vitro for *Tegenaria* exhibit a digestive capacity that will enable these spiders to quickly consume large meals. Within just 3 h some *Tegenaria* species are able to subdue prey and ingest nutrients that cumulatively constitute the equivalent of the spiders own body mass. However, because the enzymatic activity is highly influenced by pH and temperature (Mommsen 1978a, b, c), it follows that the digestive capacity will be amendable to potentially differing conditions that may arise during process of extraoral digestion (Collatz 1987).

Although the temporal absorption of nutrients has not been studied in spiders it is known from other extraorally digesting arthropods that protein and carbohydrates are extracted much faster than lipids (Cohen 1995) which is consistent with findings from *Tegenaria,* where amylase and protease activities were higher than those of lipases (Collatz 1987).

5.8 Nutrient Selection

Spiders have the capacity to extract most of the macronutrients (i.e., carbohydrate, protein, and lipid) of their prey (Lang and Klarenberg 1997; Samu and Biro 1993), but a number of studies indicate that both the extraction efficiency and

composition of the extracted food is regulated (Furrer and Ward 1995; Jensen et al. 2011; Mayntz et al. 2005). Such nutrient selection enables spiders to prioritize one macronutrient over the other and thereby regulate toward an 'optimal' diet that fits prevailing requirements (Toft 1999). Nutrient selection is attained by regulating the quantity of different types of prey, by regulating the parts of the prey digested or through modifications of the relative digestive efficiency of the different macronutrients (Toft 1999; Mayntz et al. 2003, 2005; Wilder 2011). In a classic study Mayntz et al. (2005) demonstrated that spiders (*P. prativaga*) raised on a lipid-rich and protein-poor diet had preference for protein-rich prey, while spiders raised on a protein-rich and lipid-poor diet preferred lipid-rich prey (See Fig. 5.5a, b). In the same study it was demonstrated how another species (*S. lineatus*) showed variable efficiency for protein extraction of the prey. This variable efficiency depended on the nutritional status of the spider, such that protein-deprived spiders had better protein extraction efficiency (See Fig. 5.5c). Along the same line, tarantulas (*A. geniculata*) starved for >200 days extract nutrients with a higher protein/lipid ratio than recently fed tarantulas (Knudsen 2011) (See Fig. 5.5). Thus, by virtue of these responses spiders are able to generate nutrient-specific compensatory responses that aid these predators toward an optimal dietary intake.

5.9 The Respiratory and Metabolic Response to Digestion

Spiders are capable of consuming huge meals, which may be equivalent to the spiders' body size (Anderson 1970; Nentwig 1987). Estimates of meal size may be exaggerated because extraction efficiency decreases with the meal size (Furrer and Ward 1995) and because the non-digested cuticula of arthropod prey can constitute a large proportion of the total meal mass. Nonetheless, spiders can consume large meals, and as other sit-and-wait predators, such as snakes, they display a marked metabolic transition when feeding.

The metabolic response to feeding is termed the SDA of food and it incorporates the costs of digestion, absorption, and assimilation of food as well as prey handling and any gastrointestinal growth that may be associated with feeding (McCue 2006; Secor 2008). Previous reviews of SDA in animals have not included examples of spiders, but the feeding of other arthropods (primarily insects and crayfish) show typical meal sizes of 2–10% and increases in metabolic rate in the range of 1.5–4 times resting values (McCue 2006; Secor 2008). Recent studies on spiders show that the SDA response is considerably larger than those of most arthropods (Fig. 5.6a–c). Wolf spiders (Jensen et al. 2010) increase their metabolic rate by at least 4 times upon feeding, but the maximal factorial scope was probably underestimated because measurements had to be taken over a 3 h period. Tarantulas have an even larger metabolic response with a 6.5-fold increase in *E. truculentus* (Nespolo et al. 2011) and more than 20-fold increase in *A. geniculata* (Knudsen 2011). The factorial scope of the SDA response is obviously highly dependent on resting metabolism, which as in other animals varies

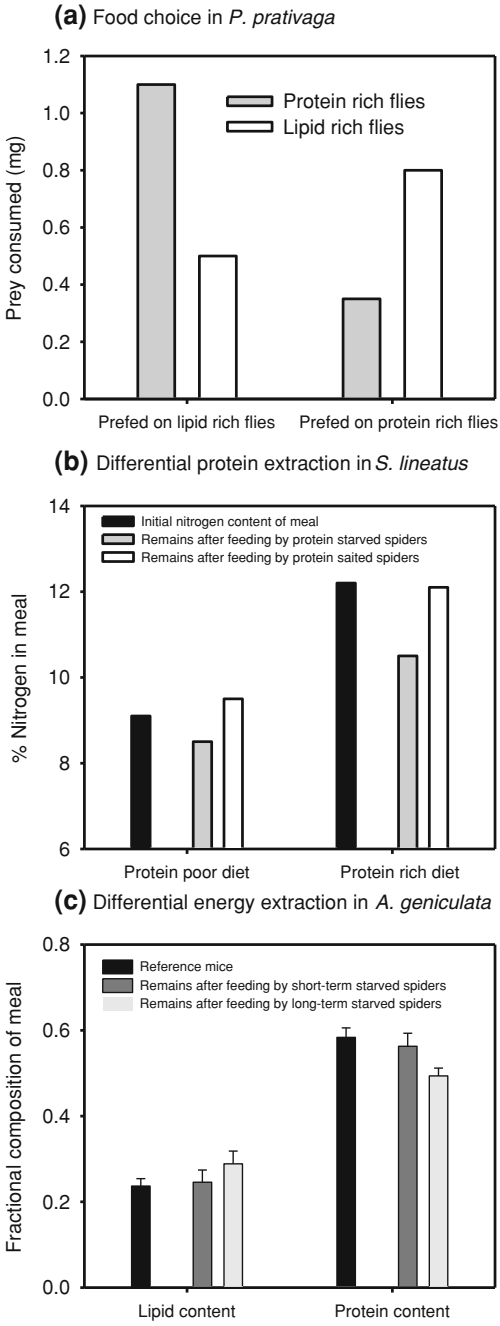

Fig. 5.5 Dietary preference in spiders. **a** The wolf spider *P. prativaga* pre-fed on a lipid-rich diet displays larger hunger for protein-rich flies than for lipid-rich flies. The converse pattern is found if the spiders are pre-fed a protein-rich diet. **b** A difference in digestive preference/efficiency is found between protein starved and protein saited specimens of the web-building spider *S. lineatus*. The difference persist regardless if the spider is fed a protein-poor (*right*) or protein-rich meal (*left*). Data are from (Mayntz et al. 2005). **c** Lipid and protein content of food remains after feeding by short- and long-term starved tarantulas (*A. geniculata*). Data are adapted from (Knudsen 2011)

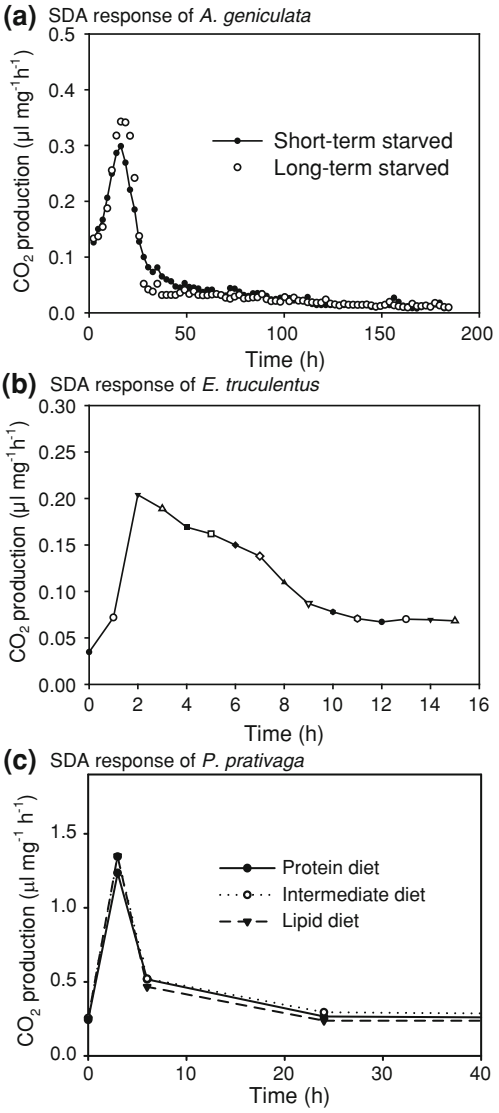

Fig. 5.6 Metabolic response to feeding in spiders. **a** Respiratory response of the tarantula *A. geniculata* feeding *ad lib* on a dead mice. The response is shown separately for a group of short-term starved spiders (<30 days of starvation) and long-term starved (>200 days of starvation). **b** Respiratory response of the tarantula *E. truculentus* feeding on a cricket (approximately 18% of spider mass). **c** Respiratory response of wolf spiders *P. prativaga* feeding on lipid-rich, intermediate, and protein-rich fruit flies (approximately 8% of spider mass). Data are compiled from (Jensen et al. 2010; Knudsen 2011; Nespolo et al. 2011)

with nutritional status (see above). Long-term food-deprived spiders do, for example, show a larger factorial response to digestion than short-term starved spiders as a consequence of their difference in resting metabolism (Knudsen 2011).

From the few published examples it is evident that the duration of the SDA response varies. It seems that smaller size of the predator and smaller relative prey size shortens SDA duration (Fig. 5.6). The maximal metabolic rate during feeding is attained in the initial phase when prey handling, enzymatic degradation, and suction feeding occurs (Jensen et al. 2010; Knudsen 2011; Nespolo et al. 2011). It

is presumably also in the initial phases of feeding that protein is extracted (Cohen 1995) and it is of general consensus that protein-based meals generate larger SDA responses than meals relatively high in carbohydrates or lipids (McCue 2006). This is probably because protein synthesis and deamination of the ingested amino acids are energetically expensive processes. Interestingly, the overall metabolic expenditure does not change with starvation duration or prey nutrient composition, although these two factors may affect the relative extraction of macronutrients (Figs. 5.5 and 5.6) (Jensen et al. 2010; Knudsen 2011). While the costs of protein feeding should be higher than feeding on other macronutrients, it is possible that differences in nutrient composition are minor relative to the other costs associated with feeding in spiders (Knudsen 2011; McCue 2006; Secor 2008). The SDA coefficient expresses the proportion of the digested energy used for the digestive process and the SDA coefficient was calculated from the three studies of spider digestion shown in Fig. 5.6. The SDA coefficient of spiders ranged from 19 to 29% in *A. geniculata* and *P. prativaga* (Jensen et al. 2010; Knudsen 2011). A low value (3%) calculated for *E. truculentus* is likely an artifact of only estimating the digestive cost during the initial 8 h (Nespolo et al. 2011) (See Fig. 5.6b) so the SDA coefficient of spiders may, in general, be higher than the average reported for other invertebrates (i.e., 11%) (McCue 2006; Secor 2008). The reasons for this large SDA coefficient are presently unclear, but may be related to the energetically expensive mode of feeding where food/digestive juice is refluxed. Alternatively it is possible that the transition between feeding and fasting in spiders involve larger physiological shifts related to their feeding ecology, where long periods of starvation is exchanged with digestion of huge meals. At least it seems that constrictor snakes also have high metabolic cost of digestion (McCue 2006; Secor 2008; Wang et al. 2001) and these snakes share feeding ecology with spiders as they also consume huge meals, they are also principally sit-and-wait predators and have also adapted to stochastic prey encounters where periods of fasting may be long compared to more regular and 'stable' feeders. At least for constrictor snakes this type of feeding ecology has been proposed to relate to the particular high costs of digestion (Secor 2008).

The factorial increment in metabolism during feeding is equivalent to or larger than metabolic scopes found during activity in spiders. Active *P. amentata* have metabolic rates that are 3–4 fold higher than resting values (Ford 1977) and a similar scope was found in active tarantulas *Aphonopelma* (Seymour and Vinegar 1973). Schmitz found higher metabolic scope for spiders running on a treadmill (scope of 4–12 in *P. lugubris* and 14–25 in *M. muscosa* (Schmitz 2004, 2005), but these levels of activity were only sustainable for seconds to minutes. In contrast the metabolic increase following feeding remains high for hours to days and it is likely that the metabolic response following feeding represents the largest sustained metabolic increment for spiders. Thus, the magnitude, scope, and duration of the SDA response of spiders is equivalent to the responses displayed by some snakes that constitute a very distant group in the evolution of animals which share many of the same characteristics of feeding ecology.

5.10 Conclusion

As sit-and-wait predators, spiders are highly adapted to prolonged food deprivation by a progressive reduction in metabolism and usage of lipid stores, while sparing protein. The capacity of spiders to subdue large prey items provides means to restore energy stores upon fasting, while the unique extraoral digestion also allows for selective extraction of nutrients according to the prevailing needs of the individual spider. Feeding on large meals causes a huge increase in metabolic rate, which likely constitutes the largest sustained increase in metabolism of spiders. Nonetheless, few species have been studied so far and many of the mechanisms underlying the regulation of the digestive responses still remain to be investigated. We suggest that this group of invertebrates is particularly amendable to studies of metabolic transitions because it is relatively easy to study the transfer of energy between prey and predator and because it is relatively easy to manipulate the food available to the spider. It is our hope that this review will inspire to further study the feeding ecology and physiology of spiders.

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Chapter 6
Adaptation of the Physiological, Endocrine, and Digestive System Functions to Prolonged Food Deprivation in Fish

Nadav Bar and Helene Volkoff

6.1 Introduction

Fishes from all types of habitats face limitations in food resources. These limitations may be characterized as local or widespread, predictable or unpredictable, direct or indirect, or acute or chronic (Table 6.1). Despite this diversity, all instances of starvation share one thing in common, namely, they all force the fish to mobilize and oxidize stored metabolic fuels to meet its energy demands. Fishes have undoubtedly faced starvation throughout their evolutionary history, and have apparently evolved different physiological strategies for coping with starvation. Understanding how different fishes respond to starvation is important to a range of biologists including wildlife ecologists, comparative physiologists, and commercial aquaculturists.

Most of the theory about starvation physiology is based on experiments of birds and mammals. Such experiments have given rise to the idea that starving animals undergo three distinct physiological and/or morphological phases. These phases are typically defined by the type of physiological fuel (e.g. carbohydrates, lipids, or proteins) that is being catabolized. Transitions from one phase to another may be indirectly identified by changes in hormone levels, enzyme activities, blood metabolites, or body mass (McCue 2010). Unfortunately, comparisons of starvation physiology across species are generally precluded because researchers rarely measure more than one or two of these variables. Such comparisons are further obscured by the fact that these measurements can be highly variable among individuals.

N. Bar (✉)
Department of Chemical Engineering,
Norwegian University of Science and Technology (NTNU), 7491, Trondheim, Norway
e-mail: nadi.bar@ntnu.no

H. Volkoff
Department of Biology and Biochemistry, Memorial University of Newfoundland,
St. John's, NL A1B 3X9, Canada

M. D. McCue (ed.), *Comparative Physiology of Fasting, Starvation, and Food Limitation*, DOI: 10.1007/978-3-642-29056-5_6,

Table 6.1 Starvation characteristics

Characteristic	Examples
Local	Within an individual's home range
Widespread	Across a species' distribution
Predictable	Caused by tidal or seasonal variations
Unpredictable	Caused by stochastic climatic events
Direct	Lack of food
Indirect	Unsuitable temperature for feeding or digestion
Acute	Lasting hours to days
Chronic	Lasting weeks to months

Starvation is not a typical focal point of studies by aquaculturists. Nevertheless, starvation represents one extreme in the nutritional continuum, and the field of aquaculture offers an abundance of data from controlled studies that can be used to examine the starvation biology of fishes. Commercial aquaculturists are concerned with optimizing the quantity and the quality of production, and measurements of the body composition of fishes are therefore routine. In contrast to the correlative measurements usually used to study starvation, measurements of tissue carbohydrate, lipid, and protein content offer unambiguous insight into the metabolic fuels that are mobilized and oxidized during food limitation. In addition to the nature of the measurements, the large sample sizes and numbers of replicates used by aquaculturists means that these data are particularly well suited for modeling purposes.

Although there are several reports describing distinct phases of starvation in fish, these reports give only evidence that the transition exist but usually do not characterize, quantify them or attempt to find the mechanistic trigger for these transitions. Our first goal is to characterize the sequential compositional changes associated with starvation in fish. These changes will allow us to model the way in which fishes mobilize and oxidize different metabolic fuels. We pay particular attention to exploring the possibility that starvation in fishes follows the same pattern exhibited by starving endotherms. We do this by identifying the transition and then measuring the fat storage level (which is usually not the whole body fat level) at that point. We found that each species exhibits a distinctive threshold.

Although the exterior morphology of fishes is generally similar and constrained by fluid dynamics, the internal morphology is quite diverse. Different species are recognized for high lipid content in the muscles, liver, or viscera. Our second goal is to examine how different strategies for storing energy affect the mobilization of endogenous energy stores during starvation. We are particularly interested in which organs are most responsive to changes in nutritional status (see also Bauchinger and McWilliams, Chap. 12).

Ambient temperature has minimal effects on the starvation responses of endotherms, but can have large effects on the metabolic processes of fishes. Consequently, our third goal is to examine the effects of temperature on starvation

dynamics. Here we present hypothesis generated by a dynamic model of fish growth (Bar et al. 2007; Bar and Radde 2009), namely that low temperatures may prolong the transition to Phase III, and present evidence for the support of this hypothesis.

The results shown here are important for aquaculture applications. We demonstrate through a quantitative model that energy value in the diet formulation is very important in lean fish with fat deposit values close to the fat threshold, common for instance in rearing of juvenile fish.

6.2 Phases During Starvation

Starvation physiology was studied extensively in birds and mammals. It has been shown that these animals undergo three distinct physiological and/or morphological phases. These phases are typically defined by the type of physiological fuel (e.g. carbohydrates, lipids, or proteins) that is being catabolized. These phases have been demonstrated in birds (Maho et al. 1981), including penguins (Robin et al. 1988, 1998; Cherel et al. 1988a; Groscolas and Robin 2001), and mammals (Cherel et al. 1988b, 1991, 1992; Cherel and Le Maho 1991; Belkhou et al. 1991). They can be described by the following properties:

1. a short transient stage where both protein tissues and fat reserves are mobilized (Phase I)
2. a longer steady state with mobilization of fat as the main source of energy, that lasts until fat stores reach a critical threshold (Phase II)
3. a state in which the mobilization of protein tissue is largely increased as this becomes the main source of energy (Phase III)

Although there are several reports describing distinct phases of starvation in fish (e.g. the well-cited work of Black and Love 1986), these reports only give evidence that the transitions exist without characterizing them. Furthermore, it appears that all the studies either concentrate on a short time scale of starvation (weeks), or very long time periods of starvation with very few measurements [e.g. 1594 starvation days in eel, studied by Boëtius and Boëtius (1985)]. In the latter case it is difficult to clearly distinguish between the phases, as the measurements are scarce, include little information on body tissues and often do not include details on endocrine changes.

6.2.1 Phase I: Hormonal and Physiological Changes

At the start of a starvation period, the concentrations of many hormones change (Table 6.2). As the published data is mostly qualitative, a quantitative comparison across species is not possible. In many species, plasma growth hormone (GH)

shows significant elevation in the first weeks of starvation. Ghrelin levels are generally high, although reported not to be affected by starvation in tilapia (Riley et al. 2008). Fasting reduces the gene expression and plasma concentrations levels of insulin-like growth factor (IGF-I) rapidly, and levels remain low throughout the starvation period. Plasma leptin has been reported to increase in salmon during the first days of starvation (Kling et al. 2009). It also appears (Table 6.2) that this phase of starvation has relatively little effect on plasma cortisol levels (Sumpter et al. 1991). The physiological changes at the start of starvation include increased catabolism, mostly of glycogen but also of protein and fat. Woo and Cheung (1980) show a strong initial decline in body weight and circulating ammonia levels in the first 15 days of starvation of sneakhead (*Ophiocephalus maculatus*). Woo and Fung (1981) report a 10% decrease in body weight in the first 7 days of starvation of red sea bream, whereas lipid levels slightly increase during this period, possibly due to excess of energy from protein catabolism. This is followed by a steady weak decline in body weight and protein tissue, corresponding to Phase I and II, respectively. Similarly, protein and lipids were rapidly consumed at the beginning of the starvation experiments on *Hoplias malabaricus* (Rios et al. 2006), immature carp *Cyprinus carpio* (Blasco et al. 1992), rainbow trout *Oncorhynchus mykiss* (Lauff and Wood 1996), *Salmo gairdneri* (Jezierska et al. 1982; Reinitz 1983; Loughna and Goldspink 1984), Atlantic salmon *Salmo salar* (Einen et al. 1998), larval *Pleuronectes platessa* (Ehrlich 1974), red porgy *Pagrus pagrus* (Rueda et al. 1998), juvenile *Ambassis vachelli* (Molony 1993), red sea bream (Umino et al. 1991), roach *Rutilus rutilus* (Van Dijk et al. 2005; Binner et al. 2008), burbot *Lota lota* (Binner et al. 2008), larval trout cod *Maccullochella macquariensis* (Gunasekera et al. 2001), and coho salmon *Oncorhynchus kisutch* (Larsen et al. 2001). In sunshine bass, liver mass and liver glycogen contents decreased after 1 week of fasting and remained low until the end of the study. Liver weight was reduced 4–5 fold and liver glycogen was essentially depleted after 2 weeks of fasting. Intraperitoneal fat was significantly lower after 2 weeks of fasting and did not recover after a week of refeeding (Davis and Gaylord 2011).

6.2.2 Phase II

After Phase I of starvation, mobilization of protein appears to be reduced and fat is the main source of energy. In starvation experiments of red sea bream (Woo and Fung 1981) lipids declined from levels of 13% body weight to 1.5% during the 34–55 days of Phase II, whereas the protein loss was relatively low, decreasing from 18.7% body weight to 17.4% (Fig. 6.1), indicating conservation mode. Similar results have been reported for many fish species (Binner et al. 2008; Black and Love 1986; Blasco et al. 1992; Van Dijk et al. 2005; Einen et al. 1998; Gunasekera et al. 2001; Jezierska et al. 1982; Kiessling et al. 1990; Larsen et al. 2001; Loughna and Goldspink 1984; Luo et al. 2009; Molony 1993; Pottinger et al. 2003; Rios et al. 2006; Rueda et al. 1998; Woo and Fung 1981).

Table 6.2 The effect of starvation on hormonal levels in different fish species at the first period (up to 6 weeks) of starvation (compared to control, non-fasting fish)

Variable	Species	Effect	Duration of effect	Duration of starvation	Reference
Plasma GH	Salmonids	Significantly elevated			Rousseau and Dufour (2007)
	eel	Elevated			Rousseau and Dufour (2007), Wilkinson et al. (2006)
	Sea Bream				Rousseau and Dufour (2007)
	Hybrid striped bass	Elevated	23 days	106 days	Picha et al. (2009)
	Mozambique tilapia	Elevated	4–8 days	8 days	Fox et al. (2010)
	Trout	Elevated	2–6 weeks	6 weeks	Norbeck et al. (2007), Valente et al. (2003)
	Steelhead trout	Up 6-fold	30 days	30 days	Barrett and McKeown (1988)
	Rainbow trout	Elevated (5–6 folds)	30–45 days	45 days	Sumpter et al. (1991)
		Elevated (3-fold)	1–4 weeks	4 weeks	Kling et al. (2009)
	Tilapia	GH No effect, muscle GH-R significantly increased	4 weeks	4 weeks	Fox et al. (2010)
	Tilapia	Elevated	Day 3		Riley et al. (2008)
	Rabbitfish	Elevated	Day 9–15	15 days	Ayson et al. (2007)
Ghrelin	Mozambique tilapia	Elevated weeks	2 weeks	4 weeks	Fox et al. (2010)
	Hybrid striped bass	Elevated (5–7 fold)	23 days	106 days	Picha et al. (2009)
	Salmon	Elevated	From day 2		Hevroy et al. (2010)
		Lower mRNA levels	From day 2		Hevroy et al. (2010)
		Elevated stomach mRNA levels		6 days	Murashita et al. (2009)
	Tilapia	No effect		7 days	Riley et al. (2008)

(continued)

Table 6.2 (continued)

Variable	Species	Effect	Duration of effect	Duration of starvation	Reference
	Goldfish	Elevated gut and brain mRNA	7 days	7 days	Unniappan et al. (2004)
		Elevated plasma levels	3–5 days	7 days	Unniappan et al. (2004)
	Seabass	Elevated stomach mRNA			Terova et al. (2008)
	Cod	No effect on gut mRNA		30 days	Xu and Volkoff (2009)
	Zebrafish	Increase in gut mRNA		3, 5, 7 days	Amole and Unniappan (2009)
	Rainbow trout	No change		3 weeks	Jonsson et al. (2007)
Ghrelin-1 mRNA	Salmon	Lower	Day 2		Hevroy et al. (2010)
IGF-1	Tilapia	Lower	4 weeks	4 weeks	Fox et al. (2010)
		Lower	3–7 days	7 days	Riley et al. (2008)
	Mozambique tilapia	Lower	4–8 days		Fox et al. (2010)
	Atlantic salmon	Lower	15 days		Wilkinson et al. (2006)
	Trout	Lower plasma levels	2–6 weeks		Norbeck et al. (2007)
	Rabbitfish	Elevated plasma and mRNA levels	Day 3–9	15 days	Ayson et al. (2007)
	Sunshine bass	Lower plasma levels	4 weeks	4 weeks	Davis and Gaylord (2011)
IGF-binding proteins	Salmon	Elevated	Day 14		Hevroy et al. (2010)
Plasma leptin	Rainbow trout	Elevated	Week 1		Kling et al. (2009)
Cortisol	Salmon	No effect			Sumpter et al. (1991)
	Sneakhead	Lower three fold	66 day	142 days	Woo and Cheung (1980)
	Seabass	No effect		31 days	Caruso et al. (2011)
	Seabream	No effect		31 days	Caruso et al. (2011)
	eel	Increase	Day 42	58 days	Caruso et al. (2010)
	Plaice	Decrease		48 hours	White and Fletcher (1986)
	Catfish	Decrease		21 days	Small (2005)
	Catfish	Increase		30 days	Peterson and Small (2004)

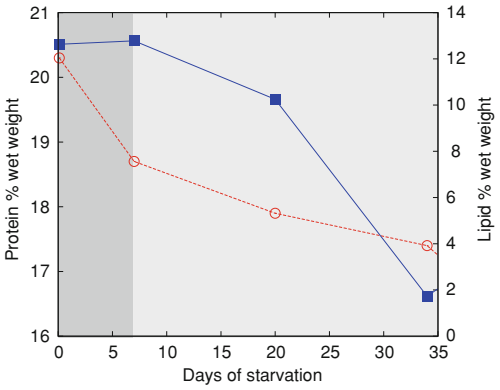

Fig. 6.1 The first phase (*dark gray*) is characterized by sharp decline of protein or/and fat storage in a short time. Phase II (*light Gray*) is apparent by a slow, linear loss of protein (*red circles*) and sharp decline of the energy storage (lipids, *blue squares*). Data acquired from Woo and Fung (1981)

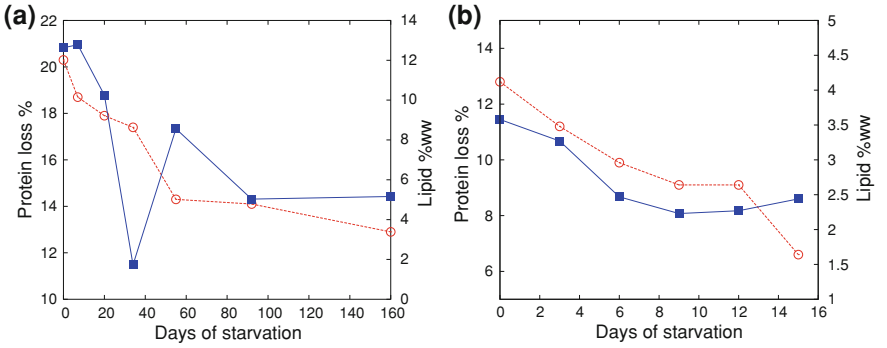

Fig. 6.2 Values of protein (% BM, *circles*) and lipid (% BM, *squares*) during **a** 160 days of starvation in red sea bream (Woo and Fung 1981) and **b** 15 days (Gunasekera et al. 2001)

The observed endocrine levels studied to date usually display a linear increase or are stable during the second phase. Plasma levels of ghrelin in hybrid striped bass increased continuously from about 7 pg/ml at the end of Phase I to approximately 75 pg/ml after 106 days of starvation, whereas the levels stayed under 2 pg/ml for fed fish (control) (Picha et al. 2009). In the same experiments, GH plasma levels were elevated in the beginning of the experiment and apparently remained constant (elevated) during the whole starvation period. In Mozambique tilapia, plasma ghrelin levels were elevated significantly after 4 weeks of fasting, but no change was detected in stomach ghrelin mRNA levels. Four weeks of fasting did not affect plasma GH levels, although plasma IGF-I, and glucose were reduced significantly (Grau et al. 2009). In Atlantic cod, ghrelin gut mRNA expression was not affected by 30 days starvation (Xu and Volkoff 2009).

Table 6.3 Critical values in various fishes. The γ value is determined as the level of fat (% BM) in which the protein loss significantly elevated

Species	γ value	Source of lipid	Data source
Roach	1.2% BM	Muscle	Binner et al. (2008)
Trout Cod	2.27% BM		Gunasekera et al. (2001)
Carp *Cyprinus carpio*	1.04% BM	Muscle	Blasco et al. (1992)
Atlantic cod	0.9–1.3% BM	Liver	Black and Love (1986)
Traira *Hoplias malabaricus*	3.5% liver mass	Liver	Rios et al. (2006)
Red sea bream	1.6% BM	Vicera	Woo and Fung (1981)
Atlantic salmon	2.10% BM	Vicera	Einen et al. (1998)
Plaice	(*Pleuronectes platessa*)	1.12–1% bm	NA
Ehrlich (1974)			
Ambassis vachelli	2.20%	NA	Molony (1993)
Rainbow trout	$<4.4\%$	NA	Reinitz (1983)
Brown trout	0.68–0.75% BM (1.14–1.36% muscle) for winter and summer, respectively	Muscle	Navarro et al. (1992)
Red sea bream	$3 < \gamma < 5.1\%$	Intraperitoneal fat and muscle	
European Eel	$\gamma < 5$	Muscle	Dave et al. (1975)

6.2.3 Transition to Phase III

Many researchers report that when the lipid reserves of the fish are near depletion, protein tissues start to be degraded more intensively (Binner et al. 2008; Black and Love 1986; Blasco et al. 1992; Ehrlich 1974; Einen et al. 1998; Gunasekera et al. 2001; Kiessling et al. 1990; Molony 1993; Rios et al. 2006; Umino et al. 1991; Woo and Fung 1981). A few researchers even reported an elevated secretion of hormones and ammonia in the plasma at this final phase for rainbow trout (Pottinger et al. 2003), taira (Rios et al. 2006), and carp (Blasco et al. 1992), similar to penguins (Robin et al. 1998; Cherel et al. 1988c; Groscolas and Robin 2001) and rats (Cherel et al. 1992; Belkhou et al. 1991). The transition to Phase III is typically followed by elevated concentrations of ammonia products in the blood (Woo and Fung 1981; Rios et al. 2006), similar to birds and mammals. This most likely reflects

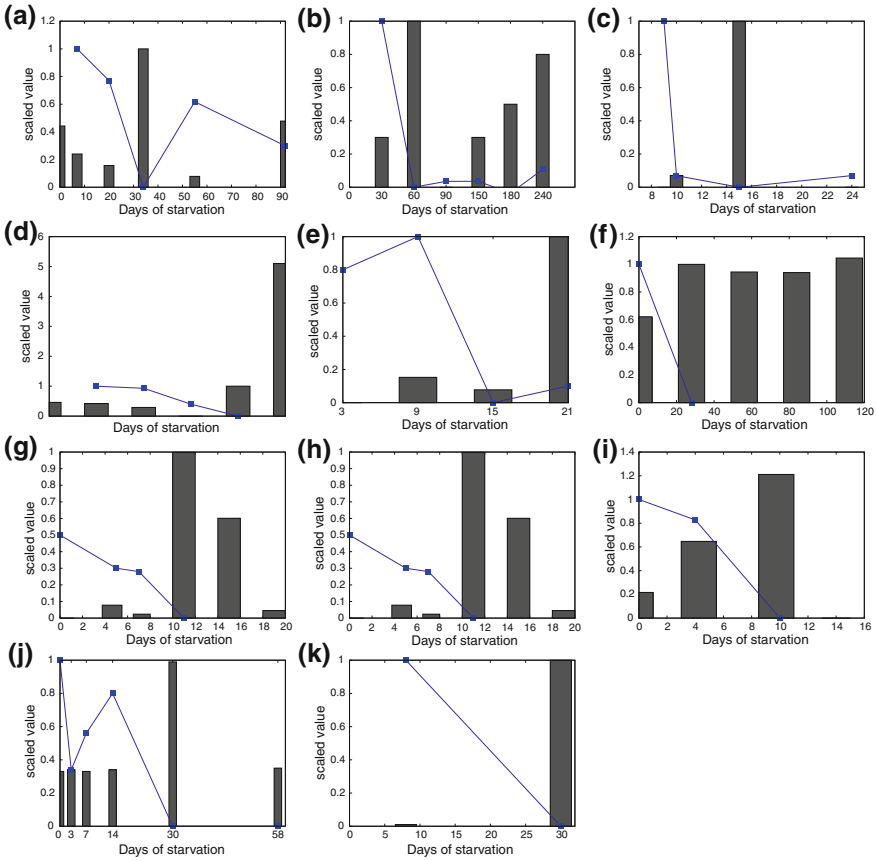

Fig. 6.3 The protein loss (columns) as calculated in Eq. (6.1) and the deviation of lipid (% BM) from the critical value γ (*lines*, see values in Table 6.3) for **a** red sea bream (Woo and Fung 1981), **b** traira (Rios et al. 2006), **c** juvenile *Ambassis vachelli* (Molony 1993), **d** trout cod (Gunasekera et al. 2001), **e** juvenile Roach/Burbot (Binner et al. 2008), **f** rainbow trout (Reinitz 1983), **g–i** three experiments on *Pleuronectes platessa* (Ehrlich 1974), **j** Atlantic salmon (Einen et al. 1998), and **k** brown trout (Navarro et al. 1992)

a significant increase of protein degradation, followed by deamination of the amino acids.

Data show that Phase II and the transition to Phase III can be demonstrated quantitatively. The protein levels (% wet weight) and the percent body mass (% BM) are gradually (almost linearly) reduced during the first 12 days of starvation of larval trout cod reported by Gunasekera et al. (2001) (Fig. 6.2a), while fat is rapidly mobilized. When fat reaches low levels, the protein mobilization increases. Similarly, in the first 10–40 days of starvation in red sea bream (Woo and Fung 1981) protein is conservatively degraded (Fig. 6.2b), whereas fat is catabolized rapidly. When the lipids reach low levels (less than 2% BM), protein is rapidly mobilized.

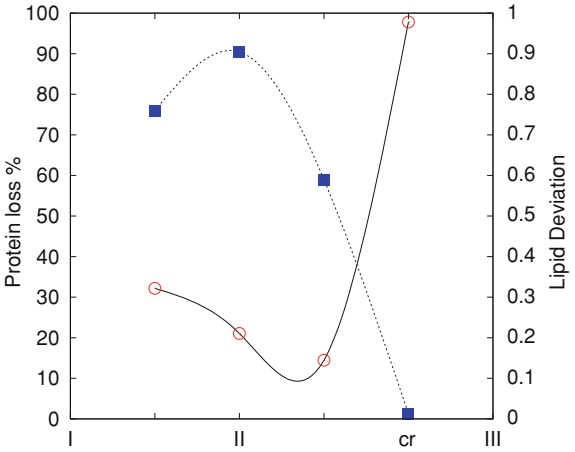

Fig. 6.4 Lipid deviation from the critical γ value (*squares*) and the protein loss (*circles*) as a function of starvation time. The starvation phases and the time point the fat reaches critical value are marked (cr). The curves were generated by spline interpolation. Protein is rapidly degraded (maximum protein loss) when the fat reserves are depleted (the lipid deviation from critical value is very small)

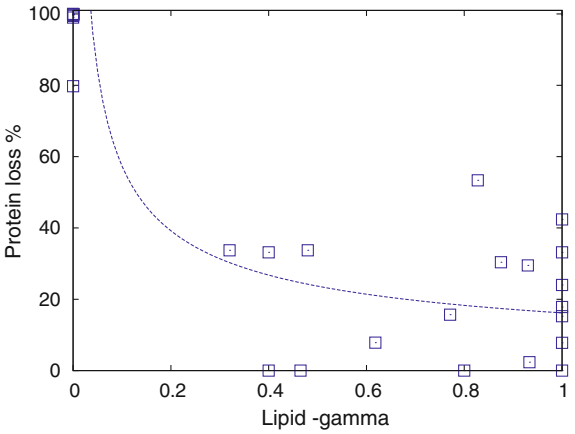

Fig. 6.5 Protein loss as a function of the lipid deviation from the critical γ of the experiments presented in Fig. 6.3 and a fitted ($f(x) = 16.178x^{-0.558}$, $R^2 = 0.072$) exponential curve (*dashed*)

To emphasize the drop in protein tissue and to be able to compare between experiments, we define the protein loss in the following manner:

$$\mathrm{P_{loss}}(t) = \left(1 - \frac{P(t)}{P(t-1)}\right) * 100 \tag{6.1}$$

with $P(t)$ is the protein value measured at time t, $P(t-1)$ is the preceding protein value. We then scale (normalize) the protein loss value:

$$\mathrm{P_{loss,S}}(t) = \frac{\mathrm{P_{loss}}(t)}{\max(P)} \tag{6.2}$$

where $\max(P)$ is the maximum value of protein measured during the starvation experiment.

We illustrate the change in the scaled protein loss as a function of the deviation of the lipid reserved value (L) from the critical level (γ in Table 6.3), i.e. $L - \gamma$ (Fig. 6.3a–k). There was a significant sudden increase in the protein loss (corresponding to a drop in the protein levels) when the lipid reserves levels reached the critical value γ in all the experiments reviewed here. After the initial protein breakdown, protein losses were either maintained at high levels (Fig. 6.3f, i) or oscillated (Fig. 6.3a, b, g, and h) as the lipid levels continued to drop. The deviation of fat from the critical levels usually remained low since the fat reserves were already depleted. To illustrate the effect of fat depletion on mobilization of protein, we plotted the protein loss (%) and the lipid deviation from the critical value γ as functions of starvation time (Fig. 6.4). Data were generated by calculating the average values of protein loss and fat in the experiments presented earlier (Fig. 6.3) at Phases I and II and at the time point the critical value is reached. The results confirm that the protein degradation is at maximum (corresponding to maximum protein loss) as the lipid reserves drop towards the critical value (lipid deviation approaches zero). Note that the figure does not incorporate values after the transition to Phase III, i.e., measurements taken after the lipid has reached its critical value. According to the data reviewed here (Table 6.3, Fig. 6.3), there is an exponential relation between the protein loss and the lipid deviation (Fig. 6.5).

6.3 Effect of Temperature During Starvation

6.3.1 Effect on Hormone Levels

Temperature appears to have an effect on the response of fish to starvation and on the levels of hormones such as GH, ghrelin, and leptin. For instance, plasma GH levels significantly increase during 3 weeks of starvation at the optimal temperature (24°C) of hybrid striped bass (Picha et al. 2009). However, despite its high levels, plasma GH does not increase farther during 3 months starvation at winter temperature (14°C). Conversely, ghrelin has a sharper increase during starvation at colder temperatures. Plasma IGF-I shows opposite pattern to GH and ghrelin, and declines continuously at both high and low temperatures. In burbot, a 2-week fasting period induces a loss in body weight at a higher rate at 10°C than at 2°C. However, it reduces the plasma leptin and ghrelin-immunoreactive peptide concentrations and the relative liver weights and the liver and muscle glycogen concentrations at 2°C but not at 10°C (Nieminen et al. 2003).

6.3.2 Effect on Body Composition

Previous studies show that protein degradation is strongly affected by temperature, and that it increases with low temperatures (McCarthy and Houlihan 1997; Van Dijk et al. 2005). The model of Bar and Radde (2009) assumes minimal

protein degradation rate at the optimal growth temperature. However, protein synthesis, which is regulated by the energy demands, linearly increases with the temperature (Houlihan et al. 1995; McCarthy and Houlihan 1997; McCarthy et al. 1999). This thermal dependency of protein metabolism can have major consequences in starvation periods, as the excess ketone bodies generated from protein degradation at low temperatures combined with low demand for fuel (due to low protein synthesis rates) can prolong the phases of starvation significantly, particularly Phase II. The excess of energy reduces fat oxidation rates and preserves adipose cells, thus increasing the time needed to reach the critical lipid level and delaying the transition to Phase III. Conversely, at warmer temperatures, particularly around the optimal temperature for growth (a species dependent factor), Phase II can be significantly shorter since the metabolic rate is high simultaneously with high rates of protein synthesis (that consumes large amount of energy) and low rates of protein degradation (that supply little energy). Body fat is then the main source of energy and rapid oxidation and depletion of adipose cells shortens the transition to Phase III.

6.4 Digestive System During Fasting

The literature provides limited evidence regarding the effect of starvation on the digestive system. It appears to vary among species probably due to adaptation to various habitat conditions, including temperature, diet type (e.g. carnivore, herbivore), and the frequency of natural starvation events. At the early stages of starvation (i.e. hours to several days), rapid changes of both gut physiology and enzyme activity can be noticed. Krogdahl and Marie Bakke-McKellep (2005) report a decrease of 20–50% of both mass and enzyme activity in the pyloric intestine, mid intestine, and distal intestine in the first 2 days of fasting of Atlantic salmon. Conversely, Mommsen et al. (2003) observed an increase in metabolic enzyme activity in the mucosa of the stomach and along the intestinal tract of Nile tilapia after a short-term starvation period. Higher protein degradation in intestinal tissue than other tissues were measured during early phases of fasting for other fish species (Houlihan et al. 1988). Fasting seemed to cause an immediate mobilization of protein resources from the intestine (Krogdahl and Marie Bakke-McKellep 2005) and histological changes in the structure of the distal intestine could already be seen in the first 2–3 days of starvation. Reduction in gut length, surface area, and cell height occurred also in other fish species (Hall and Bellwood 1995) after a short-term starvation period (see also Lignot, Chap. 14).

As the starvation period is prolonged, some degradation processes reverse their trends. Krogdahl and Marie Bakke-McKellep (2005) report that mass loss and catabolic enzyme activity slow down. The intestinal mass of starving salmon was stabilized from day 4–40, and no significant change could be observed. Stabilization of the enzyme activity may indicate shifts from luminal signals to hormonal signals and other regulatory pathways. In a number of salmonid species (Baeverfjord and

Krogdahl 1996), starvation causes alteration in the structure of the absorptive cells, characterized by reduction in cell height, amount of cytoplasm, RNA levels and the arrangement of the nuclei. In bluegills (Hossain and Dutta 1991), 50 days of starvation exerted less profound influences on the caeca than on the main tract (intestine). The intestine's mucosal complexity was diminished, and of all the microscopic layers, the submucosa was found to be the most affected by starvation in both caeca and intestine. In freshwater rainbow trout fasted for 4 weeks, mucosal weight decreased by 76% and after 8 weeks by 84% (Bogè et al. 1981). Winter flounder in comparison, lost 57% of its mucosal mass and its surface area reduced by 50% during 7 months of fasting (McLeese and Moon 1989). Mucosal folding was reduced in all sections of the intestine during the starvation period. Goblet cell number declined, but unlike the salmon, little change in enterocyte microstructure was observed. Intestinal length remained the same, although the caeca shortened. McLeese and Moon (1989) suggest that the continued demand for salt and water transport for osmoregulation maintains the proximodistal gradient of mass and surface area and ameliorates the effect of starvation on mucosal mass and area. The sparing of intestinal mucosa may also be influenced by colder temperatures.

It appears that the transformations that starvation induces in the digestive system are in accordance with the overall description of Phases I and II, at least of several fish types (mainly salmonids, which are well-studied species). Although the transition between the phases can not be read directly from the studies on the digestive tract (changes in whole body lipid and protein were not recorded), the strong decrease in enzyme activity and mass that characterizes Phase I is clearly evident in the gut. The stabilization of the mass loss after several days is also evidence for the transition to Phase II, and an almost perfect conservation of mass and protein is maintained during the following period, Phase II.

6.5 Implication of the Critical Fat Level in Aquaculture

The primary goals in aquaculture are growth, health, and sustainability. Fish should not only grow relatively fast and increase their food consumption, but also increase the efficiency at which they utilize the feed ingredients. The feed should contribute to better fish health, composed of readily available ingredients, and deliver optimal amounts of protein and fat. Fish utilize the energy from the dietary fat and protein in different manners according to biotic and environmental conditions. The aquaculturists primary aim is to reduce the utilization of dietary protein to energy, by adapting the diet to the energetic needs of the fish in such a way that fish use most of the dietary protein to build body mass, while using dietary fat to satisfy energy requirements for metabolism and protein synthesis. A less desirable, but nevertheless common, scenario occurs when the fish uses dietary protein and body mass to supply most of its energy demands, particularly if the dietary fat is insufficient. In lean fish, the latter scenario is more likely to occur if it receives high protein and low fat dietary content (Fig. 6.6).

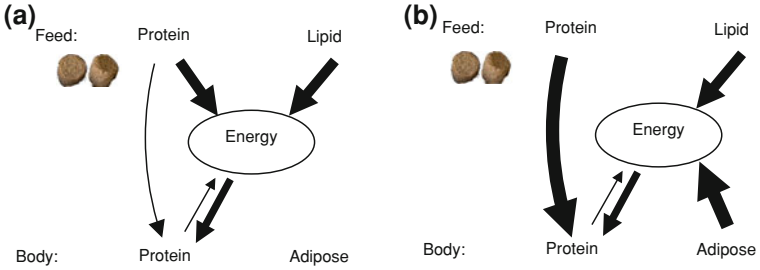

Fig. 6.6 Utilization of dietary components for **a** lean fish, where most of the energy to protein synthesis is satisfied from dietary fat and protein and **b** fish with high fat levels, which can supply the fuel to protein synthesis from its adipose cells. The width of the arrows indicates the flux size

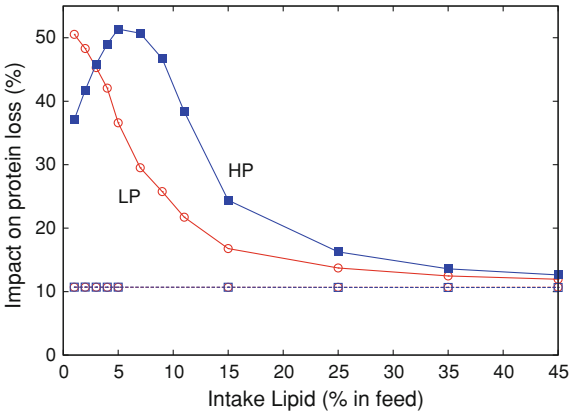

Fig. 6.7 The sensitivity (impact) of intake lipid on protein loss for high protein (HP) and low protein (LP) diet is increased as the feed intake reduces in lean fish. The protein loss has a constant linear values (*dashed lines*) for both HP and LP diets when simulated for fat fish (initial lipid values $> 10\%$ BM)

To be able to calculate a cost-effective feed composition under various conditions, a model of fish growth was developed (Bar and Radde 2009), which enables easy simulations of various feeding scenarios, including fasting and refeeding. Simulations revealed that body mass and growth rates are highly sensitive to even small reductions in dietary fat for lean fish, i.e., when the fish has low fat storage (Fig. 6.7), whereas fishes rich in adipose cells exhibit only linear growth change. According to the model, the sensitivity grows nonlinearly as the body lipid values approaches the critical level, and reaches a peak around the critical lipid level γ.

To produce cost-effective fish in rearing conditions, the aquaculturist has to consider the dietary fat levels, prices of the lipid and protein, season, and temperature, particularly at early stages when fish usually display low body fat contents. The aim is to keep the fat stores in fish above the critical lipid level by supplying sufficient dietary fat, but to use cost-effective ingredients when the body fat levels are high enough to optimize production.

6.6 Further Notes and Discussion

Plasma GH and pituitary GH-mRNA increases during starvation (Table 6.2) and remain elevated during most of the starvation period, through Phases I and II. Unlike mammals, there is often a delayed response of GH increase at the start of the starvation period up to several weeks, particularly in salmonids (Pottinger et al. 2003). This delay may be common to many ectothermic animals that are adapted to prolong periods of fasting. It is possible that GH increases after the transition to Phase II or at least at a similar time point and is a key factor for Phase II. Sumpter et al. (1991) suggested a lipolytic role of GH during starvation and a protein-sparing effect, most likely by facilitating mobilization of free fatty acids and glycerol (Picha et al. 2009; Pottinger et al. 2003). Thus, high plasma GH levels might represent an adaptive response to make available metabolic fuels during malnutrition at Phase II but, at the same time, could prevent adiposity in actively feeding fish (Pèrez-Sànchez and Le Bail 1999). There are no reports on GH levels on Phase III, it is likely that GH levels drops since lipids are not the important metabolic fuel at this stage.

Although there is a consensus regarding the role and mechanisms of GH in fish, the role of ghrelin during starvation in fish is less obvious. Most of the studies indicate elevated levels of ghrelin early in fasting stage (Table 6.2), in accordance to ghrelin response to starvation in mammals (Jonsson et al. 2007). Ghrelin levels have been reported to increase continuously during the whole starvation period (Picha et al. 2009). Experiments on tilapia, cod and rainbow trout, however, did not indicate any change in ghrelin concentrations, and ghrelin expression was even down-regulated 2 days after starvation began (Hevroy et al. 2010). Both in vivo and in vitro evidence suggest that plasma ghrelin levels rise during negative energy balance in fish (Picha et al. 2009) and this may trigger, at least partially, GH secretion. It appears also that the rise in ghrelin levels is steeper at low temperature (over-wintering periods) than at high ones in hybrid sea bass (Picha et al. 2009). Interestingly, ghrelin concentrations continued to rise during the whole overwintering starvation period, whereas GH levels remained almost constant. These results suggest that ghrelin may have other functions than GH upregulation in periods of low temperatures, common for low food availability (over-wintering). There is currently no data on ghrelin concentrations during Phase III, and this is an important area for future investigations.

In most fish species studied to date, cortisol is significantly increased at the beginning of Phase III (Dave et al. 1975), in accordance to the finding reported in birds (Jenni et al. 2000; Cherel et al. 1988c), and mammals (Belkhou et al. 1991) (see also Chapters Jenni-Eiermann and Jenni, Chap. 11; Harlow, Chap. 17; Ben-Hamo et al., Chap. 16; Champagne et al., Chap. 19). It may be a factor involved in the regulation of protein mobilization after depletion of lipid stores. However, several exceptions have been reported. Fasting has been shown to have no effect (Sumpter et al. 1991; Pottinger et al. 2003; Caruso et al. 2011) or decrease plasma cortisol (Small 2005; Woo and Cheung 1980). For example, cortisol has shown to

be down-regulated threefold after 66 days of starvation in sneakhead (Woo and Cheung 1980). However, during this period, transition to Phase III was not evident and it may be the reason we do not observe any elevation of cortisol. The variability in the response of cortisol to fasting might be species specific or might be due to different experimental conditions related to other stressors and duration of fasting. For example, in catfish, cortisol levels peak after 30 days of fasting, but no differences between fed and starved fish are seen before or after that peak (Peterson and Small 2004), indicating that the duration of the fasting influences the variations in cortisol levels. Most likely, cortisol has other functions besides protein mobilization during starvation and may respond in various manners prior to Phase III, according to the conditions and species. There is little additional information on cortisol (or corticosteroid hormones generally) during starvation and this warrants further investigation.

Laboratory experiments under controlled temperatures and light exposure conditions indicated that roach may have a sort of internal clock, and the starvation physiology changes with the seasons in addition to the temperatures (Van Dijk et al. 2005). Generally, winter fishes were better prepared to face starvation periods than summer adapted ones, particularly in cold water starvation periods. This pattern may have an evolutionary origin since winter is associated with low food availability and reduced activity. Burbot and roach developed different season-dependent strategies to minimize energy turnover during their inactive periods, which are associated with a restricted food intake (Binner et al. 2008).

There are some issues we have to consider regarding the calculation of the critical value. A large reduction in protein (which may reflect the transition to the third phase) is not always obvious as in the case of Navarro et al. (1992). The fish lost 21% of its body and muscle wet mass in the period between days 30 and 50, but muscle protein as percent of wet body mass shows only a minor decrease. This response may be explained as the relative water content in the body has increased and different results might be obtained if calculations were made using dry BM (see example in McCue 2010). Another good indication for the transition to Phase III is the significant increase of ammonia in the plasma and amino acids resulting from increased protein catabolism. Calculations of the critical lipid value can be made at that point. Additionally, it appears that the critical value is a species specific factor (Table 6.3) and the source of lipid varies among fishes. The critical values given here (Table 6.3) are approximations since most of the experiments do not provide frequent measurements of fat and protein composition, but rather measurements encompassing long time intervals. The protein degradation may already be in progress when lipid and protein measurements are taken. Therefore, the critical value used in this study may actually represent an upper bound of the critical value and serve as a comparison between the value of different species. It is important to note also that the third transition is most likely not a discrete process, and the protein loss increases continuously, long before the fat reaches the critical level (see Black and Love 1986). This is also apparent in the protein loss function generated here (Fig. 6.5). It may appear as a discrete event (occurs almost instantaneously) if the time interval in which the fat is reduced to the critical level

is short or if the frequency between measurements is low. If the fat is reduced slowly, as a result of scarce/ insufficient feeding, or low temperature, the transition is most likely more continuous and less abrupt.

6.7 Concluding Remarks

The mobilization of energy during starvation in fish follows a pattern similar to that of endotherms, which apparently have three distinctive phases. Phase I incorporates rapid tissue degradation and variations in endocrine factors. Phase II is characterized by utilization of energy from lipid stores and conservation of protein. The source of the lipid stores varies between fish species, and usually the liver and muscle are utilized. When the lipid stores reach a critical level, transition to Phase III occurs with rapid protein mobilization. The critical value changes between species and the temperature may prolong the period during which Phase II is active.

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Chapter 7
Starvation in Subterranean Species Versus Surface-Dwelling Species: Crustaceans, Fish, and Salamanders

Frédéric Hervant

7.1 Introduction

Subterranean (i.e., hypogean) ecosystems (mainly caves, karstic, and porous systems) are characterized by continuous darkness (and therefore by a lack of photosynthetic organisms), constant temperature, high moisture, low dissolved oxygen levels, and restriction in space (Malard and Hervant 1999; Culver and Pipan 2009). As primary production is absent from these hypogean biotopes, food is generally only available from the surface, during a few periods of the year (for example, after important rainfalls that drain nutrients across ground layers, or after an increase in the infiltrating stream flow). This reliance on external energy sources generally means that there are fewer energy resources in hypogean habitats and that the diversity of food sources is low (Culver and Pipan 2009). Following these allochthonous inputs, nutrients are quickly consumed by microorganisms (Gibert et al. 1994). Thus, apart from some exceptions (e.g., nutrient-rich tropical caves) hypogean organisms must cope with frequent, unpredictable, and prolonged starvation (Hervant et al. 1997; Hervant and Renault 2002). In order to survive such extreme constraints, subterranean organisms have evolved efficient behavioral, physiological, and metabolic adaptations, which extend their survival during food deprivation. Several hypogean invertebrates are able to tolerate up to one year without food, but some cave fishes and salamanders are apparently able to survive a few years of starvation (Poulson 1963, Hervant et al. 1997, 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010; Table 7.1).

F. Hervant (✉)
Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés (UMR CNRS 5023), Université Lyon 1, bât. Forel, 69622, Villeurbanne Cedex, France
e-mail: frederic.hervant@univ-lyon1.fr

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Table 7.1 Main metabolic and physiological parameters in fed and starved surface-dwelling and subterranean crustaceans, amphibians, and fishes

	Surface-dwelling species				Subterranean species					
	Gammarus fossarum	*Asellus aquaticus*	*Astyanax fasciatus* (epigean population)	*Calotriton asper* (epigean population)	*Niphargus virei*	*N. rhenorhodan*ensis	*Stenasellus virei*	*Astyanax fasciatus* (cave population)	*Calotriton asper* (cave population)	*Proteus anguinus*
Survival time	1 month	2 months	unknown	up to 5 months	up to 1 year	up to 1 year	up to 1 year	unknown	up to 8 months	1–2 years
Duration of experimental starvation (days)	28	28	60	120–60	240–180	240–180	240–180	60	120–60	240–180
Oxygen consumption (fed)	7.3	7.1	12	1.5	3.2	4.8	3.9	6.1	1.1	0.55
Starvation-induced hypometabolim (%)	−25	−21	*NS*	−30	−65	−50	−53	−29	−51	−72
Starvation-induced hypoactivity (%)	−65	−63	−16	−45	−96	−95	−98	−64	−76	−90
Glycogen stores (fed)	28	41	10	198 (liver) 38 (muscle)	65	85	78	19	251 (liver) 47 (muscle)	92 (muscle)
Glycogen utilization rate (starved)	0.7	0.8	–	1.3 (liver) 0.2 (muscle)	0.15	0.19	0	0.25	0.91 (liver) 0.05 (muscle)	0.06 (muscle)
Triglycerides stores (fed)	3.8	4.9	65	23 (liver) 7.1 (muscle)	6	5.5	7.5	41	26.8 (liver) 10.5 (muscle)	30 (muscle)
Triglycerides utilization rate (starved)	0.06	0.09	–	1.3 (liver) 0.2 (muscle)	0.008	0.01	0.01	–	0.91 (liver) 0.05 (muscle)	0.01 (muscle)
Arginine phosphate stores (fed)	2	3.6	–	–	7	9	7.3	–	–	–
Arginine phosphate utilization rate (starved)	0.07	0.06	–	–	0.006	0.008	0.007	–	–	–

Values calculated from Hervant et al. (1997, 1999, 2001), Hervant and Renault (2002), Salin et al. (2010) and Issartel et al. (2010), and personal unpublished data. Values are means (n = 10–30). Oxygen consumption was expressed in µmol O_2/g fresh mass. NS = no statistical difference with the fed value. Energy stores (glycogen, triglycerides, and arginine phosphate levels) were expressed in µ mol/g fresh mass; arginine phosphate is not observable in vertebrates. Utilization rates were expressed in µ mol/g fresh mass/day

The first part of this review focuses on the food status of subterranean ecosystems (especially groundwaters and caves). The second part examines the sensitivity and responses of hypogean organisms to lack of food, reporting recent experiments designed to elucidate whether the colonization of cave, karstic, and porous biotopes induces behavioral, physiological, and metabolic adaptations to long-term starvation and renutrition in animals. To do this, I compared the fasting responses of 10 hypogean and epigean (i.e., surface-dwelling) species or populations of fishes, crustaceans, and amphibians.

7.2 Sources of Energy and Food Limitations in Subterranean Biotopes

7.2.1 Limited Food Supply in Most Habitats

Because of the absence of sunlight, there is fundamentally no photosynthesis in subterranean biotopes. Except for a very few caves and probably some deep interstitial aquifers, all of the energy is transferred from surface to hypogean habitats, mainly by wind, gravity, percolating surface water, and/or flowing water (mainly from epigean streams). Percolating and flowing surface waters carry with them small amounts of dissolved organic matter, suspended particulate organic matter, microorganisms, feces, dead bodies, and some minute invertebrates and plant fragments transported by occasional floods. Wind and gravity bring small amounts of nutrients (e.g., larger invertebrates and plant fragments) into cave entrances (Culver and Pipan 2009). Poulson (2005) showed that mostly millimeter sized particulate organic matter can be brought into caves by percolating water, and that both amount and size of these particles are progressively reduced with depth below the surface. Therefore, the food inputs could be very low in deep groundwaters. Food can also have an internal origin (e.g., autochthonous bacteria-fungi-protozoa biofilm constitutes a significant energy source in some phreatic aquifers), but its availability is largely dependent on levels of dissolved nutrients from surface water. In addition to food limitation, Malard and Hervant (1999) showed that oxygen availability tends to be reduced in groundwaters. Overall, these features indicate that subterranean ecosystems are harsh and unpredictable biotopes where energy limitation is a constraint on life.

7.2.2 Energy-Rich Caves: The Exception

Although subterranean habitats are often considered to be extremely food-limited, not all are, and some caves harboring immense bat colonies can be extremely eutrophic (Culver and Pipan 2009). This bat guano can represent a very important

source of food, as guanobiotic bacteria and microfungi decompose it, thus building the basis for a large food chain in such 'energy rich' caves (Culver and Pipan 2009). Other caves are routinely visited by epigean animals for shelter or reproduction thereby providing a significant additional food input in the form of their feces or their carcasses. A few shallow caves (especially lava tubes) can also be energy-rich due to exudates from tree roots growing through the ceilings (Poulson and Lavoie 2000). In rare cases caves without both natural entrance and water infiltration from the surface possess chemoautotrophic primary producers (mainly from sulfur oxidation, by bacteria and/or archaea). However, conclusive evidence that the entire food webs of such ecosystems are dependent on chemoautotrophic production has only been demonstrated in a handful of caves (Culver and Pipan 2009).

7.3 How to Find Food in Energy-Limited Subterranean Habitats?

An improved food-finding ability corresponds to a functional advantage in food-limited caves and groundwaters, and may be realized through improvements both in foraging behavior and in sensory orientation/detection (Poulson 2005). Several aquatic hypogean organisms have abandoned traditional grouping or shoaling behavior and adopted a strategy of slow continuous movement that is better adapted to darkness and scarce food resources (Hüppop 2005). Based on this pattern, Hervant et al. (1997) proposed that hypogean animals use an 'exploration' tactic, that is advantageous for life in patchy habitats instead of the 'exploitation' tactic used by surface-dwelling species to forage for food. Subterranean animals (mainly blind: see Figs. 7.1, 7.2) may also compensate for the optically orientated food-searching mode of epigean species by covering a greater area using a larger amount of chemo- and mechanoreceptors, and so increase their chances of encountering an area with food (Poulson 2005).

Amblyopsid cave-dwelling fishes have larger and more neuromast cells with which to detect vibrations of prey items. Consequently the brains of cave fishes generally possess enlarged olfactory lobes. These fish also exhibit larger lateral line systems than their epigean relatives (Culver and Pipan 2009). The heads of hypogean amblyopsids are larger and displace more water which makes the detection of obstacles and prey items more efficient. Cave urodels such as the blind salamander, *Proteus anguinus*, have adopted a worm-like appearance (Fig. 7.2) and a large head that increases the efficiency of the sensory system thus improving food-searching efficiency and enabling them to search a larger area per unit of energy expended (Hüppop 2005; Poulson 2005). Similar adaptations can be observed in most subterranean insects and crustaceans that possess longer sensory appendages than surface relatives. These invertebrates often exhibit neurological differences that parallel those observed in the cave fishes and salamanders (Poulson 2005; Culver and Pipan 2009).

Fig. 7.1 The subterranean crustacean *Stenasellus virei* (photo Jacques Daffis, CNRS Moulis)

7.4 Responses to Long-Term Starvation

The primary goal of this review is to characterize the various behavioral (activity), physiological (oxygen consumption and ventilatory rates), and metabolic (energy allocation patterns, qualitative and/or quantitative changes in body composition) responses showed by subterranean animals during prolonged food deprivation (i.e., a 60/240-day fasting period) in six aquatic subterranean species: three crustaceans, (*Stenasellus virei*, *Niphargus virei* and *N. rhenorhodanensis*), two salamanders (*Proteus anguinus* and a cave population of *Euprotus/Calotriton asper*), and one fish (from a cave population of *Astyanax fasciatus*). To generalize the energy strategy for groundwater organisms, we undertook a parallel study during a 28/90-day fasting period in two morphologically similar surface-dwelling crustaceans (*Gammarus fossarum* and *Asellus aquaticus*) and in individuals from epigean populations of *Calotriton asper* and *Astyanax fasciatus* (Hervant et al. 1997, 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010; Salin et al. 2010).

7.4.1 Reduced Activity and Ventilatory Rates, "Sit-and-Wait" Strategy

Surface-dwelling crustaceans (*A. aquaticus* and *G. fossarum*) and amphibians (from the surface population of *C. asper*) responded to food deprivation (from 28 to

Fig. 7.2 The cave salamander *Proteus anguinus* (photo Patrick Cabrol, CNRS Moulis)

90 days: Table 7.1) with a marked but transitory hyperactivity associated with an increase in displacement speed that corresponds to increased food-searching behavior. Conversely, subterranean crustaceans (*N. virei*, *N. rhenorhodanensis*, *S. virei*), fish (*Astyanax fasciatus*: cave population), and urodels (*P. anguinus* and the cave population of *C. asper)* drastically reduced their energetic expenditures linked to spontaneous activity (Table 7.1) and ventilation during long-term starvation (from 120 to 240 days: Table 7.1) (Hervant et al. 1997, 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010; Salin et al. 2010). This hypometabolic response was observed after a few weeks of starvation and was manifested as a weaker ventilatory activity, a reduction in displacement speed, and a rapid onset of immobility (except for fishes). Most hypogean species spent most of the time resting in a state of temporary torpor, showing low rates of biological activities and therefore drastically lowering their energy expense. This 'sit-and-wait' strategy is considered to be an efficient energy saving adaptation (Hervant and Renault 2002; Salin et al. 2010).

7.4.2 Low Metabolism and Reduced Metabolism

When nourished most hypogean organisms exhibit a lower standard metabolic rate (SMR) than their epigean counterparts (Hervant et al. 1998; Spicer 1998; Hüppop 2005; Issartel et al. 2010). Nourished groundwater crustaceans (Hervant et al. 1997) and cave-dwelling fishes (Salin et al. 2010) exhibited oxygen consumption rates lower than surface-dwelling ones (among them hypogean and epigean populations belonging to the same species) (see Table 7.1). Low metabolism is often considered as adaptive to survive food and/or oxygen stress (Spicer 1998), and more generally to life in low energy biotopes such as subterranean and deep-sea habitats (Childress 1995; Hervant and Renault 2002), or as a response to the selective pressure on energy economy (Hüppop 2005).

Entry into an additional, stress-induced, reduced metabolism state is an important survival strategy for many organisms when challenged by environmental stress, including low oxygen, food shortage, dehydration, and cold temperatures. Therefore, a common (but not general) energy-conserving physiological response to starvation is a lowering of SMR (Fuglei et al. 2000; McCue 2010). As a result, most subterranean crustaceans and amphibians showed a large additional metabolic depression during fasting (Table 7.1), whereas surface-dwelling populations and morphologically similar epigean species showed a smaller decrease in metabolism (Hervant et al. 1997, 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010). From an ecological point of view, the low standard metabolic rates characteristic of most subterranean animals and their ability to further depress metabolism during fasting are advantageous in organisms living in energetically poor environments. Indeed, subterranean organisms can survive during long periods of food deprivation at a low energetic cost. As a result, lower metabolism and metabolic depression capacities may have been selected during the colonisation of the cave environment, inferring a reduced energy expenditure, and thus promoting the conservation of energetic stores during nutritional limitation (Issartel et al. 2010). The cave population of the tropical fish *Astyanax fasciatus* showed a smaller decrease in SMR (Table 7.1) than hypogean species from temperate biotopes (Salin et al. 2010); this taxon generally inhabits energy-rich environments and does not regularly experience food shortages. Thus, the selective pressure on energy economy described above may be drastically reduced.

7.4.3 Metabolic Adaptations

The capacity to withstand periods of inadequate/poor nutrition depends on the presence of sufficient endogenous nutritive stores, and the necessary metabolic responses (i.e., adjustments in energy and intermediary metabolism) to ensure that these stored metabolites are utilized efficiently. When animals experience periods of limited food, they must rely on endogneous body reserves to fuel metabolic

processes and maintain homeostasis. During long-term fasting, most subterranean crustaceans and salamanders showed rates of relative mass loss ranging largely slower than those of surface-dwelling ones. These results suggest that hypogean organisms utilize their endogenous energy stores at a relatively low rate. For both epigean and hypogean species, experimental data on fasting-induced changes in body composition indicated significant utilization of glycogen, triglycerides, proteins, and—in invertebrates only—phosphagen (i.e., arginine phosphate) reserves. Nourished subterranean species and populations possess larger glycogen, triglycerides, and arginine phosphate body stores than their epigean relatives (Table 7.1) (Hervant et al. 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010). Moreover, hypogean species metabolized body stores at very low rates under conditions of food deprivation (Table 7.1). Some of them metabolized glycogen only at the beginning of the fast, but surprisingly were later able to resynthesize lost glycogen stores. Overall, this drastic reduction in energy use (which was initially low) sustains the metabolic reserves of hypogean organisms therefore increasing survival time under fasting conditions.

Studies that have addressed the effects of fasting on subterranean amphibians and crustaceans have demonstrated both qualitative and quantitative changes in body composition. The relative importance of metabolic reserves and their order of utilization vary among species/populations, and most taxa switch from one stored metabolite to another as prolonged starvation continues (Hervant et al. 1999, 2001; Hervant and Renault 2002; Issartel et al. 2010). For example, epigean species showed a monophasic response to food deprivation, characterized by an immediate, linear, and large decrease in all of its energy reserves. In contrast, prolonged fasting by hypogean species was generally characterized by three successive phases: (1) an immediate, but low, depletion of both glycogen and arginine phosphate stores (during the first month), followed by (2) the utilization of triglycerides associated with glycogen resynthesis (during a few months), and finally (3) a slow depletion of both proteins and lipids, always associated with a glycogen resynthesis.

In groundwater and cave species, the rapidly usable carbohydrates and phosphagen stores served only as initial metabolic fuels, before being replaced by lipid reserves. The de novo synthesis of glycogen observed in these species after 2–3 months of fasting may be a result of an increased conversion/utilization of amino acids (originating from proteolysis) and/or glycerol (from lipolysis) to glycogen. This possibility is supported by the decrease in both arginine (originating from the utilization of arginine phosphate) and glycerol observed after a few months in some starved hypogean crustaceans and amphibians (Hervant et al. 1999, 2001; Hervant and Renault 2002). These results demonstrate that most hypogean species are generally better adapted to long-term food shortage than surface-dwelling relatives, and capable of using lipids to spare glycogen (the main fuel metabolized during oxygen deficiency in crustaceans; Hervant et al. 1996) and proteins (and therefore muscular mass). Consequently, hypogean species are also better able to withstand severe hypoxia that is characteristic of most groundwaters (Malard and Hervant 1999) and is usually associated with nutritional stress. They

also appear to be better suited to quickly resume foraging behaviors when nutrients reappear.

7.5 Responses to Renutrition

From an ecological point of view, it is important for organisms to quickly and entirely restore energy reserves and physiological parameters to be able to resist an ensuing food stress, especially in harsh and unpredictable biotopes provided by most caves and groundwaters. With the exception of the frequently feeding tropical cave fish *Astyanax fasciatus* (Salin et al. 2010), refeeding (from 7 to 14 days: Table 7.2) resulted in only a partial restoration of body stores within surface-dwelling species, but resulted in complete restoration within food-limited hypogean ones (Table 7.2; Hervant et al. 1999; 2001; Hervant and Renault 2002). Moreover, food-limited hypogean animals resynthesized body stores with production rates consistently higher than their epigean relatives (Table 7.2). Furthermore, for the hypogean crustacean *Stenasellus virei*, these resynthesis rates were 18- to 29-fold greater than utilization rates (Hervant and Renault 2002, Tables 7.1, 7.2). As a result, the rate at which fat stores were deposited while subterranean organisms fed was largely higher than fat accumulation rates measured in most wild mammals and birds, including antarctic penguins, which experience prolonged periods of starvation on land (Groscolas and Robin 2001; see also Champagne et al., Chap. 19; Jenni-Eierann and Jenni, Chap. 11).

Fasted cave amphibians consumed 50% more food than surface salamanders upon refeeding. This hyperphagia permitted an acceleration in the resynthesis of depleted energy stores. In addition, the 'digestive efficiency' (defined here as the gain in body mass per gram of O_2 consumed per day, and calculated from the extra O_2 consumed beyond SMR during realimentation) was about 20% higher in the infrequently feeding hypogean crustaceans and amphibians than in epigean relatives (Hervant et al. 1999, 2001; Hervant and Renault 2002). The presence of food immediately suppressed the fast-induced hypometabolism in cave amphibians, fishes, and crustaceans. A few hours after refeeding, all physiological and behavioral parameters did not differ from those of individuals that had not been starved. This rapid restoration of SMR and food searching activity could offer a selective advantage, allowing subterranean organisms to assimilate sporadic food inputs more effectively. Moreover, the protein sparing observed during fasting may preserve essential functions such as locomotion (Fuglei et al. 2000) so that they can rapidly resume foraging and predator behaviors when food becomes available. This strategy could be crucial in most subterranean habitats, where competition for food is intense. In other words, individuals whose locomotory capabilities are rapidly restored may have a selective advantage over others.

Table 7.2 Main metabolic and physiological responses to refeeding in surface-dwelling and subterranean crustaceans, amphibians, and fishes

	Surface-dwelling species		Subterranean species			
	Gammarus fossarum	*Asellus aquaticus*	*Niphargus virei*	*N. rhenorhodanensis*	*Stenasellus virei*	*Proteus anguinus*
Duration of experimental refeeding(days)	7	7	14	14	14	14
Glycogen resynthesis(%)	48	60	100 (*128 at Day 7*)	100 (*125 at Day 7*)	100	100 (muscle) (*123 at Day 7*)
Glycogen resynthesis rate	0.8	1.25	1.8 (*6.1 at Day 7*)	2.6 (*7.9 at Day 7*)	0 (*2.7 at Day 7*)	–
Triglycerides resynthesis(%)	75	62	100	100	100	89 (muscle)
Triglycerides resynthesis rate	0.07	0.1	0.11	0.12	0.18	0.18 (muscle)
Arginine phosphate resynthesis(%)	25	80	100	100	100	–
Arginine phosphate resynthesis rate	0.05	0.05	0.1	0.16	0.2	–

Values calculated from Hervant et al. (1997, 1999, 2001) and Hervant and Renault (2002), and personal unpublished data. Values are means (n = 10–30). NS = no statistical difference with the fed value. Arginine phosphate is not observable in vertebrates. Resynthesis rates were expressed in μ mol/g fresh mass/day, and resynthesis (ie., recovery) was expressed as percent of the fed value (see Table 7.1) for all three energy stores

7.6 Conclusion: A Proposed Adaptive Strategy for Life in Food-Limited Subterranean Habitats

Based on these results, I propose a general model of adaptive strategy for subterranean organisms, involving the ability to withstand long-term starvation and the efficient use of consumed food. Adaptation to prolonged food scarcity includes a 'sit-and-wait' behavior (i.e., a period of depressed metabolism and activity during which the subterranean species subsisted on lipid reserves) and the possession of low energetic requirements and large fuel stores. In addition, hypogean species displayed high recovery abilities during refeeding, showing optimal utilization of available food energy, and therefore rapid restoration of the body reserves depleted during nutritional stress. Collectively, these adaptations allow most hypogean organisms to tolerate a prolonged reduction in food availability by maximizing the length of time that metabolism can be fueled by a given food ration and/or a given energy reserve. These adaptive responses are thought to be an efficient energy-saving strategy in harsh environments where food is scarce and unpredictable. It is worth noting that hypogean species tend to be long-lived, with lifetimes of about 100 years for the olm, *Proteus anguinus*, and more than 150 years for a North American cave crayfish. Such extended life spans are coupled with slow growth, late maturation, and low reproduction rate, life history variables that are undoubtedly part of an adaptive complex for low energy flux. Therefore, food-limited (and hypoxia tolerant) hypogean species are excellent models to examine physiological adaptations to low energy systems.

The many behavioral, physiological, and metabolic adaptations that subterranean species have to starvation do not appear to be necessarily caused by their hypogean habitats, but rather a general strategy to coping with energy-limited habitats in general. The cave tropical fish *Astyanax fasciatus*, living in an 'energy rich environment', did not show significant response to starvation. So, it is now clear that starvation adaptations, although extremely widespread, are not necessary to cave life, but are more probably correlated to the 'energetic state' of each ecosystem (mainly food and oxygen availabilities), and that troglomorphism is not strictly linked to starvation capacities.

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Chapter 8
Physiological Responses to Starvation in Snakes: Low Energy Specialists

Marshall D. McCue, Harvey B. Lillywhite and Steven J. Beaupre

8.1 Introduction

The nearly 3,000 species of snakes (Suborder: Serpentes, Linnaeus 1758) undoubtedly differ in their abilities to tolerate food limitation; however, snakes are the only major vertebrate group with dozens of species capable of surviving complete starvation for longer than one year while remaining relatively active. This remarkable ability may have even facilitated the evolutionary radiation of these low energy specialists into environments characterized by low food availability over the past 100 million years (McCue 2007a). In this chapter we distinguish between fasting and starvation with respect to whether the cause for not eating is internal or external, respectively, to the animal (sensu McCue 2010).

We begin this chapter by describing the physiological responses of snakes starving under controlled conditions and compare them with representative species from other vertebrate classes. We also review the effects of food limitation in two wild populations of snakes from mainland and insular environments.

M. D. McCue (✉)
Department of Biological Science, St. Mary's University,
San Antonio, TX 78228, USA
e-mail: mmccue1@stmarytx.edu

H. B. Lillywhite
Department of Biology, University of Florida, Gainesville,
FL 32611, USA

S. J. Beaupre
Department of Biology, University of Arkansas,
Fayetteville, AR 72701, USA

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8.2 Individual Responses

Snakes appear to use two complementary strategies for tolerating prolonged food limitation. These have been referred to as supply-side and demand-side strategies (McCue 2007b). In short, demand-side strategies are those that involve changes in metabolic rates whereas supply-side strategies involved physiological 'decisions' to mobilize and oxidize metabolic fuels. In the following sections we discuss specific responses that are related to each of these general strategies.

8.2.1 Mass Loss

Snakes, like all vertebrates, reduce body mass during prolonged fasting. However, unlike some animals adapted to prolonged starvation (see Champagne et al., Chap. 19; Hervant, Chap. 7), their rates of mass loss tend to be relatively constant over 6 months of fasting (Fig. 8.1). On average five species of snakes lost 0.11% of their mass each day, a rate that is on average half that of other ectothermic vertebrates (McCue 2010). Rates of mass loss are reportedly higher in starving garter snakes (i.e., 0.64% day^{-1}; Starck and Beese 2002) and water snakes (0.23% day^{-1}; Pati and Thapliyal 1984): species that are considered to be frequent feeding species (Beaupre and Montgomery 2007; Secor and Diamond 2000).

Because the body is mostly water, small changes in tissue water content can have significant effects on body mass. An understanding of water flux among tissue compartments is particularly important for studies of fasting and starvation because increases in body water content can mask the losses in organic mass. Snakes increased relative body water content by 5% over 168 days of starvation, an increase similar to that reported for other vertebrates (Table 8.1). Like other animals the specific cause for increased water content in starving snakes remains unclear. It is possible that this increase in body water content is simply an artifact of the reduced lipid stores, which are low in water. In the case of snakes the losses in lipid mass are unable to explain the entire increase in water mass suggesting the possibility that other mechanisms are operating. It has been suggested that increased moisture in tissues is an adaptive mechanism to maintain cell volume amidst continual losses of organic material (McCue 2007c); however, future studies will be useful to examine differential responses in intracellular, interstitial, and vascular water volumes in snakes during prolonged starvation. Such responses can be contrasted with those documented in animals facing severe dehydration (Lillywhite and Navas 2006).

Changes in body mass represent the sum of the changes in mass of each organ. Nearly all organs reduce in mass, but different organs do not necessarily lose mass at the same rates during fasting, thus giving rise to the idea that organs are somehow prioritized (Navarro and Gutierrez 1995) (see also Bauchinger and

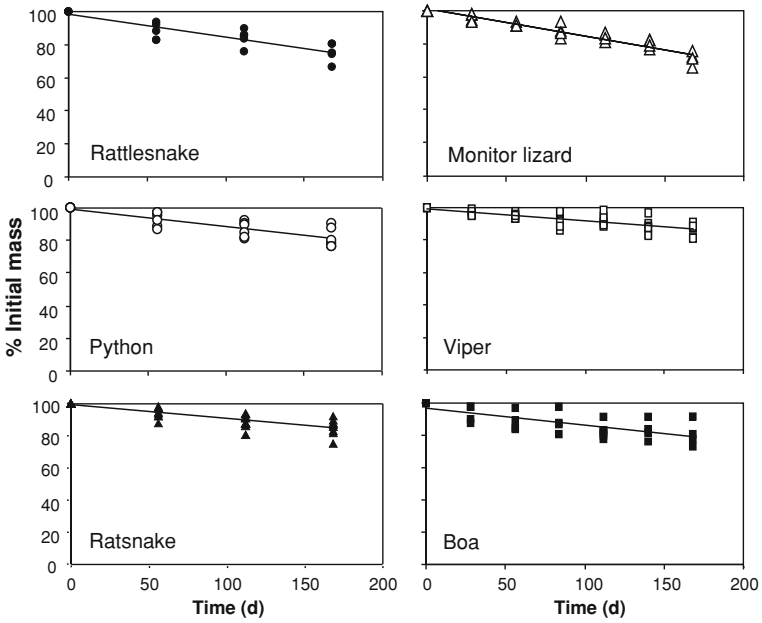

Fig. 8.1 Relative mass loss of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. *Data points* represent repeated measures on 4–6 individuals of each species: rattlesnake (*Crotalus atrox*), python (*Python regius*), ratsnake (*Elaphe obsoleta*), boa (*Boa constrictor*), viper (*Bitus gabonica*), and monitor lizard (*Varanus exanthematicus*)

McWilliams, Chap. 12). Starving snakes, like other animals exhibited large reductions in liver mass, but are unique in the degree to which heart mass decreased (Table 8.2). This decrease in heart mass may be permitted by the exceptional ability of snakes to reduce their standard metabolic rates during starvation (McCue 2010) and to rebuild cardiac tissues immediately after refeeding (Andersen et al. 2005).

8.2.2 Energetics

Starving snakes may increase or decrease their energy expenditure. Captive snakes that are habituated to a regular feeding schedule often become visibly more active in their cages if they miss a meal (MDM personal observation). This quasi-foraging behavior typically ceases after a few days. In the wild, increased foraging behavior could result in a meal, but almost certainly involves tradeoffs including increased energy expenditure and risk of predation. Alternatively, if opportunities are available, starving snakes may adopt a strategy to reduce energy expenditure by seeking cooler microhabitats (Bicego et al. 2007; McCue 2004). The 'decisions' to

Table 8.1 Changes in the water content in the bodies of starving animals with ad libitum access to water

Animal	Days	Water increase (%)	References
Aves			
Chicken	20	1	Yokota et al. (1992)
Mammals			
Rat	15	9	Cherel et al. (1992)
Reptiles			
Boa constrictor	168	1	McCue (2008a)
Monitor lizard	112	5	McCue (2008a)
Python	168	5	McCue (2007b)
Ratsnake	168	8	McCue (2007b)
Rattlesnake	168	4	McCue (2007c)
Viper	168	4	McCue (2008a)
Amphibians			
Xenopus	360	8	Merkle and Hanke (1988)
Fishes			
Brook trout	84	5	Phillips et al. (1960)
Carp	30	0	Shimeno et al. (1990)
Catfish	28	1	Shoemaker et al. (2003)
Cyprinid	49	5	Mendez and Wieser (1993)
Eel	90	0	Inui and Oshima (1966)
Herring	129	17	Wilkins (1967)
Porgy	28	1	Rueda et al. (1998)
Rainbow trout	147	9	Simpkins and Hubert (2003)
Sea bass	60	2	Stirling (1976)
Tilapia	82	5	Satoh et al. (1984)
Trout	84	6	Kawatsu (1966)

increase foraging activity or retreat to conserve energy are probably dependent on species and duration of starvation (Beaupre and Montgomery 2007).

In a controlled laboratory setting where opportunities to forage or seek cooler microclimates are unavailable, snakes are still able to reduce their overall energy expenditure (Table 8.3). Snakes subjected to 168 days of starvation exhibit metabolic rates that are 22–78% lower than well-nourished individuals, postabsorptive individuals. When metabolic rates of fasted snakes are appropriately corrected for concomitant reductions in body mass (McCue 2010; assuming an allometric mass exponent of 0.75; Andrews and Pough 1985; Bennett and Dawson 1976) these reductions in metabolic rate fall to 5–71% of their initial postabsorptive values. Similar reductions in metabolic rate are often reported for starving birds; however, in contrast to snakes, birds only achieve this feat through adaptive reductions in core body temperature (Ben-Hamo et al. 2010; Geiser 2004; McKechnie and Lovegrove 2002) (see also Hohtola, Chap. 10). The mechanisms responsible for the starvation-induced hypometabolic responses among snakes remain generally unexplored.

Table 8.2 Changes in relative organ mass during starvation

Animal	Organ	Days	Initial (index)	Final (index)	Relative loss (%)	References
Aves						
Warbler	Liver	2			36	Karasov et al. (2004)
Warbler	Intestine	2			45	Karasov et al. (2004)
Chicken	Kidney	8	0.190	0.128	33	Brady et al. (1978)
Chicken	Liver	8	2.52	1.53	39	Brady et al. (1978)
Owl	Liver	5			48	Thouzeau et al. (1999)
Owl	Heart	5			4	Thouzeau et al. (1999)
Owl	Digestive tract	5			29	Thouzeau et al. (1999)
Owl	Pectoral muscle	5			12	Thouzeau et al. (1999)
Quail	Liver	2	2.00	1.49	26	Lamsova et al. (2004)
Quail	Spleen	2	0.035	0.025	29	Lamsova et al. (2004)
Quail	Testes	2	2.86	2.89	−1	Lamsova et al. (2004)
Mammals						
Rat	Gastrocnemius	4	0.54	0.55	−2	Goodman et al. (1984)
Rat	Heart	4	0.30	0.30	0	Goodman et al. (1984)
Rat	Kidney	4	0.43	0.42	2	Goodman et al. (1984)
Rat	Large intestine	4	1.36	0.82	40	Goodman et al. (1984)
Rat	Liver	4	5.16	2.55	51	Goodman et al. (1984)
Rat	Small intestine	4	3.78	1.89	50	Goodman et al. (1984)
Reptiles						
Anole	Liver	20	2.90	2.30	21	Gist (1972)
Anole	Fat body	20	0.39	0.09	77	Gist (1972)
Boa constrictor	Liver	168	2.05	1.50	27	McCue (2008a)
Boa constrictor	Heart	168	0.19	0.19	3	McCue (2008a)
Garter snake	Liver	28	3.30	2.50	24	Starck and Beese (2002)
Monitor lizard	Liver	168	2.25	1.73	23	McCue (2008a)

(continued)

Table 8.2 (continued)

Animal	Organ	Days	Initial (index)	Final (index)	Relative loss (%)	References
Monitor lizard	Heart	168	0.20	0.26	−27	McCue (2008a)
Python	Liver	168	1.94	1.87	4	McCue (2007b)
Python	Heart	168	0.25	0.16	37	McCue (2007b)
Ratsnake	Liver	168	1.99	1.31	34	McCue (2007b)
Ratsnake	Heart	168	0.21	0.17	19	McCue (2007b)
Rattlesnake	Liver	168	2.88	2.20	24	McCue (2006)
Rattlesnake	Heart	168	0.70	0.56	20	McCue (2006)
Viper	Liver	168	2.90	1.84	36	McCue (2008a)
Viper	Heart	168	0.25	0.19	25	McCue (2008a)
Amphibians						
Frog	Heart	540	0.16	0.18	−13	Grably and Peiery (1981)
Frog	Liver	540	2.55	1.17	54	Grably and Peiery (1981)
Xenopus	Heart	365	0.23	0.24	−4	Merkle and Hanke (1988)
Xenopus	Kidney	365	0.35	0.35	0	Merkle and Hanke (1988)
Xenopus	Liver	365	2.65	1.86	30	Merkle and Hanke (1988)
Fishes						
Carp	Intestine	30	2.6	1.9	27	Shimeno et al. (1990)
Carp	Kidney	30	0.7	0.45	36	Shimeno et al. (1990)
Carp	Liver	19	2.45	1.09	56	Blasco et al. (1992)
Catfish	Liver	28	1.31	0.56	57	Shoemaker et al. (2003)
Catfish	Viscera	66	1.75	0.45	74	Zamal and Ollevier (1995)
Salmon	Brain	42	0.57	0.57	0	Soengas et al. (1996)
Salmon	Liver	42	0.82	0.57	30	Soengas et al. (1996)

Table 8.3 Changes in metabolic rates of starving animals

Animal	Days	Change (%)	Mass specific change (%)	References
Aves				
Garden warbler	3	−36	−18	Klaassen and Biebach (1994)
Goose	32	−39	−18	Boismenu et al. (1992)
Kestrel	3	−13	10	Shapiro and Weathers (1981)
King penguin	45	−58	−29	Cherel et al. (1988)
Pigeon	10	−28	−1	Smith and Riddle (1944)
Stork	5	−27	−20	Mata et al. (2001)
Mammals				
Dog	15	−16	4	Anderson and Lusk (1917)
Fur seal (pup)	5	−24	−8	Arnould et al. (2001)
Gray seal	70	−25	56	Worthy and Lavigne (1987)
Harp seal	56	−7	220	Worthy and Lavigne (1987)
Human	31	−25	−5	Benedict (1915)
Opossum	3	0	11	Weber and O'Connor (2000)
Rat	12	−34	0	Westerterp (1977)
Reptiles				
Lizard	19		0	Roberts (1968)
Python hatchlings	114	−68		Bedford and Christian (2001)
Python adults	56	−25		Bedford and Christian (2001)
Rattlesnake	168	−78	−71	McCue (2006)
Ratsnake	168	−44	−34	McCue (2008a)
Boa constrictor	168	−59	−49	McCue (2007b)
Python	168	−22	−5	McCue (2008a)
Monitor lizard	168	−37	−11	McCue (2008a)
Viper	168	−68	−63	McCue (2007b)
Tortoise	5	0		Hailey and Loveridge (1997)
Turtle	90		−75	Seidel (1978)
Amphibians				
Salamander (epigean)	90	−18	−4	Hervant et al. (2001)
Salamander (hypogean)	240	−55	−34	Hervant et al. (2001)
Fishes				
Cyprinid spp.	28	−34	−24	Wieser et al. (1992)
Eel	60	−32	8	Inui and Oshima (1966)
Perch	13	0	14	Mehner and Wieser (1994)
Salmon	70	7		Cook et al. (2000)
Snakehead	140	35	91	Woo and Cheung (1980)

8.2.3 Changes in Size

Although many large adult snakes can apparently tolerate more than 2 years of starvation (Wang et al. 2006), we are unaware of any controlled studies that formally document such events as have occasionally been reported for fish (see Boetius and Boetius 1985). Consequently, most of our understanding about

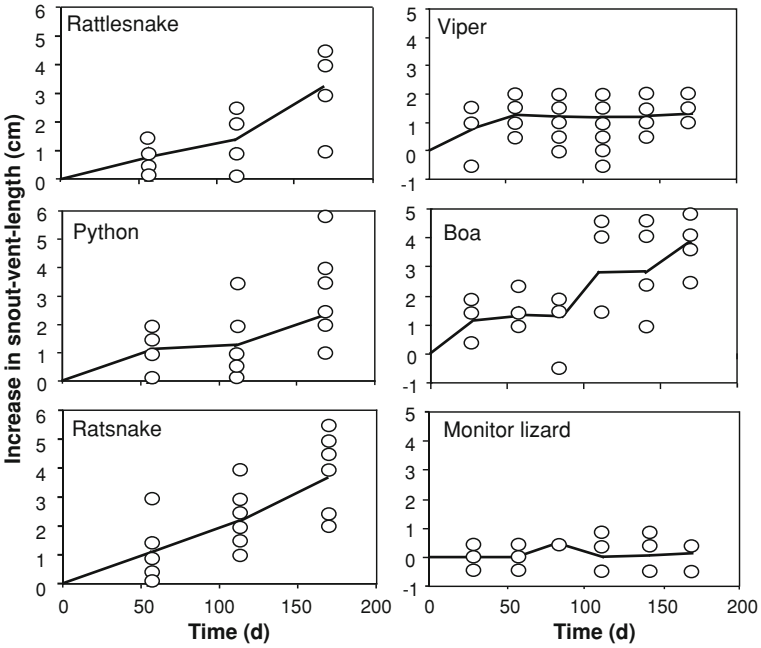

Fig. 8.2 Changes in snout-vent-length of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. Simultaneous reductions in mass for each species are illustrated in Fig. 8.1

responses to starvation in snakes comes from controlled studies of subadults and young adults. In the face of chronic food limitation but not total starvation, juvenile and adult marine iguanas respond by reducing their body length by up to 20%, presumably as a mechanism to reduce energy expenditure (Wikelski and Thom 2000). Despite anecdotal reports from hobbyists (reviewed in McCue 2007b), controlled studies have been unable to document significant reductions in body lengths of snakes (Luiseli 2005; Madsen and Shine 2001; Taylor et al. 2005). In fact, juveniles of five species subjected to starvation were found to increase their snout-vent-length (SVL; Fig. 8.2). Interestingly no such changes were documented in monitor lizards. Presently, nothing is known about the specific structural mechanisms by which snakes are capable of changing their body length during complete starvation. Studies involving histological or imaging technologies will be useful to explore the mechanisms by which these changes occur.

Most species of snakes are capable of indeterminate growth, meaning that they will grow asymptotically (e.g., body mass and SVL) over the course of their life as long as food is available (Shine and Charnov 1992). Larger body sizes tend to confer fitness advantages such as a wider prey base (Forsman 1996; Miller and Mushinsky 1990; Shine 1991), combat success among males (Shine 1994; Shine et al. 2004), increased reproductive output among females (Madsen and Shine 1993;

Taylor et al. 2005), and increased survivorship in general (Blouin-Demers et al. 2002; Shine and Charnov 1992). The fact that juvenile snakes increased their SVL in the complete absence of food underscores the possibility that subadults, may be under strong selective pressure to increase body length even when food is unavailable. These 'growing' subadults must therefore reallocate endogenous resources. Unfortunately, the finding that SVL is confounded with both age and nutritional status in subadults complicates traditional interpretations of body condition alone as a proxy of feeding history, particularly across a wide range of body sizes.

8.2.4 Carbohydrate Dynamics

Glucose is the most commonly measured blood metabolite in fasting and starving animals. In some mammals blood glucose levels decrease in a predictable manner during fasting thereby allowing researchers to use this metabolite as a proxy for starvation time. However, in snakes, as in birds (Jenni-Eiermann and Jenni 1998) (see also Jenni-Eiermann and Jenni, Chap. 11) this is not necessarily the case (Fig. 8.3). Of five snake species examined by MDM only three significantly reduced blood glucose during prolonged starvation. Ketone bodies (e.g., β-hydroxybutyrate) are carbohydrates that are produced as a by-product of catabolism of ketogeneic amino acids and β-oxidation of fatty acids. In endotherms, levels of ketone bodies in the blood tend to increase by 2- to 4-fold during starvation (Table 8.4), but in snakes significant increases were only detected in two of the five species that have been examined (Fig. 8.3). A similar lack of congruence with respect to ketone bodies is reported among fishes (Black and Love 1986; Frick et al. 2008; Soengas et al. 1998; Wood et al. 2009; Zammit and Newsholme 1979); however, unlike fishes the absence of consistent increases in ketone bodies of fasting snakes is likely related to their comparatively high prefasting levels (Table 8.4). Overall, these observations suggest that commonly measured blood metabolites may have limited utility in estimating the nutritional status of snakes.

8.2.5 Lipid Dynamics

Like most animals, snakes mobilize and subsequently oxidize endogenous lipids during starvation. Over 168 days of starvation five species of snakes reduced their lipid content as a proportion of dry body mass by nearly half from 27.0 to 14.7% (McCue 2008b). If these lipids and their constituent fatty acids were mobilized and oxidized at rates dependent on their initial abundance, the relative amounts of the different fatty acids would remain constant. Interestingly, analyses of fatty acid composition of the bodies of snakes reveal that fatty acids are not used

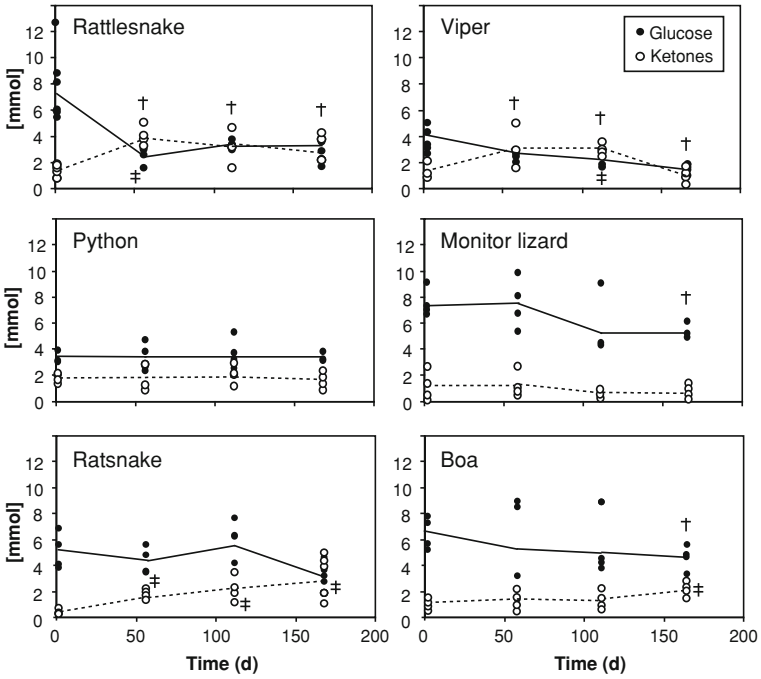

Fig. 8.3 Plasma metabolites of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. *Daggers* and *double-daggers* refer to statistically significant differences from day zero. The species are the same as in Fig. 8.1

indiscriminately (Fig. 8.4; see also Price and Valencak, Chap. 15). All five species of snakes examined show characteristic increases in the ratios of linoleic: palmitoleic acid ratios and oleic: palmitic acid ratios, and a general increase in the mean number of double bonds in the fatty acids (i.e., unsaturation index). Starvation-induced changes in fatty acid composition have been reported in the bodies of fishes (Jezierska et al. 1982; Luo et al. 2009; Tandler et al. 1989; Tidwell et al. 1992; Zamal and Ollevier 1995); however, the specific changes are much more variable than those documented among snakes. Overall, because the different species of snakes show such similar changes to one another, it has been suggested that fatty acid composition of fresh carcasses opportunistically collected on roadways can be used to gain insight into the nutritional status of wild populations of snakes (McCue 2008b).

8.2.6 Protein Dynamics

Fasting snakes continually oxidize endogenous protein as a fuel, albeit at much lower rates than carbohydrates and lipids. Over 168 days of starvation, protein content in the bodies of five species of snakes increased from 55.1 to 63.9% of dry

Table 8.4 Changes in ketone bodies of starving animals

Animal	Days	Initial (mmol/L)	Final (mmol/L)	Change (mmol/L)	References
Aves					
Chicken	1	0.2	5.1	4.9	Linares et al. (1992)
Emperor penguin	15	0.7	1.4	0.7	Groscolas (1986)
Goose	70	0.1	0.7	0.6	Cherel et al. (1988)
Herring gull	6	0.4	6.3	5.9	Totzke et al. ((1999))
King penguin	25	0.7	0.3	−0.4	Cherel et al. (2005)
White-throated sparrow	0.5	1.0	3.1	2.1	Smith et al. (2007)
Mammals					
Dog	22	0	0.4	0.4	de Bruijne and van den Brom (1986)
Goat	3.5	2.5	4.7	2.2	Radloff et al. (1966)
Guinea pig	3	1.4	2.7	1.3	Stephens and Fevold (1993)
Hamster	1	1	2.2	1.2	Rowland (1983)
Human	42	0.1	5.9	5.8	Owen et al. (1979)
Marten	5	0.5	0.8	0.3	Harlow and Buskirk (1991)
Rat (lean)	20	0.1	0.4	0.3	Goodman et al. (1980)
Rat (obese)	20	0.3	1.3	1.0	Goodman et al. (1980)
Sheep	11	0.2	1.2	1.0	Bouchat and Paquay (1981)
Reptiles					
Lizard	114	2.7	0.2	−2.5	Pontes et al. (1988)
Boa constrictor	168	1.3	2.3	1.0	McCue (2008a)
Monitor lizard	168	1.3	0.7	−0.6	McCue (2008a)
Python	168	1.76	1.65	−0.1	McCue (2007b)
Ratsnake	168	5.1	3.7	−1.4	McCue (2007b)
Rattlesnake	168	1.8	3	1.2	McCue (2006)
Viper	168	1.6	1.2	−0.4	McCue (2008a)
Fishes					
Bass	150	0	0	0.0	Zammit and Newsholme (1979)
Carp	60	0	0	0.0	Segner et al. (1997)
Cod	155	0	0	0.0	Black and Love (1986)
Dogfish	150	0.1	2.3	2.2	Zammit and Newsholme (1979)

mass. Analyses of amino acids in body protein showed that, unlike fatty acids described above, amino acids are not differentially oxidized during starvation (Fig. 8.5). It is difficult to speculate about whether the conservative nature of amino acid composition during starvation in snakes is unique because data about the amino acid composition of other vertebrates is scant. Nevertheless the different patterns of amino acid and fatty acid composition during starvation are probably rooted in the biochemistry of each polymer. For example, during starvation fatty acids are mobilized by hydrolysis from their parent glycerol groups at different rates (reviewed by Price and Valencak, Chap. 15). In contrast, when proteins are degraded into the constituent amino acids the mobilized monomers are ultimately

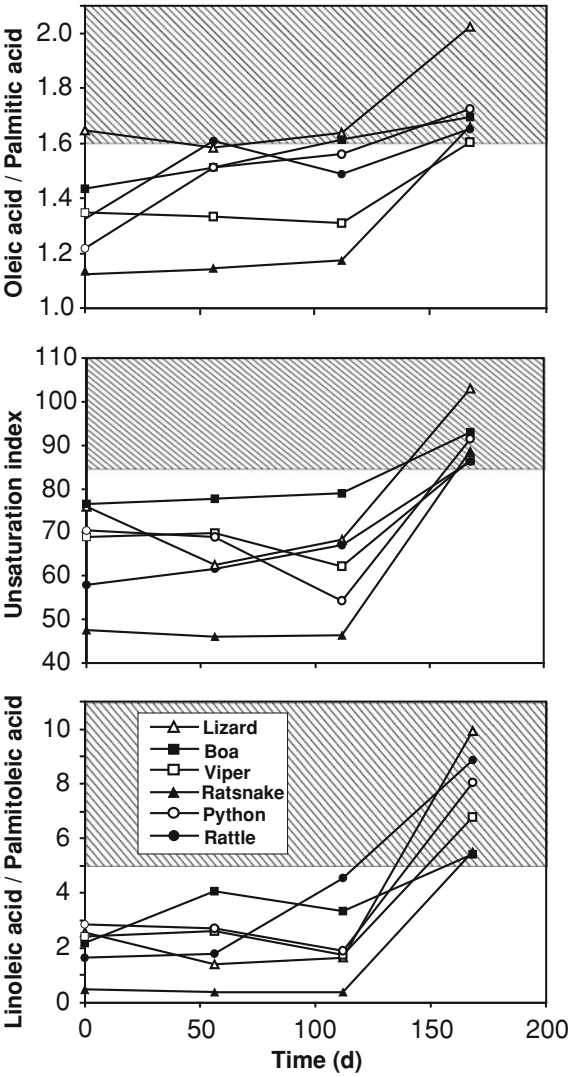

Fig. 8.4 Changes in the fatty acid composition in the bodies of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. *Cross-hatched* region refer to values above which nutritional stress may be inferred. The species are the same as in Fig. 8.1

oxidized because animals do not have a mechanism to sequester specific amino acids.

Starving snakes continually produce uric acid, but because uric acid voiding is a discrete event rather than a continuous one, information about the specific rates of nitrogen excretion cannot be described with the same precision seen in studies of fishes and mammals (see Bar and Volkoff, Chap. 6; Hall, Chap. 22). However, uric

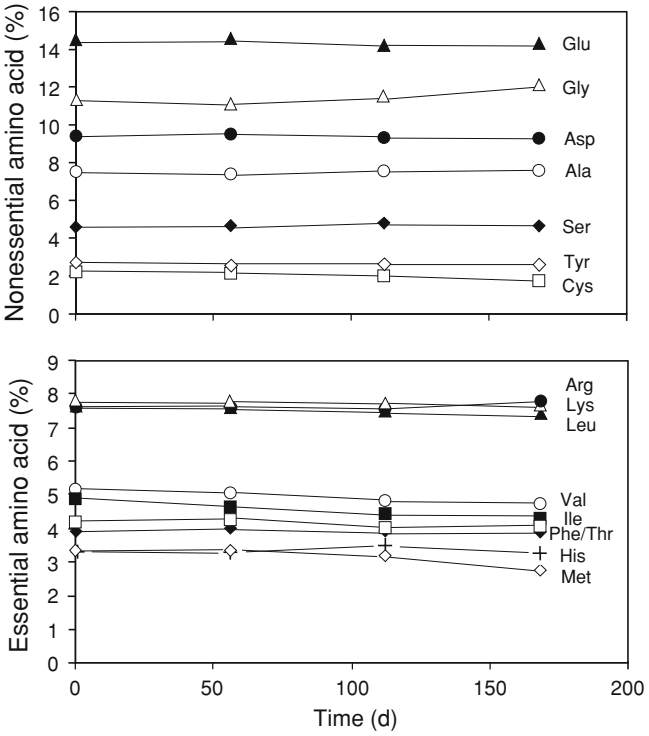

Fig. 8.5 Amino acid composition in the bodies of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. The species are the same as in Fig. 8.1

acid samples collected over a 28-day interval revealed a characteristic pattern of ^{15}N enrichment with starvation (Fig. 8.6). This isotopic enrichment is thought to result from the gradual accumulation of endogenous ^{15}N-amino acids to levels sufficient to overcome isotopic discrimination by transaminases and deaminases (see Hatch, Chap. 20). It has been suggested that the difference in ^{15}N values between the body tissues and the uric acid of snakes can be a useful indicator of nutritional stress among wild populations of snakes (McCue and Pollock 2008).

8.3 Insular Snake Populations: Boom and Bust

Islands are interesting systems in which to explore starvation due to their relative isolation and the nature of resource availability, which can be very different from mainland conditions even if nearby. Energy from insular food resources can be limiting relative to that on the mainland, and can influence the attributes of insular

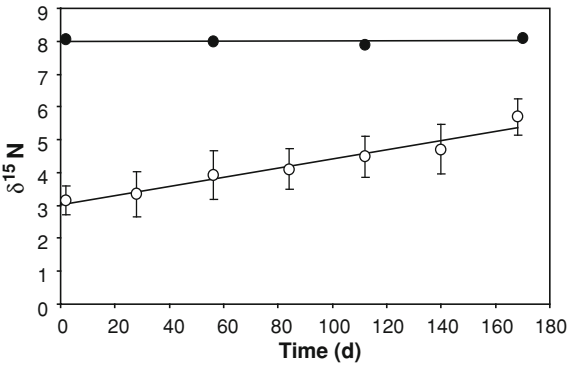

Fig. 8.6 ^{15}Nitrogen values of tissues of five snake species and a lizard subjected to complete fasting for 168 days at 27°C and 12L:12D. *Closed circles* refer to the whole body and *open circles* refer to voided uric acid

fauna as in the case of changes in body size (Wikelski et al. 1997). On many islands ectotherms have replaced endotherms as dominant herbivores or predators, attributable to lower rates of resource use (McNab 1994). Such shifts have permitted ectotherms to achieve both large populations and large body size, although evolutionary trends in insular body size are variable and dependent on the types of prey available (Shine 1987).

Ectothermic vertebrates such as snakes may survive well due to low rates of metabolism and abilities to withstand variable periods of starvation. Importantly, these characteristics allow exploitation of ephemeral or unpredictable food resources. Islands can also be very productive systems that enable snakes to achieve large body size and population densities depending on the type, size, and abundance of prey (Bonnett et al. 2002; Sun et al. 2001). Moreover, attributes related to body size and reproductive potential are important when food resources are saturating but highly seasonal (Schwaner and Sarre 1988). Insular populations can also benefit from allochthonous resource input from the surrounding marine or aquatic environment (Anderson and Polis 1998). Common examples are islands that are nesting sites for seabirds that contribute fish scraps, carcasses, feathers, and nutrient-rich guano that enriches the environment for both invertebrate and vertebrate consumers (Polis and Hurd 1996).

8.3.1 Snakes in the Pacific

Many islands commonly support snake populations, as in the case of amphibious sea kraits (*Laticauda* spp.) which depend on islands of the Indo-Pacific for seclusion, digestion, and reproduction. In these cases, foraging and consumption of food resources occur offshore in the marine environment. In other examples, islands support populations of snakes which associate with insular resources in unusual ways. The island of Shedao off the eastern coast of China supports an endemic pit viper (*Gloydius shedaoensis*) that relies on two pulses of resources per year when migrating passerine birds stop on the island and are preyed upon by the snakes (Shine et al. 2002; Sun et al. 2001). Regular spring and autumn migrations

of birds support a remarkably dense population of the endemic snakes. The juveniles feed less often than adults and consume invertebrates in addition to birds. However, the adults prey almost entirely on birds which they ambush from trees and shrubs as well as from the ground.

Reproductive output of *G. shedaoensis* is high because of the assured food supply, even though it is ephemeral. The snakes display high philopatry, and their assimilation efficiency is high. However, the periods of bird migration are short (6 weeks), and the snakes are inactive for most of the year. Thus, if something goes wrong with the bird migration this endemic snake population faces possible starvation and lives 'on the edge' in this sense. Inactivity includes brumation during colder months of the year, however, and the lowered metabolic demand of snakes during this period helps to prolong survival without food. Snakes retreat to deep burrows, and the ground freezes to depths of 1 m.

Shedao Island pit vipers produce very heavy litters of young compared to maternal mass, and the neonates are large. This favors survival of the young which are dependent on invertebrate prey during early life (passerines are too large for ingestion by neonates). With respect to females, parturition occurs just before the autumn bird migration, so postpartum snakes have normally little risk of death from starvation before they can obtain food (Sun et al. 2002).

8.3.2 Snakes in the Gulf of Mexico

Another instructive example of 'boom or bust' living is the population of insular Florida cottonmouths (*Agkistrodon piscivorus conanti*) living on the Gulf coast island of Seahorse Key. This species typically is a generalist feeder characterized by considerable breadth of diet, which includes terrestrial, aquatic, and carrion prey (Gloyd and Conant 1990; Lillywhite et al. 2002a; Lillywhite and McCleary 2008; Savitzky 1992). The cottonmouths living on Seahorse Key feed largely or exclusively on marine fishes that are dropped or regurgitated by colonial water birds—including brown pelicans, double-crested cormorants, white ibis, and various herons and egrets—that collectively nest on the island in tens of thousands generally from March through October (Lillywhite et al. 2008; Wharton 1969).

The insular cottonmouths are entirely terrestrial and characteristically occupy upland hammock, where they associate with the bird rookeries. The snakes tend to remain at or near the bird rookeries, and generally do not range widely (Wharton 1969; Lillywhite et al. 2008; McCue and Lillywhite 2002). However, the bird rookeries are concentrated at the western end of the island. Snakes at the east end of the island have access to less resources, range more broadly, and are generally found in lower body condition than are snakes at the western half of the island (Lillywhite and McCleary 2008) (Fig. 8.7). Generally, cottonmouths at Seahorse Key have achieved large population size and large body size related to abundant carrion (Wharton 1969). As with the Shedao Island pit vipers, ephemeral resources

Fig. 8.7 Comparison of body condition in two Florida cottonmouths (*Agkistrodon piscivorus conanti*) from Seahorse Key, Levy County, Florida. The snakes were roughly the same length. The snake in the *upper photo* has a robust body condition typical of snakes that forage beneath colonial water bird rookeries, where there are abundant resources in the form of fish carrion (dropped or regurgitated by the nesting birds). In contrast, the snake in the *lower photo* is from part of the island away from the bird rookeries and has a mass/total body length ratio that is half that of the snake in the *upper photo*. Onset of emaciation is indicated by the thinning of subcutaneous tissue at the head, dorsal vertebral column (*arrows*), and caudal tissues posterior of the cloaca (*arrows*). Compare these features in the two snakes, especially the tail condition. Photographs are of living but anesthetized snakes (HBL)

are available to insular cottonmouths following parturition, for females give birth to young in early September, and there is fish carrion available from nesting birds (especially Brown pelicans) until as late as November.

The population of cottonmouths on Seahorse Key differs from many other insular snake species in that Seahorse Key cottonmouths are dependent on nesting birds for resources but do not prey on these birds directly. There is scant evidence that cottonmouths prey on the nesting water birds or their eggs, probably because (i) the snakes are entirely ground-dwelling, (ii) the birds and their chicks are too large for snakes to ingest (except at birth), and (iii) the shape of these birds makes ingestion difficult. The cottonmouths do, however, prey on smaller passerine birds largely during the biannual migratory 'fallout' when unfavorable winds render migratory birds weak and dehydrated during spring and fall migrations. These occasional fallouts provide a brief 'pulse' of extra resource (Lillywhite and McCleary 2008). Thus, snakes at Seahorse Key are provisioned with predictable resources in the form of fallen fish, supplemented by relatively unpredictable pulses of passerines in certain years. Snakes also prey on occasional invasive rats *Rattus rattus* that are also found on the island, and a fraction of the population sometimes scavenges for dead fish along the intertidal zone of the south facing beach.

Dependence on ephemeral resources of course means the snake population is vulnerable to interruption of these resources and might experience starvation periodically. Wharton (1969) concluded that starvation was 'probably' the principal cause of death, although dehydration might also be important. He also concluded based on fat body mass, body condition (see Sects. 8.4.1 and 8.4.2), and feeding behaviors that Seahorse Key cottonmouths were at a 'critical survival level.' This is perhaps why selection has favored snakes ingesting diverse and sometimes inanimate objects (i.e., geophagy) related to carrion scavenging, which can yield variable amounts of extractable energy (Lillywhite and McCleary 2008; Lillywhite et al. 2002b, 2008).

Despite the dynamic nature of food resources on the island, a dense population of cottonmouths and their association with colonial water bird rookeries has persisted for at least 70 years (Carr 1937; Lillywhite and McCleary 2008; Wharton 1969), probably because aspects of the association are mutualistic. In fact, the avian colony at Seahorse Key has been relatively more stable than many others along the Gulf coast of Florida. Any factor that might negatively impact the bird colony, however, would have a similar negative effect on the snakes, because fish carrion from the nesting birds provide more than 90% of the food resource. One such factor is drought, which affects nesting success of the birds and also directly impacts the snakes. Even if resources provided by the birds in terms of fish fall are present, the snakes become reclusive and cease to forage once they lose a critical amount of body water (HBL, unpublished data). There is no permanent source of water on Seahorse Key, so the cottonmouths are dependent on rainfall for water. In dry years, snakes might therefore cease to forage (remaining in underground refugia) even if fish carrion and other resources are present.

8.3.3 Water Stress and Starvation

Selection might also act with respect to dehydration/desiccation as well as starvation, or indeed a combination of the two, and smaller life stages might be especially vulnerable. Emaciated snakes have been found periodically on Seahorse Key, and they are commonly seen on Shedao. Wharton (1960) reported that young insular cottonmouths born in early September have the ability to survive on yolk reserves well into the following spring. Laboratory experiments have demonstrated that neonatal pit vipers (*Gloydius shedaoensis*) can survive for very long periods (up to 392 days) without food, provided they have access to water (Wu 1977b). Starving neonates without water die much more quickly. In any event, experiments using artificial hibernacula suggested that >40% of neonatal Shedao Island pit vipers die during winter (Wu 1977a, b). This could be possibly due to low energy reserves prior to brumation, but negative water balance is also a conceivable contributing factor. Management strategies including food and water supplementation have led to increased numbers of Shedao Island pit vipers and to an increase in the proportion of juvenile snakes in the population (Shine et al. 2002).

8.3.4 Starvation and Reproduction

Studies of reproduction in insular snakes indicate that clutch size and/or mass per young are significantly higher during years with higher prey density or female body condition, a conditional proxy for availability of food (Andren and Nilson 1983; Shine et al. 2002). However, in snakes generally, the relations between fluctuations in resources and fluctuations in reproductive output are complex and variable, and measures of reproductive output can bear little relationship to the costs of reproduction in a given system (Sun et al. 2002). Assuming that resources are adequate or 'normal,' other factors can intervene to reduce body condition and/or reproductive output. For example, a temporal dissociation between foraging and reproduction occurs in many ectotherms, owing to their low metabolic rates and abilities to survive on stored reserves (reviewed in Pough 1980; Bonnet et al. 1998; Brischoux et al. 2010). Thus, in many snake species reproduction reduces feeding rates in females, and gravid females may exhibit anorexia. In some species, anorexic females may be unlikely to survive after parturition because of depletion of energy reserves (starvation) or predation (e.g., Madsen and Shine 1993).

Various interpretations of this phenomenon have focused advantages of not feeding on facilitating careful thermoregulation, enhancing locomotor performance, or physically constraining these and other attributes due to bodily distension from oviductal eggs (e.g., Daltry et al. 1998; Gregory et al. 1999; Shine 1988). Female reproductive sea kraits (*Laticauda* spp.) in New Caledonia cease feeding as their eggs develop, seemingly because bodily distension impedes locomotor ability and renders them less effective at foraging and more vulnerable to aquatic predators. The feeding rate decreases progressively with increasing follicle size, suggesting a lack of

'threshold' effect but increasing degrees of burdening that render foraging less productive and increasingly risky (Brischoux et al. 2010).

8.4 Timber Rattlesnakes in a Degraded Ozark Forest

Since 1995, the third author has conducted mark-recapture and radio telemetry studies of the timber rattlesnake (*Crotalus horridus*) in northern Madison County, Arkansas (Beaupre 2002, 2005, 2008; Beaupre and Montgomery 2007; Beaupre and Zaidan 2001; Browning et al. 2005; Wills and Beaupre 2000). Land holdings of the Arkansas Game and Fish Commission (McIlroy Madison County Wildlife Management Area), Arkansas Natural Heritage Commission (Bear Hollow Natural Area), and the Ozark Natural Science Center comprise the nearly 6000 ha study site. Oak-hickory hard wood forest dominates the site, punctuated by limestone outcrops, xeric limestone prairies (cedar glades), intermittent streams, and wildlife feed plots. Forest quality at the site is degraded, consisting of closed canopy even-aged stands of hardwoods; the result of over 80 years of post-logging fire suppression. Canopy closure has reduced ground-level productivity (due to lack of sunlight penetration), and resulted in mast crop (primarily acorns) dependence of wildlife (Spetich 2002). Thus, rattlesnakes at the site are subjected to boom and bust resource dynamics associated with swings in acorn mast crop that affect abundance of their primary prey—small mammals (Beaupre 2008; Beaupre and Douglas 2009; Douglas 2010). As we show below, in some cases, low food abundance can persist for several consecutive years, subjecting the snakes to periods of significant weight loss, and even extreme starvation.

Snakes are captured at the site during intensive spring and fall surveys and visual sweeps of the habitat during the summer active season. Frequently, radio-tagged snakes lead us to new individuals, especially during the late summer mating season. All captured snakes were transported to the laboratory at the University of Arkansas where they were individually marked (by PIT tag), sexed (by caudal probe), weighed ($\pm$0.01 g), and measured (snout-vent length [SVL], head length [HL], head width [HW] and tail length [TL]; cm) in a squeeze box (Beaupre 2008; Quinn and Jones 1974). After processing, all snakes were released at their point of capture. As of this writing, a total of 415 individuals have been captured, marked, measured and released, yielding 471 recaptures.

Two lines of evidence are available to infer the presence and influence of long-term starvation stress among individuals in the focal population. First, morphometric data (body mass and length) can be used to compare body girth relative to length (i.e., body condition) among years using analysis of covariance. Such comparisons document significant among-year variation in body condition. Second, long term body mass fluctuations of individual radio-tagged snakes can be used to document both extended periods of starvation and the overall proportion of body mass lost over time. We consider each of these lines of evidence in turn.

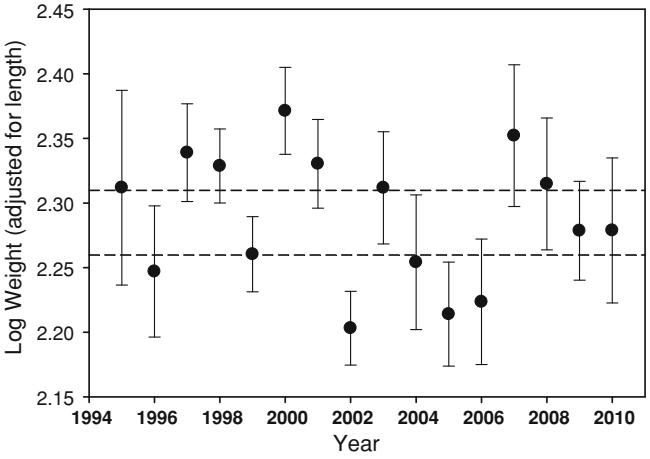

Fig. 8.8 Least squares adjusted mean log-body mass (with 95% CI) by year of timber rattlesnakes from a population in a degraded Ozark ecosystem. *Dotted lines* divide the plot into three zones. Means above the *upper dotted line* are significantly different from means below the *lower dotted line*. However, neither the upper nor the lower group is significantly different from means near the center of the plot

8.4.1 Body condition

Body condition can be compared among years by analysis of covariance (ANCOVA). Mean body weights (response) of snakes can be compared among years (treatment) after adjustment for body length (covariate). Prior to analysis, both length and body mass were log-transformed to achieve linearity. In poor years, during which prey abundance is low, and snakes are losing mass to starvation, we expect average length-adjusted mass to decrease. Conversely, during good years with abundant food, length-adjusted body mass is expected to increase. We constrained our analysis to a single body mass—SVL pair per individual, per year. Thus, although single individuals may be represented by multiple data points, the observations are in separate calendar years, and are presumed independent. Five hundred and eighty-eight observations were available for analysis, spread out among 16 years (1995–2010 inclusive). Slopes of the lines relating log-SVL to log-body mass were homogeneous among years. The overall ANCOVA model (SAS PROC GLM, SAS Institute, 1985) was significant ($P < 0.0001$). A significant effect of log-SVL on log-body mass was detected ($P < 0.0001$), along with a significant effect of year ($P \leq 0.0168$). Log-SVL adjusted means of log-body weight (Least Squares Means) were compared by PDIFF post hoc comparison procedure (SAS, PROC GLM, SAS Institute, 1985) to determine which years differed from others. Examination of a plot of length-adjusted means by year documents shifts in body condition attendant to annual variation in food acquisition (Fig. 8.8). In general, 1997, 1998, 2000, 2001, and

2007 were good years wherein snakes achieved increases in body mass relative to length, whereas, 1996, 2002, 2005, and 2006 were relatively poor years, during which snakes lost significant body mass. Several years tended toward average, including 1995, 1999, 2003, 2004, 2008, 2009, and 2010. These shifts in body condition demonstrate the boom and bust nature of resource dynamics in the degraded Ozark ecosystem and have been attributed to shifts in prey (small mammal) availability driven by annual variation in acorn mast crop (Beaupre 2008; Beaupre and Douglas 2009).

8.4.2 Body Mass

Perhaps more informative are individual body weight dynamics in the face of sometimes extended starvation events. Radio-tagged snakes provide long-term records of SVL and body weight that describe growth and body weight dynamics over multiple years. A typical trace of a female rattlesnake (ID: 023367888) monitored intermittently between September 30, 1997 and May 28, 2010 shows clear periods of body mass increase and decrease (Fig. 8.9a), spanning a range of 457.6–716.0 grams (258.4 g) while growth in SVL was negligible. As of this writing, this particular female survives in the population. The trace of this surviving female is starkly contrasted with that of three other snakes that starved to death. A male (ID: 035865037, Fig. 8.9b) continuously lost body mass (from 577.9 to 393.7 g) over the course of 455 days; a drop to 68.1% of initial body mass. After evidence of ingesting a small meal (ca. 20 g), this male entered hibernation, survived to spring 2010, but was found dead on May 28, 2010. Likewise, a female (ID: 024308878, Fig. 8.9c) underwent a 405-day starvation event losing 65% of her initial body mass (395.2 to 256.8 g) to be found dead on the surface on September 20, 2009, 45 days after her last mass-length measurement on August 16, 2009. Similarly, another female (ID:025267096, Fig. 8.9d) dropped to 58% of initial body mass (530 to 309 g) over the course of 753 days. This individual was extreme, both in the duration of the period of starvation, and in the proportional mass loss prior to death. In this case, the snake entered hibernation alive in fall of 2004, but failed to emerge in spring of 2005. We presume that the cause of death in all three of these individuals was starvation.

Annual variation in body condition and long-term decreases in body weight of individual timber rattlesnakes document the unpredictable nature of food resources and frequent exposure to extended periods of starvation in the degraded Ozark ecosystem. Boom and bust resource variation significantly influences body mass dynamics and starvation mortality. As further support that these shifts are indeed related to boom and bust prey dynamics, evidence from supplemental feeding experiments (Beaupre in progress) demonstrates severe food limitation in this system. Likewise, habitat manipulations (forest thinning and prescribed burning) aimed at enhancing ground-level food production results in increases in small mammal populations and body condition of snakes at the site (Douglas 2010,

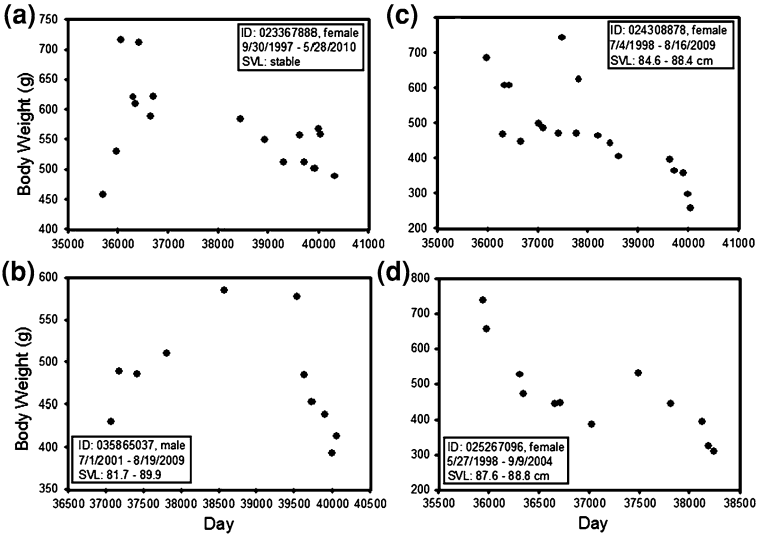

Fig. 8.9 Body weight versus day (since January 1, 1900) for four radio-tagged timber rattlesnakes. **a** Periods of mass loss and gain are apparent, but this female continues to survive in the population as of this writing. **b** Mass loss occurs between day 39539 (577.9 g) and 39994 (393.7 g), a span of 455 days during which body mass dropped to 68.1% of initial size. The snake was found deceased on May 28, 2010. **c** Significant mass loss occurs between day 39636 (395.2 g) and 40041 (256.8 g), a span of 405 days during which body mass dropped to 65% of initial size. The snake was found deceased on September 20, 2009. **d** Mass loss occurs between day 37486 (530 g) and 38239 (309.5 g), a span of 753 days during which body mass drops to 58% of initial size. The snake entered hibernation in fall of 2004, but failed to emerge the following spring

Beaupre and Douglas 2012). We conclude that extended bouts of starvation are typical of timber rattlesnakes living in the degraded Ozark ecosystem.

8.5 Synthesis

We have shown that snakes are capable of both supply-side and demand-side regulation in response to food deprivation. These combined strategies result in flexibility and starvation times that are extreme among vertebrates. Extended periods of food deprivation (including natural hibernation, infrequent pulsatile resources in insular snake populations, and unpredictable food resources in degraded forest ecosystems) are prominent features of snake populations in nature. We suggest that like their extreme trophic morphology (Cundall and Greene 2000), the ability of snakes to resist food deprivation may be a key adaptation that contributed to their successful radiation. The possibility of an adaptive trait complex related to starvation tolerance is worthy of intensified study in both a physiological and comparative context.

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Chapter 9
Cardiovascular Circuits and Digestive Function of Intermittent-Feeding Sauropsids

Rike Campen and Matthias Starck

9.1 Introduction

Our world is inhabited by a plethora of heterotrophic organisms with diverse patterns of food consumption that are determined by (i) environmental food availability, (ii) energy and nutrient demands of the animal, and (iii) feeding strategy (e.g., intermittent sit-and-wait food ingestion, grazing or browsing, 'constant' feeding). Except for some endoparasites and some filter feeding organisms living in nutrient-rich habitats, this world appears to provide only habitats with fluctuating food supply, i.e., changing food quality and quantity. Environmental food supply is affected by predictable changes such as circadian or seasonal variations in light and temperature as well as unpredictable environmental disturbances (see Bar and Volkoff, Chap. 6). Animals can prepare for these predictable changes by switching to an alternative diet or escaping local shortages by migrating to other feeding grounds (e.g., Piersma 1998; Piersma and Drent 2003; Piersma and Lindström 1997), or by hibernating/aestivating (see Harlow, Chap. 17). Short-term fluctuations in food supply are generally unpredictable in timing and duration and thus require rapid and flexible physiological adjustments (Barboza 2009; Barboza and Hume 2006; Starck 2005). Even energy-rich habitats that provide a continuous flow of food to the organism may be limiting in certain aspects of nutrition. A striking example of nutrient, but not food limitation, is the growing young of petrels and albatrosses (Procellariiformes). Parents provide large quantities of energy-rich but nutrient-poor food to offspring. To obtain the necessary amount of the rare required nutrients, the offspring need to ingest more total energy

R. Campen · M. Starck (✉)
Department of Biology II, Ludwig-Maximilians-University Munich, Munich, Germany
e-mail: starck@lmu.de

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than necessary and deposits surplus energy in large quantities of adipose tissue (energy sink hypothesis; Ricklefs 1976; Ricklefs et al. 1980).

9.1.1 Intermittent Feeding?

Continuous feeding and intermittent feeding are usually considered contrasting feeding strategies. However, at a closer look the picture becomes increasingly fuzzy because neither 'continuous' nor 'intermittent' can be clearly defined. Does continuous feeding, i.e., without any interruption, occur anywhere outside Cockaigne in the living world? Maybe some endoparasites (e.g., flukes, tapeworms, *Sacculina,* or blood parasites like trypanosome) feed continuously during certain stages of their life cycle, but even those endoparasites experience periods when they do not feed (e.g., as encysted persistence states in flukes and tapeworms; see McCue, Chap. 24). Mammals and birds are usually considered to be continuous feeders, but they often have intermittent periods of nonfeeding. Even humans who typically ingest three meals per day interrupt these feedings with periods of fasting (see McCue, Chap. 1). Some mammals (e.g., polar bears, wolf, reindeer) and birds (e.g., penguins, hornbills) periodically experience long fasting periods (up to several weeks) with no food intake. Finally, there are life cycle stages of many metazoans, including a considerable number of vertebrates that do not feed at all, but fuel their metabolism by energy stored during previous life cycle stages (e.g., numerous fish species, chelicerates, insects, etc.; see Kirk, Chap. 3). Because of this natural diversity an explicit and clear distinction must be made between 'fasting' and 'starvation' (McCue 2010). Fasting and starvation are different physiological conditions with different consequences for the organism. Fasting animals utilize internal energy stores (mainly adipose tissue; see Price and Valencak, Chap. 15) to fuel maintenance and activity metabolism. During fasting they slowly lose weight, indicating the constant decline of the energy stores. Fasting ends and starvation begins when the animal's internal energy stores become depleted and muscular tissue and other organ systems begin to rapidly degrade. At that point circulating levels of creatine phosphate, urea, and uric acid soar (Le Maho et al. 1976, 1977). Extended starvation, in contrast to fasting, may result in irreversible damage to the organism (Starck 2005; Wang et al. 2006). In this chapter, we focus on fasting as a natural, reoccurring situation in an animal's life.

If we exclude the rare extremes, i.e., continuous feeding and nonfeeding, we find that all metazoans more or less feed intermittently. What makes the difference among these situations is the size of the endogenous energy depots, the duration of the fasting interval, and the energetic demand for maintenance and activity during that period. A 12 h fasting period may not challenge a large mammal like humans (although we might think so), but it can be fatal for small mammals like shrews and bats (see Ben-Hamo et al., Chap. 16) that run on much higher mass-specific

energetic costs of maintenance. A five-month fasting period of a hibernating arctic ground squirrel or edible dormouse may not even exhaust its energy depots, because the metabolic demands of the entire organism are reduced so much (see also McCue et al., Chap. 8; Overgaard and Wang, Chap. 5). Continuous feeding and intermittent feeding seem to represent to two poles of a continuum of life-history strategies that are evidently modulated by an interaction of body size, fasting time, and energy demand. Therefore, broad comparisons among different 'intermittent feeding' organisms should account for the length of the nonfeeding period to mass-specific daily energy expenditure, and activity state. Although obvious, such scaling of fasting periods is rarely applied in comparative studies (but see Hohtola, Chap. 10; Overgaard and Wang, Chap. 5).

A discussion of all taxa is clearly beyond the scope of this overview so we restrict this chapter to the standard view of intermittent feeding in reptilian sauropsids. In doing so, we acknowledge that all reptilian sauropsids are intermittent feeders, but that they differ greatly in the length of fasting intervals, food quality, quantity and quality of energy stores, mass-specific energy demands, and intestinal passage times and digestive efficiency. Infrequent feeding species often are sit-and-wait hunters or actively hunting predators. Frequently feeding sauropsids tend to be grazing species; however, the association between feeding strategy and feeding interval is artificial and not unequivocal. Among grazers we may find species that feed only during short periods of the year, and there might be sit-and-wait hunters that feed quite frequently. For example, grazing green turtles (*Chelonia mydas*) experience fasting periods or periods when they find only small quantities of low quality vegetable food during migration or in their nesting habitats (Anton and Read 2001; Carr et al. 1974; Mortimer 1981).

Intermittent feeding is probably the ancestral pattern of food ingestion in jawed vertebrates (Osteognathostomata; Gaucher et al. 2011; Starck 2005), yet reptilian sauropsids have become the most popular models to examine the morphological and physiological changes associated with the transition from fasting to digesting (e.g., Hicks et al. 2000; McCue et al. 2005; Secor 2001; Secor et al. 1994; Starck and Beese 2001; Starck and Wimmer 2005). It is noteworthy that feeding and digesting are processes that also affect the cardiovascular system (conductive transport of absorbed nutrients, pH-buffer system, bicarbonate balance, associated blood gas changes in O_2 and CO_2); however, little attention has been directed to exploring the relationship between feeding and cardiovascular function, in particular the anatomy of its circuits. A growing body of knowledge indicates that cardiovascular circuits and patterns of food ingestion are tightly integrated (Andrade et al. 2004; Busk et al. 2000; Farmer 2011; Farmer et al. 2008; Hicks and Bennett 2004; Secor et al. 2000; Secor and White 2004, 2006; Starck and Wimmer 2005). In this chapter, we critically review the current state of knowledge, and we present ideas about how gastrointestinal anatomy, cardiovascular circuits, and feeding patterns are functionally integrated among reptilian sauropsids.

9.2 Responses of the Gastrointestinal Tract Following Functional Demands from Digestion and Fasting

Digestion and fasting impose contrasting functional demands on the gastrointestinal tract. During fasting, the gastrointestinal tract of infrequently feeding snakes is quiescent (Secor 2003, 2005). Compared to the functional, digestive condition, the measurable small intestinal nutrient uptake of some fasting snake species is only 10% of that during digestion. Similarly, intestinal mass is only 50%, and mass-specific rates of transepithelial nutrient transport are only 30% of those measured in digesting snakes (Secor 2005; see also Lignot, Chap. 14). Immediately after feeding, digestive and absorptive functions are upregulated by: (1) increasing the size of the digestive tract (including absorptive intestinal surface), (2) increasing the secretion of gastric acid and digestive enzymes, and (3) by increasing the activity (and availability) of membrane-bound transporters in the intestinal mucosa. These changes, in particular morphological changes in response to feeding, can be observed in many vertebrate species when fasting and digesting conditions are compared (Cramp and Franklin 2005; Cramp et al. 2005, 2009; Secor 2005; Starck 2005). They are most pronounced in sit-and-wait foraging species, which have therefore become popular model systems to study physiological and morphological responses to feeding. Nevertheless, these changes can also be observed in active hunters, e.g., garter snakes, though to a lesser degree (Secor 2001; Secor and Lignot 2005; Starck and Beese 2002).

Organs grow either by cellular hypertrophy, i.e., existing cells increase in size thus causing organ size to increase, or by hyperplasia, i.e., cells proliferate and their multiplication leads to an increase of organ size. So far, the morphological changes observed in the small intestine of fish, amphibians, and reptilian sauropsids appear to be largely based on hypertrophy of intestinal cells because of the incorporation of lipid droplets as originally reported by Starck and Beese (2001, 2002). The mechanisms underlying ultrastructural changes like the pertinent elongation of the microvilli (Cramp et al. 2005; Lignot et al. 2005; Lignot and Secor 2002; Secor 2001, 2003, 2005, 2008; Starck and Beese 2001, 2002; Starck et al. 2007) have not yet been analyzed in detail. The elongation of microvilli (in mammals) is based on adding of actin subunits to the actin filaments in the microvilli resulting in a rapid elongation of the microvilli (Mooseker et al. 1982). If the same mechanism applies for the microvilli elongation of other vertebrates, the ultrastructural increase of the absorptive surface area is based on restructuring of the apical enterocyte surface. Starck and Wimmer (2005) and Secor (2005) also reported increased blood flow to the small intestine. Starck and Wimmer (2005) showed that one half of the overall size increase of the small intestine in ball pythons was based on hypertrophy of the cells while the other half could be correlated to the increased blood flow to the gut. They suggested that the increased blood flow acts like a hydraulic pump by inflating the intestinal villi.

Crocodiles show, in principle, the same pattern of intestinal size changes in response to feeding as reported for snakes (Starck 2005; Starck et al. 2007) and

therefore apparently follow the same ancestral vertebrate pattern. It should be noted, however, that the hydraulic pump mechanism of small intestinal plasticity has not been studied in any other vertebrates besides boid snakes, so it is unclear whether this phenomenon occurs in sauropsids, generally.

In contrast to reptilian sauropsids, the flexibility of the digestive tract of birds and mammals is based on a dynamic balance of cell proliferation at the basis of the villi and cell death at the tip of the villi (Altmann 1972; Boza et al. 1999; Dunel-Erb et al. 2001; Iwakiri et al. 2001; Karasov et al. 2004; Raab et al. 1998; Starck 1996). Hypertrophy of cells caused by the absorption of lipids has been reported occasionally in mammals (Buschmann and Manke 1981a, b), but no data are available that suggests an integration with the cardiovascular system.

9.2.1 Effects of Feeding on Blood Acid–Base Balance

After consuming a large meal, the oxyntic cells in the stomach of reptilian sauropsids produce huge quantities of gastric acid by dissociating protons from water and pumping them into the stomach lumen, where they associate with Cl^- and form hydrochloric acid. Catalyzed by carbonic anhydrase, residual OH^- ions in the oxyntic cells associate with CO_2 forming HCO_3^- (hydrogen carbonate; bicarbonate) that must be removed from the cells to prevent alkalization. Upon release into the blood HCO_3^- causes in a rapid increase in blood pH, i.e., 'alkaline tide' (e.g. Coulson et al. 1950; Niv and Fraser 2002). Farmer et al. (2008) suggested that, driven by the hydrogen carbonate buffer system, CO_2 diffusion into oxyntic cells is the rate-limiting factor in gastric acid secretion. Thus, theoretically, directing hypercapnic blood to the stomach could increase the rates of gastric acid secretion over what would be possible from glands perfused with arterial blood. Evidence provided by Farmer et al. (2008) supports that view and shows that the pattern of blood flow to the gut is significantly correlated with digestive efficiency in alligators.

The alkaline tide ends when gastric acid production ceases and the food leaves the stomach into the duodenum. Hydrogen carbonate and sodium hydrogen carbonate are secreted from the gall bladder, respectively, into the lumen of the duodenum to neutralize the acidic chyme, (e.g., Hofmann 2010; Secor 2008). Reptilian sauropsids that are sit-and-wait predators generally need to produce large amounts of gastric acid due to the relatively large meal sizes they ingest. Additionally, the food stays in the stomach of these animals longer than those that feed more frequently on smaller meals. Therefore, the alkaline tide is more pronounced and long lasting in sit-and-wait predators than in more frequently feeding vertebrates (e.g., Coulson et al. 1950; Wang et al. 2001).

By contributing to the hydrogen carbonate buffer, CO_2 can be an essential factor in regulating blood pH. About 50% of CO_2 produced by the metabolic activity of tissues binds directly to hemoglobin or is sequestered in erythrocytes as H_2CO_3/HCO_3^-. Another 50% finally dissolves in blood plasma and contributes to

the hydrogen carbonate buffer (e.g., Penzlin 1991; Schmidt and Thews 1980). Theoretically, reptilian sauropsids have two options to increase the CO_2 content of their blood to balance metabolic alkalosis: (1) by hypoventilation like in mammals and birds (Andrade et al. 2004; Hicks et al. 2000); and (2) by bypassing the pulmonary circulation and re-directing CO_2-enriched blood directly back into the systemic circulation.

9.3 Cardiovascular Anatomy and Feeding

New evidence suggests that the digestive tract and the cardiovascular circuits of reptilian sauropsids are more tightly integrated than previously thought. This is mainly based on three observations: (1) the size increment of the small intestine after feeding is partially based on increased blood flow volume and lymph flow to the gut (hydraulic pump mechanism; Starck and Wimmer 2005); (2) the heart anatomy of reptilian sauropsids provides the possibility of central shunting, i.e., redirecting blood returning from the systemic circulation into the systemic circulation and bypassing the pulmonary circulation. Redirecting of CO_2-rich blood into the systemic circulation after feeding apparently maximizes gastric acid production and buffers alkaline tide (Farmer et al. 2008); and (3) the peripheral circuitry of blood supply to the stomach and the intestine suggests a tight integration of morphology and digestive function. In an insightful and detailed comparative morphological analysis, Farmer (2011) recently re-analyzed the pattern of major arteries in reptilian sauropsids and presented convincing functional explanations for a so far unresolved topographic pattern when digestive functions are included into the interpretations.

9.3.1 Hearts of Reptilian Sauropsids and Digestive Function

9.3.1.1 Chelonia, Rhynchocephalia, Squamata

The hearts of Chelonia, Rhynchocephalia, and Squamata consist of left and right atria and a single ventricle. This ventricle is functionally subdivided into the cavum arteriosum, the cavum venosum, and the cavum pulmonale; thus, there are five functional chambers of the heart (Fig. 9.1). The cavum arteriosum is separated from the two other ventricular chambers by the horizontal septum. It receives inflow from the left atrium and its outflow is through the interventricular canal into the cavum venosum. The cavum venosum and the cavum pulmonale are incompletely separated by the muscular ridge and the 'Bulbuslamelle', leaving an opening between the two chambers that closes during the systole. There is no direct connection between the cavum arteriosum and the cavum pulmonale (Farrell et al. 1998; Jensen et al. 2010b; Mathur 1944, 1946; Starck 2009; Webb et al. 1971, 1974; Webb 1979).

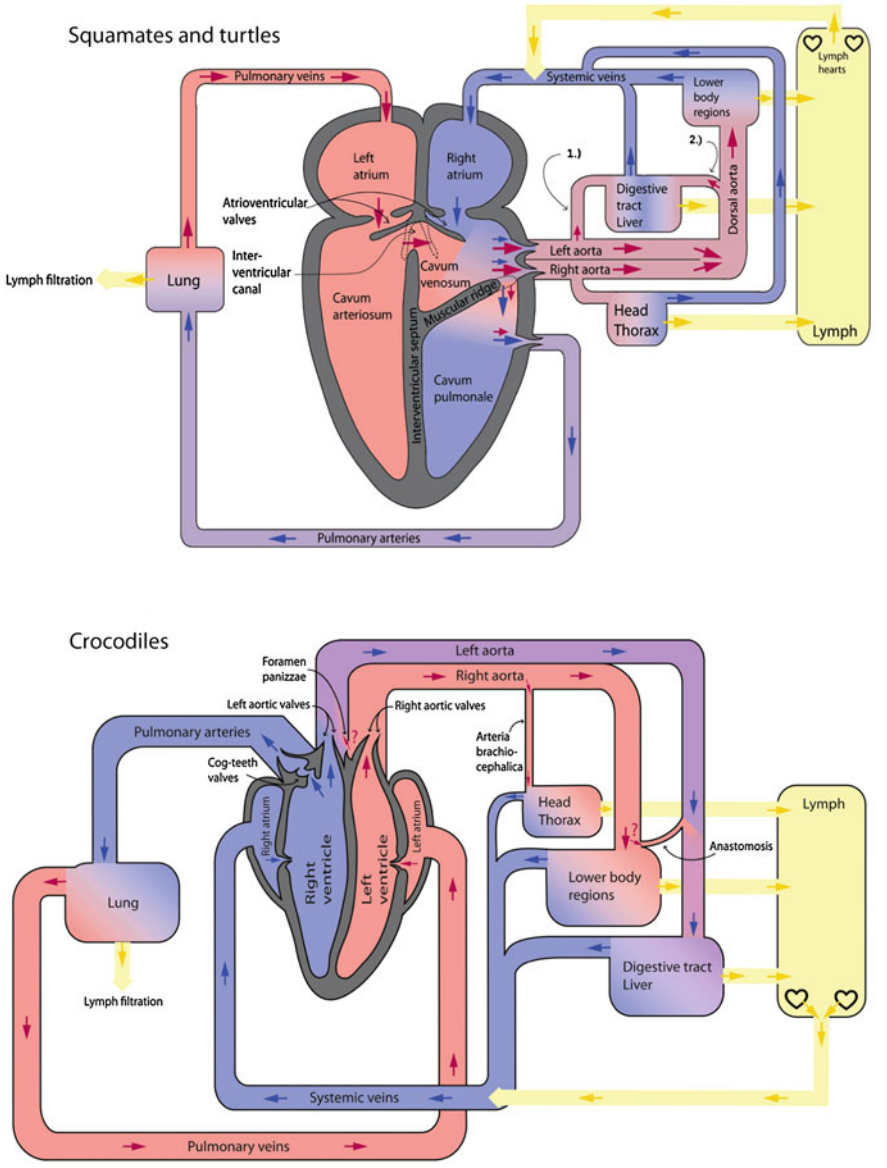


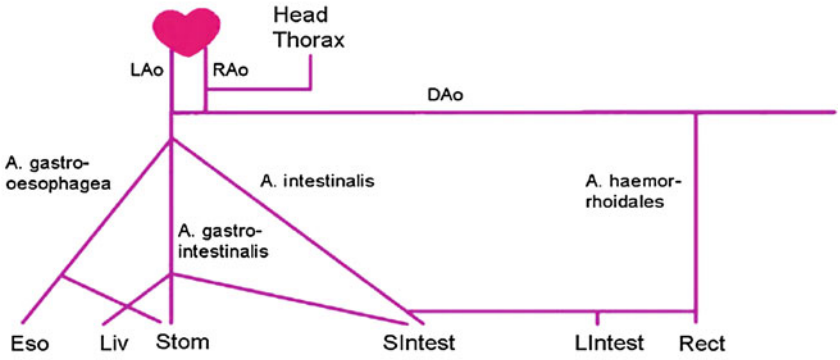
Fig. 9.1 Schematic illustration of the circulatory and lymphatic systems in squamates/turtles and crocodiles. Red color indicates O_2-rich, CO_2-poor blood, blue color indicates O_2-poor, CO_2-rich blood. Different shades of violet stand for different degrees of mixing. Lymph is drawn in yellow. Arrows indicate the direction of blood/lymph flow. The illustration for squamates and turtles (*top*) shows the blood supply of the digestive tract via the left aorta in turtles and some varanids. The alternative blood supply of the digestive tract via the dorsal aorta in squamates except varanids (*bottom*). For details see Fig. 9.2

Fig. 9.2 Ramification patterns of the major systemic arteries in different clades of reptilian sauropsids. In turtles, varanids and crocodiles, gastrointestinal arteries emerge from the left aorta. In squamates except varanids, the arteries supplying the digestive tract emerge from the dorsal aorta. It is unknown whether blood in the two aortae of squamates and turtles has the same blood gas content, it is therefore plotted in pink, indicating any degree of mixed blood. In crocodiles blood from the left ventricle is plotted in red, and blood from the right ventricle in blue indicating O_2-rich and O_2-poor blood, respectively. Exchange of blood between the aortae via the Foramen Panizzae or anastomosis is not reflected in the colour-coding. Abbreviations: *Eso* Esophagus, *DAo* Dorsal Aorta, *LIntest* Large Intestine, *Kidn* Kidneys, *LAo* Left Aorta, *Liv* Liver, *Ov* Ovary, *RAo* Right Aorta, *Rect* Rectum, *SIntest* Small Intestine, *Spl* Spleen, *Stom* Stomach. All figures redrawn after Hafferl (1933)

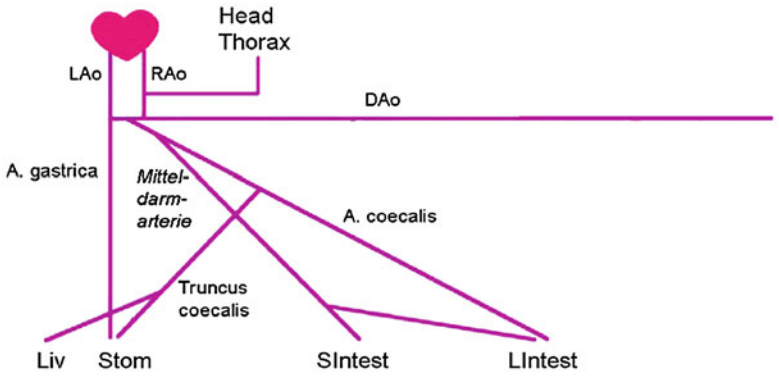
The atrioventricular valves direct the blood flow into the different chambers during ventricular diastole by closing the interventricular canal so that blood from the left atrium enters the cavum arteriosum and blood from the right atrium enters the cavum venosum and cavum pulmonale. During ventricular systole, the atrioventricular valves close the atrial openings and open the interventricular canal so that blood from the cavum arteriosum can enter the cavum venosum through the interventricular canal. The muscular ridge separates the cavum venosum and the cavum pulmonale during ventricular systole thus directing flow from the cavum venosum into the aorta and blood from the cavum pulmonale into the truncus pulmonalis (Jensen et al. 2010a; Starck 2009; Webb et al. 1971; White 1959, 1968). Therefore, Starck (2009) suggested that the timing of the opening of the atrioventricular valves and the muscular ridge's closure as well as the capacity of the cavum pulmonale determine the volume of blood that is directed into the pulmonary versus systemic circulation. If the muscular ridge closes early during the ventricular systole, not all blood returning from the systemic circulation has left the cavum venosum; thus it bypasses the pulmonary circulation and is redirected into the aortae. If the muscular ridge closes later, blood returning from the pulmonary circulation has entered the cavum pulmonale; thus, it bypasses the systemic circulation and is redirected into the pulmonary arteries. A no-shunt situation could be produced if the muscular ridge closes at an intermediate point of time (Starck 2009). Jensen et al. (2010a) suggest that the architecture of the python heart with a remarkably small cavum venosum does not allow for large volumes to be shunted into one or the other direction.

The heart anatomy is intimately associated with the pattern of the major arteries emerging from left to right ventricle and supplying the different body regions (Farmer 2011; Hafferl 1933; Hochstetter 1898). As a synapomorphic character of amniotes, reptilian sauropsids have two aortas emerging from the ventricle (a pattern that is independently lost in birds and mammals which have only one aorta; Gegenbaur 1901). As reviewed by Farmer (2011), the left and the right aorta of squamates and turtles, emerge from the cavum venosum. The right aortic arch branches off the carotid arteries, the subclavian arteries, and several smaller arteries that ramify in the thoracic musculature and supply the thymus. The left aortic arch does not branch off major vessels. Further caudad, the right and the left aorta fuse to form the dorsal aorta.

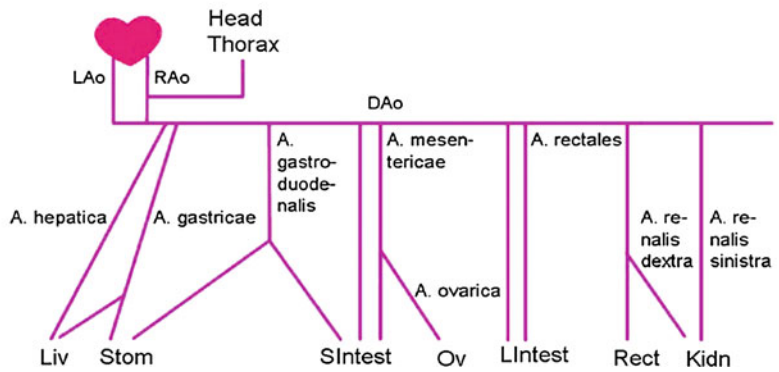
Clemmys spec.



Varanus niloticus



Boa constrictor



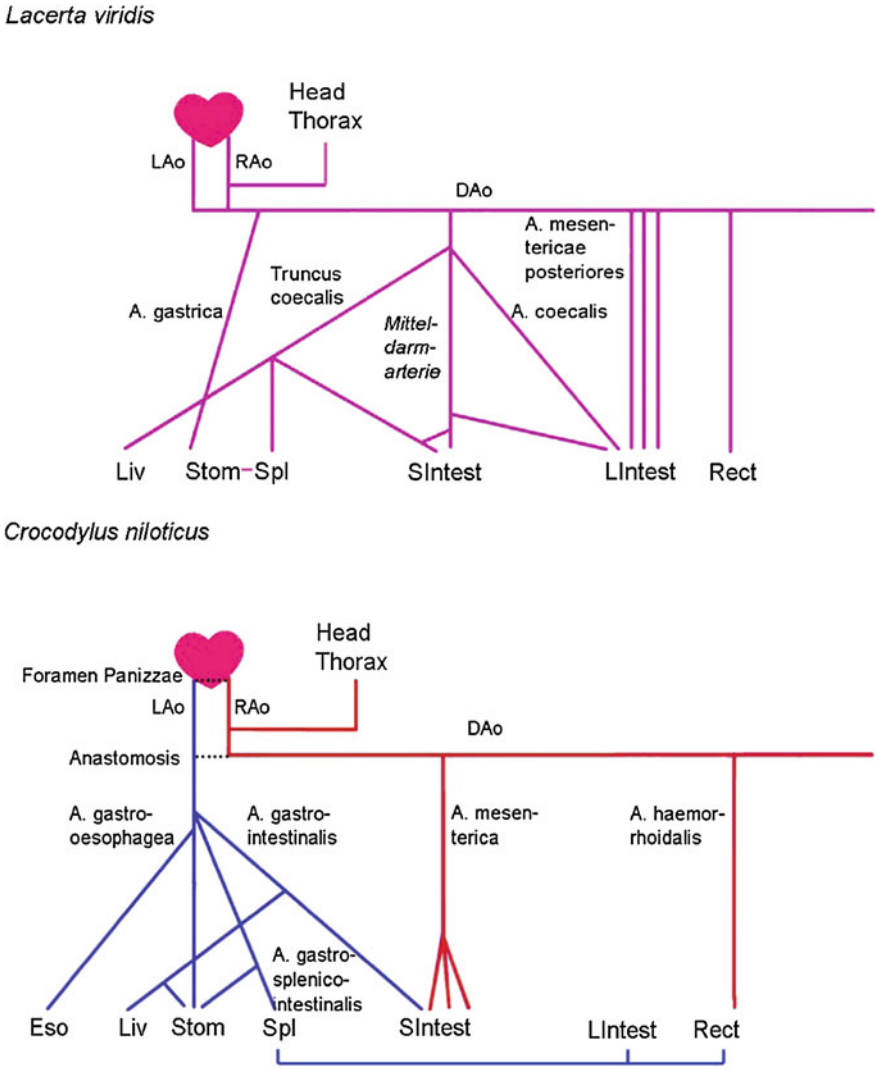


Fig. 9.2 (continued)

Blood supply to the gastrointestinal tract can either branch off the left aorta or the dorsal aorta formed by the merging of the left and right aortae. The pattern of blood supply to the stomach and the small intestine differs in major clades of sauropsids (Farmer 2011; Hafferl 1933; Hochstetter 1898). In most Testudines (Fig. 9.2a), monitor lizards (Fig. 9.2b), and some agamids the blood supply to the stomach and the anterior part of the small intestine *(Aa. coeliaca mesenterica)* branches off from the left aorta, before left and right aorta merge to form the dorsal aorta. In other reptilian sauropsids, visceral arteries emerge serially from the dorsal

aorta (Farmer 2011; Hafferl 1933), like in *Python regius* (Starck and Wimmer 2005), *Boa constrictor* (Hafferl 1933; Fig. 9.2c), and *Lacerta viridis* (Hafferl 1933; Fig. 9.2d). Though both aortae emerge from the cavum venosum, it might be possible that they receive blood with different O_2 and CO_2 content. Deducing function from the topography of the major vessels, Hochstetter (1898) suggested that the pattern of vascular supply might bring more CO_2-rich blood into the stomach. No empirical data yet support this hypothesis; but it is reasonable to assume that the timing of closure of the muscular ridge, the volume of the cavum venosum, and the dynamics of mixing blood in the ventricle may create a pattern of blood flow that would result in CO_2-rich blood delivered to the digestive tract. Although the original hypothesis was posed more than a century ago, it has not yet been tested by measuring directly blood gas pressures in the left and right aortae of non-crocodilian reptilian sauropsids under different physiological conditions (e.g., fasting vs. digesting).

9.3.1.2 Crocodylia

In crocodiles, the heart has four separated chambers (Fig. 9.1). The anatomy of the outflow vessels is unique among vertebrates and certainly an autapomorphy of crocodiles: the right aorta originates from the left ventricle, and the left aorta emerges from the right ventricle together with the pulmonary trunk. In crocodilians (and some varanids; e.g., *Varanus niloticus*; Fig. 9.2b), the blood supply to the stomach and the anterior part of the small intestine branches off from the left aorta (*A. gastro-intestinalis*; Fig. 9.2e) while the more distal part of the small intestine and the large intestine are irrigated by vessels emerging from the dorsal aorta. As noted by Hochstetter (1898), the topographic arrangement of the major vessels suggests that the left aorta receives O_2-poor and CO_2-rich blood from the right ventricle.

At its origin from the right ventricle, the inner wall of the Truncus pulmonalis is equipped with thickened tissue nodes, i.e., the cog-teethed valve, which are surrounded by a constrictor-like muscle (Fig. 9.3). Contraction of this sphincter muscle presses these cog-teeth valves against each other thus occluding the pulmonary trunk and increasing the resistance of the pulmonary circulation (Axelsson et al. 1996). Under these circumstances, ventricular blood pressure increases and opens the left aorta. Right ventricular blood then enters the left aorta instead of the pulmonary arteries, thus bypassing the pulmonary circulation. However, it is generally assumed that under standard conditions (i.e., normal breathing) the left aorta receives no blood from the right ventricle but receives O_2-rich blood from the right aorta through the Foramen Panizzae.

The Foramen Panizzae connects the right and the left aorta at their origin (Panizza 1833). More distally, both aortae are connected by the anastomosis. Both structures allow exchange of blood between left and right aorta. The detailed morphology of the Foramen Panizzae is poorly studied, and was not illustrated in the original description (Panizza 1833). Greenfield and Morrow (1961) presented

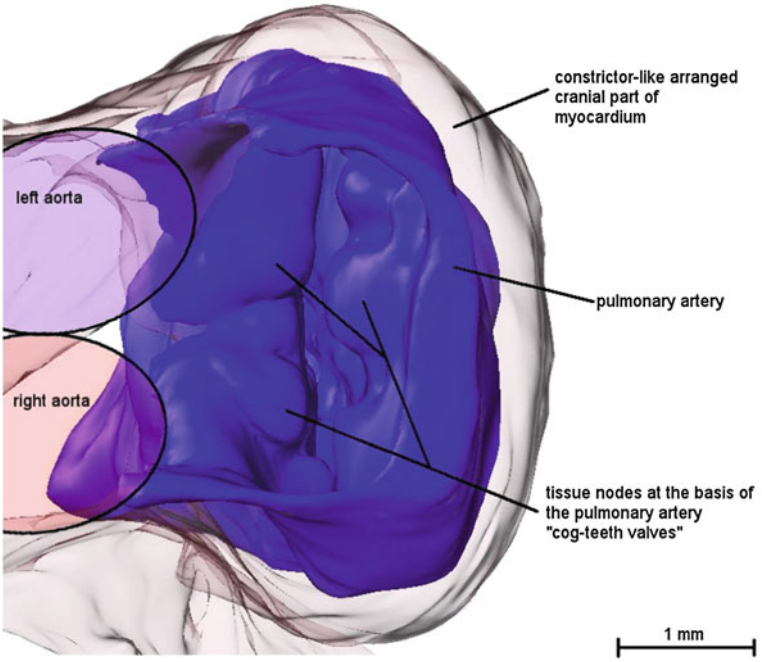


Fig. 9.3 *Crocodylus niloticus*, 3D-reconstruction of the pulmonary trunk with the cog-teeth valves at the basis of the pulmonary artery. The constrictor muscle surrounds the pulmonary trunk and its contraction supposedly presses the valves together. The reconstruction is made from histological serial sections from a juvenile Nile crocodile (Campen and Starck, unpublished material)

a semi-schematic drawing showing that the bicuspid valves of left and right aorta reach far into the aortae, overlapping the Foramen Panizzae. Opening of the valves during ventricular systole potentially obstructs flow through the Foramen Panizzae because in *Crocodylus niloticus* the valves at least of the left aorta cover it completely when they are pressed against the aortic walls (Fig. 9.4; unpublished material). In contrast to this, Axelsson et al. (1996) and Axelsson and Franklin (1997) described that the left aortic valves were unable to cover the Foramen Panizzae during any part of the cardiac cycle, while the right aortic valves obstructed the Foramen during most of systole in in situ perfused, beating *Crocodylus rhombifer* hearts. Additionally, they found its diameter to be 35–40% of the right aorta's diameter, allowing significant blood flow through the Foramen. Based on an immunohistochemical test, Karila et al. (1995), Axelsson (2001), and Axelsson and Franklin (2001) suggested that the Foramen Panizzae and the anastomosis are under adrenergic control and proposed an active role of the Foramen in exchanging blood between the right and the left aorta.

The circuits of the major vessels in crocodiles are similar to those found in monitor lizards and turtles. The left aorta branches off the coeliac artery and the

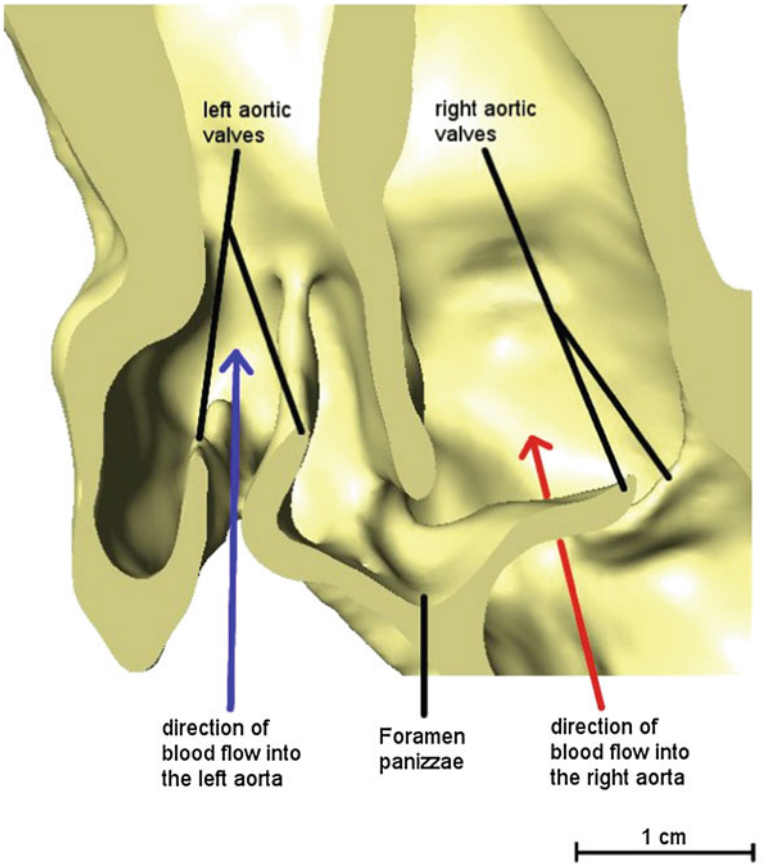


Fig. 9.4 *Crocodylus porosus,* 3D-reconstruction of the root of the aortae with the Foramen Panizzae and the left and the right aortic valves, virtually cut longitudinally. Note that the aortic valves are so large that they possibly obstruct the blood flow through the Foramen during ventricular systole. The reconstruction is made from magnetic resonance tomography serial sections through the heart of a salt water crocodile (Campen and Starck, unpublished material)

gastrointestinal artery shortly after the anastomosis connects it with the right aorta. Posterior to the anastomosis the right aorta is called the dorsal aorta, while the left aorta becomes the *A. coeliaco-mesentericae* (Hafferl 1933). Only a relatively small *A. mesenterica* emerges from the dorsal aorta to supply the more distal segments of the small intestine. The large intestine is supplied by the *A. haemorrhoidalis*, which also emerges from the dorsal aorta (Hafferl 1933). Thus, supposing that the left aorta receives a major portion of blood from the right ventricle, the stomach and anterior segments of the small intestine are flushed with CO_2-rich blood from the right ventricle; all other parts of the body would receive O_2-rich blood from the left ventricle. Using non-invasive Doppler-ultrasonography on resting Nile crocodile we found evidence that the left aorta received blood from the right

ventricle during resting as well as during diving and that flow through the Foramen Panizzae does not play a major role under those conditions (Starck 2007).

9.4 Functional Hypotheses on Cardiovascular Circuits and Digestive Function in Reptilian Sauropsids

Several functional hypotheses about central shunting and the functioning of the Foramen Panizzae in crocodiles have been proposed. Only a few of these have been tested explicitly but results of these tests led to contrasting interpretations.

9.4.1 The Text Book Paradigm

Traditionally, bypassing the pulmonary circulation by shunting blood from the right ventricle back into the systemic circulation in crocodiles has been interpreted in the context of diving apnea (Grigg and Johansen 1987; Hicks and Wang 1996; White 1969). According to the textbook paradigm (e.g., Kardong 1995), pressure differences between pulmonary (low pressure) and systemic (high pressure) circulation direct blood from the right ventricle into the pulmonary or systemic circulation. Under breathing conditions, pulmonary pressure is low and systemic pressure higher, thus blood from the right ventricle automatically enters the pulmonary trunk with lower pressure and resistance while the left aorta remains closed and does not receive blood from the right ventricle.

Bypassing the lungs during diving results in increased tissue and blood CO_2 content; this lowers blood pH and consequently improves O_2 delivery to the tissues (Bohr effect). Although the diving paradigm dominates textbooks, no unequivocal empirical data support that view. In contrast, published data are consistently inconsistent. For example, Axelsson et al. (1991) studied blood flow in the salt water crocodile *(Crocodylus porosus)* and reported that short fright dives led to a decreased flow in the right aorta, the coeliac artery, and the common carotid, but no clear effect on blood flow in the left aorta could be detected. Grigg and Johansen (1987) studied the cardiovascular dynamics in *Crocodylus porosus* breathing air and during voluntary aerobic dives. By measuring blood pressure they found evidence for a pulmonary bypass via the left aorta, occurring frequently as an aerobic dive progressed. Nevertheless, blood O_2 saturation in the left aorta suggested mixed blood content from the right and left ventricle, with the greater portion of blood coming from the right aorta even during diving. Söderström et al. (1999) showed, also in *Crocodylus porosus*, that induced anoxia (in a N_2-atmosphere, not diving) caused cerebral blood flow velocity to temporarily increase by 125%. Increased cerebral blood flow during hypoxia, however, is indicative of increased blood flow in the right, not in the left aorta.

9.4.2 Cardiovascular Circuits and Digestion

While there is some logic in the textbook paradigm, it provides functional explanations of the heart anatomy only for amphibious lifestyle of crocodiles which undergo long periods of apnoea during diving. It does not explain why the majority of land living sauropsids are equipped with an equally complicated heart anatomy and the ability to bypass the pulmonary or the systemic circulation. This is where we think the functional integration of the cardiovascular system and the digestive system comes into play, particularly among intermittent feeding sauropsids. Swallowing a large meal after an extended fasting period initiates the production of large quantities of gastric acid, followed by an alkaline tide in the blood. By bypassing the pulmonary circulation, the digesting individuals might respond to changes in the pH-balance of the blood very quickly. The alkaline tide occurs early during digestion when O_2 demand increases during specific dynamic action. Bypassing the pulmonary circulation to balance blood pH by retention of CO_2 therefore potentially conflicts with the increasing energetic demand of the body. However, if bypassing of the pulmonary circulation occurs only on a beat-by-beat basis (Campen and Starck, unpublished data) it may still buffer the alkaline tide yet also provide sufficient O_2 to tissues. While this particular response has not been demonstrated, it would explain the contrasting results of published studies; most studies assume an extended period of shunting and they may actually have missed the fine-tuned beat-by-beat bypassing of the pulmonary circulation. If shunting occurs in response to feeding and if it is a rapid response, one will need to follow patterns of blood flow on a beat-by-beat basis. The greatest challenge to this end will be to detect a clear signal that triggers the response (i.e., blood pH).

Evidence for the integration of digestive and cardiovascular performance comes from studies on python snakes. (1) Starck and Wimmer (2005) and Secor and White (2006) showed that shortly after feeding, blood flow volume to the gastrointestinal tract increases. While Starck and Wimmer (2005) measured significantly increased blood flow in the mesenteric artery (ca. 30% increase), the liver portal vein (3-fold increase) and the liver vein, Secor and White (2006) reported an 11-fold increase in blood flow in the superior mesenteric artery compared to predigesting values. Because blood flow volume was measured in the periphery, provisioning more blood to the systemic circulation may have been the result of bypassing the pulmonary circulation, or it may have resulted from an overall increased cardiac output. Starck (2009) compared changes of pulmonary and systemic blood flow during fasting and after feeding, and measured an increase in systemic blood flow, indicating such a pulmonary bypass. It therefore seemed likely that increased systemic blood flow serves digestive tract flexibility and/or CO_2 retention after feeding. (2) Jones and Shelton (1993) suggested that shunting might play a role for digestion balancing blood pH during the alkaline tide in crocodiles, but did not test it. (3) Axelsson et al. (1991) measured such low left aortic blood flow in fasting animals and concluded that it could not explain the permanent pulsatile anterograde blood flow in the coeliac artery. Therefore, they

suggested that in fasting animals right aortic blood enters the left aorta through the Foramen Panizzae, but that during fasting most of the blood in the coeliac artery comes from the right aorta through the anastomosis. This would mean that the gastrointestinal tract is exclusively supplied by O_2-rich, CO_2-poor blood from the left ventricle during fasting. After feeding, blood flow in the left aorta and coeliac artery increased, whereas right aortic blood flow decreased thus supporting the idea that CO_2-rich blood is pumped to the stomach and anterior part of the small intestine. (4) Farmer et al. (2008) showed that in *Alligator mississippiensis*, bypassing the pulmonary circulation increased after feeding. Alligators that were surgically disabled to shunt, digested bones more slowly than animals that could shunt. (5) Starck et al. (2007) suggest that blood flow into the mucosa of the small intestine increased in *Caiman latirostris*, because structural changes after feeding are identical to those in pythons, which partly depend on increased blood flow. (6) Digestion and associated elevated metabolic rates result in enhanced cardiac activity (Hicks et al. 2000; Secor et al. 2000). Following feeding, a 40% increase in ventricular wet mass was measured in *Python molurus* (Andersen et al. 2005). Increased levels of mRNA for cardiac myosin heavy chains after feeding suggest cardiac muscle protein was synthesized. Additionally, Riquelme et al. (2011) found that ventricular growth after digestion in pythons is accompanied by increased transport and oxidation of special fatty acids and increased activity and expression of the cardioprotective enzyme superoxide dismutase. While these results do not inform about a targeted navigation of blood into the digestive organs, they underscore the importance of the integration the cardiovascular system with the digestive system.

9.4.3 Other Possible Functions of the Cardiovascular Circuits not Related to Digestion

If the functioning of the cardiovascular circuits in reptilian sauropsids is related to regulating the acid–base balance after feeding, one may expect integration in other functional systems that have the potential to affect the acid–base system. For example, anaerobic exercise leads to a rapid acidification of the muscles and blood by production of lactate (Hicks and Bennett 2004). In particular, ectotherm sauropsids rely on anaerobic muscle power output and possess only small aerobic muscular capacities. In numerous species of ectothermic sauropsids considerably more than 50% of the energy required for intense muscle work is derived from glycolysis (Bennett 1994; Bennett et al. 1975; Bennett and Licht 1972; Gatten 1984; Ruben 1976). Although the protons leading to a decreased pH in the muscle and blood do not originate from the lactate itself (Lindinger et al. 2005), lactate production and acidification of muscle cells and blood coincide (Robergs et al. 2004). Acidification of skeletal muscles during exercise in mammals is buffered mainly by the amino acid histidine, and to a lesser extent carnosine. The hydrocarbonate buffer and inorganic phosphor play a minor role in maintenance

of acid–base balance during exercise in humans (Jones et al. 2004). Nevertheless, bypassing the systemic circulation and redirecting blood into the lungs during or after exercise could improve CO_2 disposal in the lungs of reptilian sauropsids, thus antagonizing a metabolically induced acidosis by a cardiac and respiratory alkalosis. Because of the heart anatomy (see above) bypassing the systemic circulation may occur in squamates and turtles but not in crocodiles.

9.4.4 Separating Blood Streams to Support Growth

The digestive tract of crocodiles, turtles, and varanids is supplied by arteries emerging from the left aorta; in all other reptilian sauropsids the digestive tract receives blood from the dorsal aorta. Considering the ecology of these groups, there is no obvious condition shared by those clades that would explain this specific vascular arrangement. However, Farmer (2011) suggested a connection between the arterial pattern, growth rate, and absolute adult size because all three clades contain comparatively large species; e.g., *Varanus komodoensis*, the extinct turtle *Archelon*, many of the living crocodile species and the extinct crocodile *Sarcosuchus imperator*. Because of higher absolute growth rates in larger animals, she suggested that selection might have been caused vascular topography to separate blood flow to the bones from that to the digestive tract. While the gut of digesting animals requires acidic blood to buffer the alkaline tide, acidic blood decreases osteoblast activity and increases osteoclast activity. Therefore, it is plausible to assume that a topographic separation of the blood flow to the stomach and intestines on the one side, and the body (= bones) on the other side might favor growth. This hypothesis by Farmer (2011) is provocative and difficult to test but provides stimulating ideas for future research on cardiovascular anatomy and patterns of feeding and growth in reptilian sauropsids.

9.5 Summary and Future Research Avenues

Chelonia, Squamata, and Crocodylia are infrequently feeding reptilian sauropsids that show the same principle pattern of gastrointestinal plasticity to adjust to fluctuating functional demands. Scattered data on constrictor snakes and crocodilians suggest a functional integration of the digestive system's functional plasticity and digestive function with the cardiovascular system. Both systems appear to be integrated on different levels, i.e., conductive (increased blood volume), mechanical (hydraulic pump), and acid–base balance (bicarbonate buffer and alkaline tide). Central cardiac shunting and circuitry of the major arteries suggest that morphological features evolved specifically to serve these functions. However, despite many careful studies, it is so far not possible to unequivocally explain patterns of cardiovascular dynamics in reptilian sauropsids and their response to feeding.

We have discussed several possible reasons for the conflicting outcome of carefully conducted experimental studies. We conclude that (1) the timing of shunting during digestion has not been fully explored. If shunting provides a beat-by-beat regulation of the acid–base balance one needs to apply fine-tuned measurements with the appropriate timely resolution. All published studies (including our own) are rather long-term studies. Thus each study may report accurate results but reflect animals in different physiological states (which have not been recorded) thereby precluding direct comparisons among them. (2) Many important quantitative data are completely missing. We lack information about ventricle and blood volume, and shunting volume under different physiological conditions. (3) Experimental studies are dominated by highly invasive studies, including open heart surgery or perfused heart models. While those models provide important basic information, it is unclear how much they reflect a normal physiological condition. We acknowledge the difficulty of measuring pattern of blood flow non-invasively. However, in non-invasive studies (e.g., Doppler ultrasonography) low levels of stress or lack of acclimatization may still change the pattern of blood flow, albeit to a lesser extent than that caused by invasive surgical procedures. (4) Capillary filtration rate and the role of the lymphatic system as a CO_2 sink have been neglected. The movement of lymph against gravity is closely connected to movements of skeletal muscles and the lung. Anesthesia therefore leads to drastically reduced backflow of lymph to the lymph hearts and therefore into the systemic veins in frogs (Hillman et al. 2010). If this is also case in ectothermic sauropsids, anesthesia during experiments would not only disturb normal flow and shunting because of general depression of the cardiovascular system. As 50% of the CO_2 produced in tissues dissolves in the plasma, it can leave the blood vessels and be temporarily stored in the lymph system. In contrast, O_2 is for the most part bound to hemoglobin and remains in the blood vessels. Therefore, lymph storage uncouples O_2 and CO_2 and thereby influences blood gas composition. (5) Volume compensation and compensatory shunts have largely been neglected. Following a shunting situation, it is necessary that compensatory shunts shift the blood volume back into the circulation from which it was previously subtracted.

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Chapter 10
Thermoregulatory Adaptations to Starvation in Birds

Esa Hohtola

10.1 Endothermy and Starvation in Birds: A Challenging Equation

Birds are endothermic and generally maintain a body temperature (T_b) of 40–42°C (Prinzinger et al. 1991). This situation requires a constant energy supply to sustain thermogenesis along with other vital functions thereby leading to avian metabolic rates that are significantly higher than those of mammals of similar body mass (Peters 1983). Moreover, many small nonmigratory birds in high latitudes experience severe cold combined with food limitation and a short feeding time in winter (e.g. Broggi et al. 2004; Reinertsen and Haftorn 1986; Steen 1958). Nonhibernating small mammals often avoid extreme temperatures by living under the snow cover (see Zhang et al., Chap. 13). Some birds, however, use a similar strategy and roost in the snow (Sulkava 1969).

Given their costly lifestyle, starvation and fasting pose a formidable energetic problem for birds. The depletion of body energy reserves induces a host of thermoregulatory, metabolic, and behavioral consequences (review, McCue 2010). Most small birds accumulate only enough fat to sustain overnight metabolism (Blem 1976) and thus enter a state of starvation rapidly after food deprivation. Some bird species are able to accumulate extensive fat depots to allow prolonged fasting (e.g. Le Maho 1983; Stokkan 1992; see also Jenni-Eiermann and Jenni, Chap. 11). Most birds are small making the problem of energy limitation even more acute because small size increases the relative surface area for heat loss. Assuming that the ability to carry fat depots increases isometrically with body mass, and using an overall allometric metabolic exponent of 0.681 for avian field metabolic rate (Nagy 2005), we see that fasting resistance in birds increases with an mass exponent

E. Hohtola (✉)
Department of Biology, University of Oulu, 90114 Oulu, Finland
e-mail: esa.hohtola@oulu.fi

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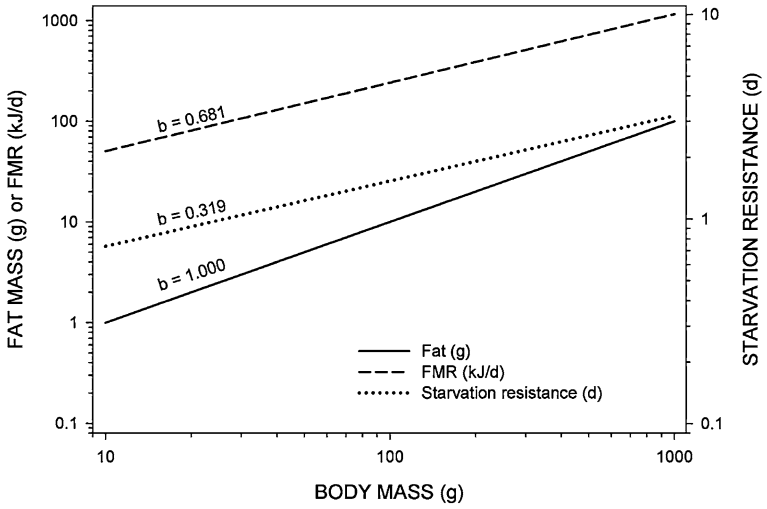

Fig. 10.1 The relationship between starvation resistance and body size in birds, assuming reliance on fat stores comprising 10% of body mass and a metabolic rate equaling the estimated field metabolic rate of birds (Nagy 2005)

of 0.319 (Fig. 10.1) (see also Overgaard and Wang, Chap. 5). This relationship is conservative in that it is based on field metabolic rates that are usually not measured during thermal stress. With decreasing temperature, this relation becomes even steeper because to maintain their T_b, small birds must increase their metabolic rate to a relatively higher multiple of basal metabolic rate (BMR) (Reinertsen and Haftorn 1986). Thus, with the same relative fat stores, a small bird succumbs to starvation more rapidly than a large bird. Moreover, many birds, most notably insectivorous and nectarivorous species (Downs and Brown 2002) rely on food sources that fluctuate temporally and spatially (see also Bar and Volkoff, Chap. 6). Species that regularly experience food shortages may cope by accumulating fat depots to buffer against periods of starvation or by reducing their energy use by reducing activity or the need for active heat production. Birds also fast voluntarily. For example, incubating birds may stay on the nest for days without a feeding bout, a phenomenon called voluntary anorexia (Sherry et al. 1980) and displaying birds may starve during long bouts of courtship activity (Hutchinson et al. 1993).

Feathers provide excellent thermal insulation but thermoregulatory costs never fully disappear. From simple thermophysiological principles, it can be inferred that the costs of maintaining homeothermy below thermal neutrality can be reduced if the bird is able to allow a (regulated) drop in T_b or if the bird can increase thermal insulation, or both. The ability to lower T_b during food limitation in certain avian groups has been known for a long time (Chossat 1843), but only one species, the North-American common poorwill (*Phalaenoptilus nuttalli*) is known to exhibit true hibernation, i.e. prolonged torpor that lasts for weeks (Jaeger 1948, 1949). It has been shown that torpor can occur without energetic stress caused by starvation

or cold exposure. For example, hummingbirds and blackcaps (*Sylvia atricapilla*) use nocturnal torpor during premigratory fattening to save energy during the night and thus to speed up the accumulation of fat reserves (Carpenter and Hixon 1988; Wojciechowski and Pinshow 2009) and barnacle geese show a regulated hypothermia during their migratory flight (Butler and Woakes 2001).

Although only some avian groups are able to enter deep torpor, recent studies have shown that most species are able to enter a mild state of hypometabolism if subjected to natural or experimental food limitation (for a compilation and review, see McKechnie and Lovegrove 2002). Such metabolic states have been referred as regulated (nocturnal, rest-phase) hypothermia or shallow torpor. Along with the various forms of hibernation and torpor in mammals (see Harlow, Chap. 17), the physiology and evolution of hypothermia in birds has attracted a great deal of interest recently (Geiser 2008; Körtner and Geiser 2000; McKechnie and Lovegrove 2002; Reinertsen 1996; Schleucher 2004).

10.2 Avian Torpor as a Response to Starvation

10.2.1 Deep Torpor

Deep torpor, where birds remain unresponsive to environmental stimuli is known in only a few avian taxa (McKechnie and Lovegrove 2002), although it must be emphasized that out of almost 10 000 bird species, most remain unstudied in this respect. Classic examples of bird families exhibiting deep torpor include hummingbirds (Trochilidae) (e.g. Bech et al. 1997; Hainsworth et al. 1977), swifts (family Apodidae) (e.g. Koskimies 1950), nightjars and their allies (Caprimulgidae) (e.g. Brigham et al. 2000; Peiponen 1966), and mousebirds (Coliidae) (e.g. McKechnie and Lovegrove 2001a; Schaub et al. 1999). It is an interesting point that hummingbirds, swifts, and hummingbirds are phylogenetically closely related and are also the best known examples of species exhibiting torpor (McKechnie and Lovegrove 2002; Sibley and Ahlquist 1990). Deep torpor has been described in at least one Passerine species, the insectivorous house martin, *Delichon urbicum* (Prinzinger and Siedle 1988). The possibility that deep torpor is a plesiomorphic character that has been lost in some endothermic taxa has been discussed in literature (Geiser 2008).

During torpor, the T_b of hummingbirds may decrease to 6.5°C and in nightjars to 4.3°C (see McKechnie and Lovegrove 2002). Hummingbirds and mousebirds regularly enter torpor during the night at mild ambient temperatures even without apparent starvation or daytime food restriction. Hummingbirds are so small that they cannot maintain an endothermic nocturnal T_b with the nectar collected during the day in cool environments and must enter torpor during the night (Bech et al. 1997; Hainsworth et al. 1977; Hiebert 1991). Continuous measurements of T_b are technically difficult in hummingbirds and thus few data exist on the effect of

starvation in controlled conditions. Nevertheless, the existing data show that hummingbirds are apparently unique in that they exhibit multiple, short torpor bouts during a single scotophase (Bech et al. 1997; Hiebert 1990). The adaptive benefit of this strategy remains unclear.

For the common poorwill, the only bird species known to enter true hibernation (Jaeger 1948), the evidence for hibernation comes from single individuals in the wild. Controlled experiments demonstrating hibernation are lacking although they have shown that the common poorwill is capable of "normal" daily torpor (Brigham 1992). Several other caprimulgid species can enter short-term deep (or shallow) torpor that can last beyond the diurnal rest-phase of these nocturnal birds (e.g. Brigham et al. 2000; Körtner et al. 2000; Lane et al. 2004; McKechnie et al. 2007). Surprisingly, owls (Strigiformes) that are often classified as being phylogenetically close to caprimulgid birds either lack a hypothermic response to fasting (Hohtola et al. 1994), or show a very small response (Chaplin et al. 1984; Thouzeau et al. 1999).

10.2.2 Shallow Torpor

Most birds have a clear daily cycle of body temperature. Diurnal birds typically allow their T_b to decrease by 1–1.5°C during the dark phase (scotophase) when they are resting, even in ad libitum conditions (Aschoff 1981). When deprived of food, many bird species resort to shallow torpor: they allow their body temperature to fall even further during scotophase (e.g. Graf et al. 1989; Hohtola et al. 1991; Rashotte et al. 1995; Walker et al. 1983). A drop in T_b of 1–10°C is now established as a general response of birds to food deprivation (reviewed in McKechnie and Lovegrove 2002). The lower T_b decreases the thermal gradient between the body and the environment and is thus an efficient way to decrease heat loss and, consequently, the need for active thermogenesis. Theoretically, energy savings should be directly proportional to the decrease in T_b (but see Sect. 10.5). The term shallow torpor is appropriate in the sense that birds remain responsive to their environment despite the lower T_b. The daily light–dark cycle seems to be necessary for the expression of rest phase hypothermia (Underwood et al. 1999). In continuous dim light, fasting birds maintain an intermediate, relatively constant T_b.

In laboratory experiments, where food deprivation is continued for several days, the nocturnal drop of T_b typically increases each night, while photophase T_b does not change (e.g. Ben-Hamo et al. 2010; Graf et al. 1989; Hohtola et al. 1991; Phillips and Berger 1991; Prinzinger et al. 1992; Rashotte et al. 1995; Walker et al. 1983). Thus, birds seem to be able to sense the depletion of their energy reserves as fasting continues and to adjust the level of hypothermia accordingly. Hypothermia is apparently not a failure of thermoregulation, as assumed in early studies (Chossat 1843), but rather a regulated mechanism as shown by thermal stimulation experiments in pigeons (Graf et al. 1989). When the thermosensory areas of the spinal cord (spinal cord is a major thermosensory in birds, Simon 1974)

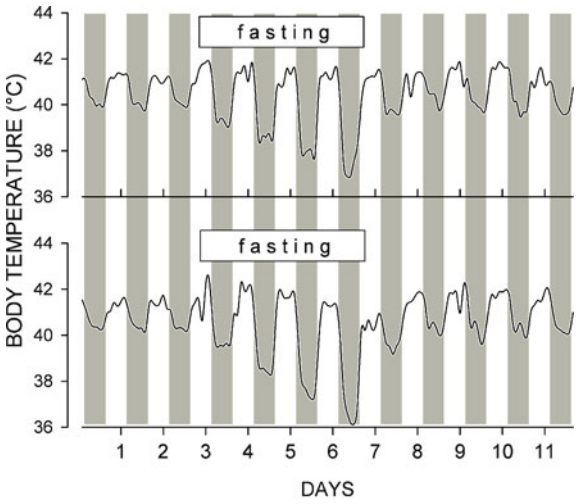

Fig. 10.2 Shallow torpor as a response to 4-day fasting in two representative Japanese quail. The vertical bars depict 12:12 h *light:dark* cycle. Each night of fasting results in a deeper hypothermia. Redrawn from Hohtola et al. (1991)

were cooled in fasting pigeons they defended their T_b through shivering thermogenesis. The spinal temperature threshold for the initiation of shivering moved progressively lower each night as fasting continued, but the daytime threshold did not change. Multiday T_b recordings in fasting birds are rare and are not feasible in small species, so few datasets are available, mainly from quail and pigeons. An example of starvation-induced multiday torpor response in laboratory conditions is shown in Fig. 10.2.

There are very few studies examining controlled food deprivation in wild birds. In radio-tagged willow tits (*Parus montanus*) returning to roost in nest-boxes equipped with receivers, the nocturnal T_b was correlated with the evening mass at roosting: the lower the evening body mass (indicating food shortage during the day), the deeper the hypothermia on the following night (Reinertsen and Haftorn 1984). Reinertsen and Haftorn (1986) studied the occurrence of torpor in three arctic small passerines, the willow tit, the great tit (*Parus major*), and arctic redpoll (*Carduelis flammea*). Each of these species exhibited nocturnal shallow torpor when subjected to moderate fasting, but only the willow tit entered shallow torpor in ad libitum conditions. Red-tailed hawks (*Buteo jamaicensis*) and great horned owls (*Bubo virginianus*) showed slightly lower T_b's during winter days when fasted for 2 and 4 days, respectively, resulting in 5% mass loss (Chaplin et al. 1984). Pigeons living in seminatural conditions in outdoor aviaries in winter showed a clear-cut nocturnal hypothermic response to 2 days of fasting (Laurila and Hohtola 2005, Fig. 10.3). Australian owlet nightjars (*Aegotheles cristatus*) use torpor more often during periods of low insect activity (Doucette et al. 2012) and the frequency of torpor increases in hummingbirds if their daytime nectar intake is decreased (Hiebert 1991).

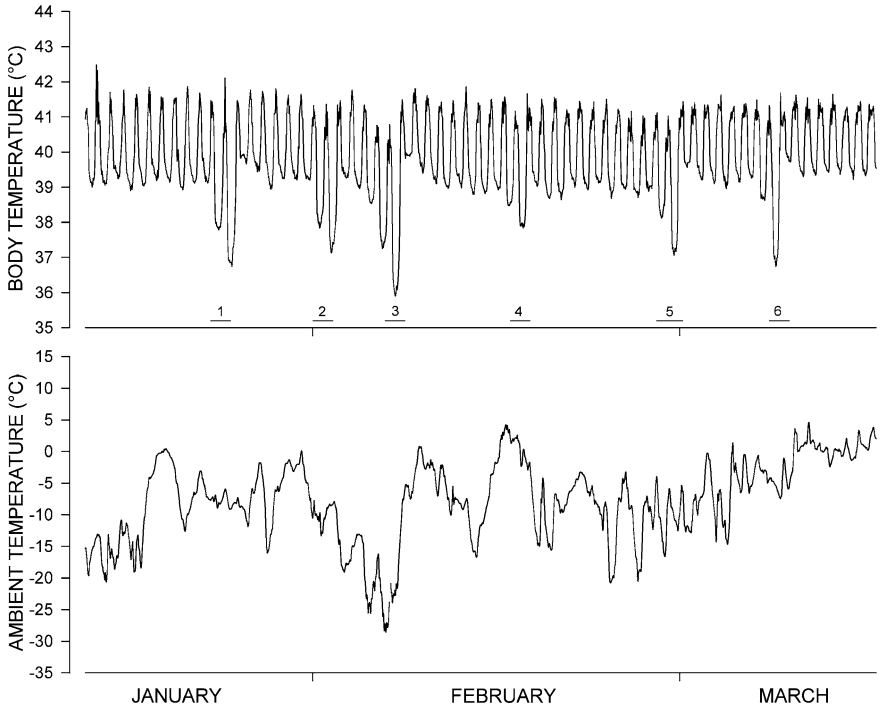

Fig. 10.3 Small modulation of the depth of fasting-induced shallow torpor by ambient temperature in the pigeon (mean of 4 birds) maintained in large outdoor aviaries in winter. The numbers show six occasions of 2-day fasts. Redrawn from Laurila and Hohtola (2005)

10.3 Regulation of Shallow Torpor and Hypometabolism by External and Internal Factors

Little is known on the internal signaling that is responsible for fasting-induced hypothermia in birds. Based on the studies available, it appears that both metabolite sensing and neurohumoral factors are involved in the control of shallow torpor.

10.3.1 Sensing Body Energy Reserves

Because the fasting-induced drop in T_b is greater during each successive night of long-term fasting, it is clear that fasting birds are able to sense the amount of remaining energy reserves. In birds that can fast only for one night, such as willow tits (*Parus montanus*), the evening of body mass of roosting birds predicts the level of hypothermia during the following night (Reinertsen and Haftorn 1984). Similarly, in long-term fasting experiments in laboratory birds, the decrease of T_b is tightly coupled with body mass loss (e.g. Rashotte et al. 1999). These observations

suggest that sensing of the remaining energy reserves occurs and that birds adjust their T_b accordingly. Whether the signal driving the hypothermic response is a so-called extensive variable (directly related to mass, for example, sensing the amount of fat directly) or an intensive variable (metabolite or hormonal concentrations related to fat or body mass) has been discussed in literature (Hohtola et al. 1991; Reinertsen 1996), and most studies suggest control by intensive (i.e. concentration-based) signals.

The metabolic consequences of fasting and starvation can be divided in three phases (Boismenu et al. 1992; Cherel et al. 1988; Le Maho 1983; Wang et al. 2006). In phase I, the bird is mainly using carbohydrates as metabolic fuel and loses body mass rapidly. In phase II, fat replaces carbohydrate as metabolic fuel (see also Price et al., Chap. 15), and body mass loss is slower (see also, Bauchinger and McWilliams, Chap. 12). Simultaneously with fat combustion, the concentration of ketone bodies in blood plasma increases (e.g. Cherel and Le Maho 1985). In phase III, fat stores have been depleted, and the bird has to resort to protein catabolism, which results in rapid body mass loss and an increase in uric acid excretion. A host of metabolic and hormonal changes thus accompany these phases and may be used by the thermoregulatory system as signals to drive hypothermic states. The sequence of these phases has been documented in various species such as penguins, geese, and quail (Le Maho 1983; Sartori et al. 1995). Such patterns are not, however, directly observed in the thermoregulatory response to starvation, as T_b decreases steadily with decreasing energy reserves. On the other hand, the increased motor activity during extended fasting, obviously related to food searching, seems to be connected to the metabolic shift for protein use in phase III of fasting (Koubi et al. 1991) (see also, McCue et al., Chap. 8).

10.3.2 Neurohumoral Signals of Feeding State

In pigeons, intravenous glucose infusion decreased the depth of fasting-induced hypothermia (Phillips and Berger 1991). The effect was apparently not specific to the hypothermic phase of the daily cycle (scotophase) because daytime T_b was also slightly increased by the infusion. Other potential metabolic signals, such as leptin, fatty acids, bile acids, and AMP, some of which are known to influence torpor occurrence and depth in mammals (Geiser et al. 1998; Swoap et al. 2007; Watanabe et al. 2006; Westman and Geiser 2004), have not been examined in fasting birds.

Fasting is known to induce a moderate increase in the concentration of corticosterone in several passerine species (Astheimer et al. 1992), and exogenous corticosterone increases food searching activity during fasting and the amount of food consumed after a 24-h fast (Astheimer et al. 1992; Wall and Cockrem 2009). Exogenous corticosterone also induces a metabolic shift that mimics the physiological state and increased motor activity attained during prolonged fasting in penguins (Spée et al. 2011). Whether corticosterone has a role in mediating the

physiological control of hypothermia during fasting is, however, not known (see Jenni-Eiermann and Jenni, Chap. 11).

Interestingly, a role of gastrointestinal filling in the regulation of body temperature has been shown in pigeons. Birds fed with noncaloric cellulose pellets after a 3-day fast increased their nocturnal T_b in direct proportion to amount of cellulose ingested (Reinertsen and Bech 1994). Similarly, in a restricted feeding regime, birds randomly receiving a daily meal of 0, 100, 150, 350, or 450 non-nutritive cellulose pellets or the same numbers of normal food pellets had a similar course of nocturnal T_b during the following scotophase. Hypothermia ensued with low pellet numbers and normothermia with high pellet numbers irrespective of whether the "food" had nutritive value or not (Rashotte and Geran 1997); if anything, T_b was higher after the cellulose treatment. As pigeons cannot digest cellulose, they were, in energetic terms, food deprived when receiving cellulose pellets but still remained normothermic. This result suggests that mechanical stimuli from the gastrointestinal tract (e.g. crop), alone, can affect the T_b set point. The more potent effect of cellulose may due to its swelling in the gut. Similarly, during refeeding after a fast, the body temperature of pigeons during the first night is higher than in ad libitum conditions (Laurila et al. 2005) due to the postprandial thermogenesis of compensatory overeating after the fast (McCue 2006). These data point to a mechanosensory input from the gastrointestinal tract to the thermoregulatory brain areas. This mechanism cannot, however, explain the continuing decrease of scotophase temperatures during prolonged fasting since the gastrointestinal tract typically empties within 24 h of food removal in pigeons.

10.3.3 Modulation of the Hypothermic Response

In many laboratory studies with prolonged fasting (e.g. 2–4 days), the hypothermic response has been repeatable, showing only minor adjustments to ambient conditions. Ambient temperature (21 vs. 4°C) did not modulate the hypothermic response in Japanese quail during a 4-day fast (Hohtola et al. 1991). In another study on quail (Ben-Hamo et al. 2010), hypothermia was slightly (ca 1°C), but significantly deeper on the third and fourth day of fasting in Japanese quail fasting at 13 versus 32°C. Similarly, a 2-day fast at ambient temperatures of 0, −10, and −25°C resulted only in minor differences in the hypothermic responses of pigeons during a 2-day fast in outdoor winter conditions: T_bs' of birds subject to ambient temperatures below 0°C decreased only by 1°C at the two colder temperatures (Laurila and Hohtola 2005, Fig. 10.3).

In Japanese quail the hypothermic response is constant during repeated fasting trials. Furthermore, the depth of hypothermia is not modulated by the duration of the scotophase ranging from 12 to 4 h (Laurila et al. 2005). Thus, quails were not able to compensate for the decreased duration of scotophase by increasing the depth of hypothermia and, consequently, lost more body mass during short scotophase. Instead, they showed a moderate decrease of T_b during the photophase

that started immediately after food removal. This adaptive strategy alleviated body mass loss to some extent in the shortest scotophase conditions and might be interpreted as (1) a weakening of the masking effect of light on hypothermia in diurnal birds (see Underwood et al. 1999) or (2) a conditioned response anticipating food deprivation. Whether such anticipatory hypothermia before actual depletion of body energy reserves really exists, has not been studied in detail. In one study, chickens were not able to anticipate repeated food deprivations by preemptive overeating after the conditioning signal (Petherick and Waddington 1991); unfortunately, T_b was not measured in that study. The rather small effect of ambient temperature on the level hypothermia in many species raises questions of the energetic significance of a response that is rigid in nature. A one degree drop in T_b in response to a 20°C decrease in ambient temperature results in a negligible decrease in the thermal gradient, which would incur very small energetic benefits (but see Sect. 10.5). This brings us to the other important aspect of energy conservation during fasting, namely that of thermal conductance.

10.4 Insulating against Starvation?

The hypothermic responses to starvation have received much more attention than changes in thermal insulation. This probably results from the basic assumption that resting birds use their total insulative capacity before resorting to thermogenesis or hypothermia (Irving and Krog 1954). Indeed, birds seem to maximize the thermal insulation of feathers by piloerection during sleep (Hohtola et al. 1980). Thus there would be minimal opportunity for further increasing feather insulation during starvation.

Many metabolic studies have shown that the inflection point of the temperature metabolism curve is not very sharp, which indicates overlapping insulative and thermogenic response to cold. In line with this idea, some studies suggest that birds are able to increase the efficiency of thermal insulation during starvation. A study using direct calorimetry revealed that pigeons fasting or maintained at 80% of the normal ration at an ambient temperature of 21°C had 50% lower dry thermal conductance than birds fed ad libitum (Phillips et al. 1991). Despite the shallow torpor induced, calculations showed that most of the energetic savings came from decreased heat loss. The reduced T_b was seen only during the scotophase, but reduced heat loss was seen throughout the 24-h cycle. Because the insulation through piloerection is probably maximal even in ad libitum fed resting birds (Hohtola et al. 1980) the reduced heat loss must be occurring elsewhere. One possible mechanism is regional heterothermy: constricting peripheral circulation in tissues beneath the immediate subcutaneous area more rigorously in the fasting state.

Extensive peripheral vasoconstriction should result in decreased peripheral tissue temperatures and less steep thermal gradient in tissues underlying the insulative layers of skin and feathers. This cardiovascular change would decrease heat loss even without a major change in actual surface temperatures. Such regional

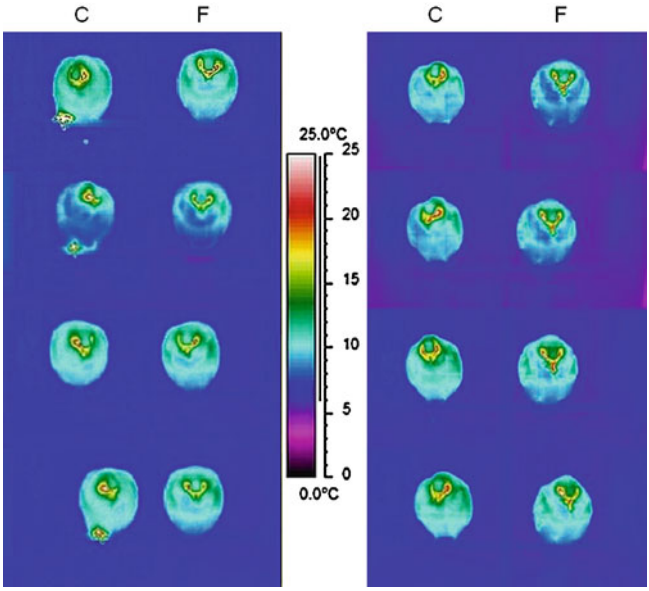

Fig. 10.4 Infra-red thermographs of the effects of fasting on surface temperatures in pigeons roosting on a perch in adjacent compartments in a cold room at 4°C. Each panel shows time-lapse images of a pair of control (C) and fasting (F) pigeons at approximately 1-h intervals. Control birds show a higher heat loss from the leg area either by exposing leg skin more frequently (*left panel*) or by using a perching posture that does not protect the legs as well as in fasting birds (*right panel*; Hohtola and Rintamäki unpublished)

heterothermy during starvation has, indeed, been shown in king penguin (*Aptenodytes patagonicus*) chicks (Eichhorn et al. 2011). Interestingly, fasting adult king penguins do not show regional heterothermy in air, but when they swimming or diving they do (Fahlman et al. 2005). As a consequence of such regional heterothermy, surface temperatures can also change in response to fasting. In one study, night-time leg skin temperature was 9–15°C lower in fasting pigeons than controls at an ambient temperature of 21°C (Ekimova 2005), suggesting an increased vasoconstriction and decreased surface temperatures over bare skin areas. Similarly, a preliminary study of surface temperatures by infrared imaging in fasting pigeons suggests that the surface temperatures and heat loss are lower especially in the legs during fasting (Hohtola and Rintamäki, unpublished, Fig. 10.4).

The changes in thermal conductance during fasting certainly need more study. Regional (peripheral) heterothermy may explain why calculations based on core T_b do not accurately predict energy savings. Energy can be saved even when maintaining almost normothermic T_b in the core because low peripheral tissue temperatures: (1) reduce heat loss and (2) reduces oxygen consumption due to the lower temperature itself ("Q_{10}-effect"). Indeed, in some studies, significant energy savings in fasting birds have been observed with no change core T_b (e.g. Boismenu et al. 1992). Typically, large birds do not show hypothermic responses, or show a

very small response, to starvation (McKechnie and Lovegrove 2002). Large birds tend to have greater starvation resistance (see Fig. 10.1) and thus may not be as dependent on hypometabolic adaptations as small birds; but regional heterothermy (reduced body core) maybe a "hidden" component of fasting resistance in these larger species. The possibility that "exceptional" species that do not exhibit fasting-induced torpor (Fahlman et al. 2005; Hohtola et al. 1994; McKechnie and Lovegrove 1999) are able to save energy through alterations in blood supply should be investigated.

10.5 Integration of Feeding, Fasting, and Thermoregulation

Thermoregulation is only one component among the physiological and behavioral array of mechanisms that birds use to survive starvation. Feeding, fasting, and thermoregulation interact in multiple ways. For example, when birds are fasting, they lack an important source of obligatory thermogenesis, namely the heat increment of feeding (see Secor 2009). For this reason, they must rely more on active thermogenesis by shivering, even when the shallow torpor decreases the need for overall heat production (Hohtola et al. 1998; Marjoniemi 2000; Rashotte et al. 1999). The magnitude of this increment also depends on food type (Kaseloo and Lovvorn 2003; Bech and Praesteng 2004). Several external factors influence the use of torpor, and a number of models have been developed to predict when a bird should use torpor to save energy (Grubb and Pravosudov 1994; Hainsworth et al. 1977; Welton et al. 2002). These models consider factors such as body mass, risk of predation, daylength, and social status of the bird. While body mass and daylength can easily be manipulated experimentally, predation risk as a factor is more difficult to study. Nevertheless, one study showed a small effect of predator presence on the hypothermia of pigeons fasting in aviary conditions (Laurila and Hohtola 2005).

The energetic benefits of torpor are easily modeled, but the ultimate liabilities are more difficult to calculate. The very fact that most birds use torpor only when their energy reserves are low indicates that hypothermia carries a cost. The relative energetic benefit of hypothermia (lowering of metabolic rate per unit of decrease in T_b) decreases with decreasing body mass (Chaplin et al. 1984). Yet starvation resistance is lower in small birds (Fig. 10.1) who must resort to torpor more frequently than larger birds. The energetic benefits can be increased by further lowering the body temperature during torpor. However, many studies show that ambient temperature has a rather small effect on T_b during hypothermia, especially in shallow torpor (e.g. Ben-Hamo et al. 2010; Hohtola et al. 1991; Laurila and Hohtola 2005). However, as explained above, the actual savings may not be predicted well by deep body temperature, as enhanced regional peripheral heterothermy may incur additional energy savings.

The physiological costs of hypothermia have not been studied in detail in birds. Hibernating mammals arouse for 2 days approximately every 2 weeks, and thus

compromise some of the energy savings of hibernation. Several hypothetical costs of torpor have been proposed to account for these bouts of arousal. As hibernators typically spend the arousal time sleeping as though they were sleep deprived and sleep-related disorders (sleep deprivation, memory loss) have been proposed as a factor explaining these arousals (Heller and Ruby 2004). The effects of avian deep torpor on sleep states have not been studied. In pigeons and penguins, shallow torpor during long-term fasting increases the amount of slow sleep (SWS), and decreases the amount, or changes the 24-h distribution of paradoxical sleep (Walker et al. 1983; Dewasmes et al. 1989; Rashotte et al. 1998). The role of SWS may be permissive, as birds showing the most SWS also showed the lowest T_b's during torpor (Walker et al. 1983). Other potential costs include metabolic imbalance, for example, respiratory acidosis (Jensen and Bech 1992).

One indication of the fact that torpor incurs costs is that birds avoid torpor if behavioral means to save energy are available. Singly housed mousebirds use torpor when energetically stressed, but in the wild they avoid torpor and roost huddled in groups (McKechnie and Lovegrove 2001b). Experiments in food-restricted pigeons in a fixed-ratio schedule (pigeons had to increase the number of key pecks to obtain food) in a cold ambient temperature (10°C) where birds could obtain warm air by breaking an infrared beam illustrates the intricate relationship between physiology and behavior (Ostheim 1992). With increasing workload for a fixed amount of food, birds first increase their work to avoid starvation. When the work load was increased further, body mass loss and nocturnal hypothermia ensued. Simultaneously, the birds started to modify the ambient temperature by obtaining warm air more frequently, thus behaviorally increasing their ambient temperature to 25°C.

10.6 Prospects

Avian torpor responses represent an important physiological adaptation against starvation. More basic studies are needed on the occurrence of torpor in various avian taxa and on the interspecific variation of torpor responses. For example, a clear difference in the level of torpor T_b is seen when comparing experiments made on two different quail stocks (Hohtola et al. 1991; Laurila et al. 2005). Is the hypothermic response as rigid as suggested by some experiments (Laurila et al. 2005), or is the response flexible with respect to season, age, etc.?

Finally, several studies suggest that the energetic savings are greater than predicted by the reduction of deep body temperature. More studies on the role of peripheral heterothermy are needed to shed light on how the energetic savings are obtained. The physiological costs of torpor in birds have not received much attention. The effect of repeated torpor periods on sleep homeostasis, metabolic balance, oxidative stress, and aging are promising avenues of research.

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Chapter 11
Fasting in Birds: General Patterns and the Special Case of Endurance Flight

Susanne Jenni-Eiermann and Lukas Jenni

11.1 Occurrence of Natural Fasting Periods in Birds

Birds are homeotherms with the highest basal metabolic rate and the highest metabolic rates attained during endurance exercise, but little scope to reduce metabolism by hypothermia or torpor (Schmidt-Nielsen 1984; McKechnie and Lovegrove 2002) (but see Hohtola, Chap. 10). Therefore, in comparison with other animals, we would not expect birds to fast for long periods. Surprisingly, however, birds undergo among the most extraordinary fasting events, for example, the 4–6 months fast of king penguin chicks (*Aptenodytes patagonicus*) during the subantarctic winter during which they lose 70% of body mass (Cherel and LeMaho1985) or the fast during more than 100 h of flapping flight by bar-tailed godwits (*Limosa lapponica)* migrating from Alaska to New Zealand across the Pacific ocean (Gill et al. 2009). In this review, we will show that it is their extraordinary ability to extensively store and use lipids as the main energy source that allows birds to fast for such long periods despite their high metabolic rates.

Birds go through periods of fasts and starvation which vary in length and predictability. Some periods of starvation occur unpredictably, for example, when bad weather events prevent food intake, and may lead to large-scale mortality. Although often unpredictable at short notice, birds generally adapt body energy stores to the prevailing risk of starvation in a trade off with the risk of predation (as a higher body mass hampers predator escape) and flight costs (e.g. MacLeod et al. 2005; van den Hout et al. 2010). The more predictable periods of starvation risk are, the more likely birds are adapted by accumulating appropriate amounts of energy stores in anticipation. For example, the regular fasts of day-active small passerines during long winter nights are buffered by finely tuned deposition of

S. Jenni-Eiermann (✉) · L. Jenni
Swiss Ornithological Institute, Seerose 1, 6204 Sempach, Switzerland
e-mail: susi.jenni@vogelwarte.ch

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energy stores (mostly lipids, but also protein) during the day (e.g. Swain 1992; Cresswell 1998). The increase and decrease of body energy stores for predictable fasting periods is usually part of the endogenous regulation of body mass and even occurs during *ad libitum* feeding in both songbirds and shorebirds (Berthold 1996; Piersma et al. 2008). Predictable fasting periods of several days to several months occur predominantly during three phases of the annual cycle: breeding, moult, and migration.

Breeding fasts typically occur when food is not available in the surroundings of the nest and/or when only one sex is attending the nest more or less continuously and is not fed by the partner. Typical examples are birds feeding over wide areas at sea far from the land-based breeding site, such as albatrosses, petrels, and penguins. During incubation, albatrosses and petrels go on foraging trips for up to 1 month over up to 9,900 km (3,500 km from the nest) while the partner incubates and fasts (Catard and Weimerskirch 1999; Waugh and Weimerskirch 2003). The most extraordinary breeding fast is performed by the emperor penguin *(Aptenodytes forsteri)* which breeds on Antarctic sea-ice. After mating and egg-laying the male takes over the single egg and incubates it during the Antarctic winter with ambient temperatures down to −50°C and wind speeds up to 200 km/h, a period of 115 days of fasting followed by a walk across the sea ice to the ocean of up to 200 km, resulting in a loss of body mass of 20 kg from 38 to 18 kg (Groscolas 1986). In smaller penguin species, the incubation fast is about 30 days and in the king penguin 54 days.

In waterfowl and galliforms, the female generally incubates the eggs alone and is not fed by its mate. Food intake during incubation is greatly reduced (e.g. chicken) or ceases altogether (common eider *Somateria mollissima*) (Mrosovsky and Sherry 1980; Parker and Holm 1990). In larger arctic breeding birds, body stores are also used to produce partly or entirely the eggs when food at arrival in spring is still lacking (Gauthier et al. 2003; Klaassen 2003). Typically, energy expenditure during breeding fasts is close to resting metabolic rate, but includes the energy needed to produce or incubate the eggs (Williams 1996). Male emperor penguins also produce a secretion by the oesophagus to feed the chick during the first days (Groscolas 1990).

Moult fasts occur when birds are so severely hampered by a massive loss of feathers (usually a simultaneous moult of body feathers or wing feathers) that they are unable to feed or to escape predators. Moult fasts occur predominantly in penguins which lose waterproofing and insulation and thus need to remain on land; in large species moulting can require a fast of up to 40 days (Groscolas and Cherel 1992; Williams et al. 1992; Cherel et al. 1994). Waterfowl and grebes with a simultaneous wing feather moult are flightless for up to 7 weeks and hence very vulnerable to predators. If food is scarce at safe moulting sites or if the risk of breaking growing feathers needs to be minimised, these birds have to rely at least partly on body energy stores; that some may stop feeding altogether is likely, but remains to be shown (e.g. Piersma 1988; Fox and King 2011). Compared to breeding fasts, moult fasts involve a higher metabolic rate because of feather synthesis and the decreased thermal insulation (Cherel et al. 1994).

Fasts during migration occur in almost all migratory bird species, but to widely different extents. Within an annual cycle migratory birds exploit geographically separated places, the breeding and the wintering grounds, and often staging areas in between. In some species this takes place on a global scale and involves the crossing of large inhospitable areas such as deserts and oceans. If at all, only a few species may feed while on migratory flight (e.g. swallows, swifts, seabirds). Hence, the length of migratory fasts depends on the duration of nonstop flights and on whether stopover sites provide food. Nonstop flights of several thousand kilometres are known from land birds and shorebirds crossing oceans (Gill et al. 2009). Most species crossing deserts cannot feed although they usually stop over during the day (Schmaljohann et al. 2007; Jenni-Eiermann et al. 2011).

Fasting during migratory flight is extraordinary in two respects (Jenni and Jenni-Eiermann 1998). First it is performed at a very high metabolic rate. In contrast to walking, the relationship between speed and energy expenditure during flapping flight is U-shaped. Therefore, birds cannot escape a certain level of power output, determined by the lowest point of the U-shaped power curve. But even at this minimum, the metabolic rate is about twice the maximum rate of exercising small mammals, thus among the highest in vertebrates (Butler and Woakes 1990). Moreover, this metabolic rate is maintained for hours (normally a whole night) and up to 100 h in certain species. Second, birds during endurance flight not only fast, but also do not drink, thus have to rely exclusively on body energy stores and on body and metabolic water (see also Bauchinger and McWilliams, Chap. 12).

11.2 General Features of Fasting in Birds

The metabolism during fasting has been extensively studied in resting birds in the lab (e.g. domestic goose, quail), as well as in wild birds fasting spontaneously during part of their annual cycle (e.g. emperor and king penguins) (e.g. LeMaho et al. 1981; Groscolas 1990, Sartori et al. 1995; reviewed in Cherel et al. 1988a). Similar to mammals, long-term fasting birds show three phases of fasting (Fig. 11.1), characterised by the composition of fuel types used and concomitant changes in body mass loss, energy expenditure, blood metabolites, and circulating hormones (summarised in Cherel et al. 1988a).

Phase I is an initial transient phase of adaptation to long-term fasting with a high rate of body mass loss. Basal metabolic rate is reduced (in penguins this occurs through a decrease in peripheral body temperature, Groscolas 1986) and the proportion of protein and carbohydrates on total energy expenditure decreases while fat stores are increasingly mobilised. Phase I lasts a few hours to several days depending on the species.

During phase II of fasting the metabolism is in a steady state. The specific rate of body mass loss and the specific metabolic rate is constant and low. A maximum of energy used is derived from lipids and a minimum proportion from protein while carbohydrates are negligible. Consequently, plasma concentrations of free

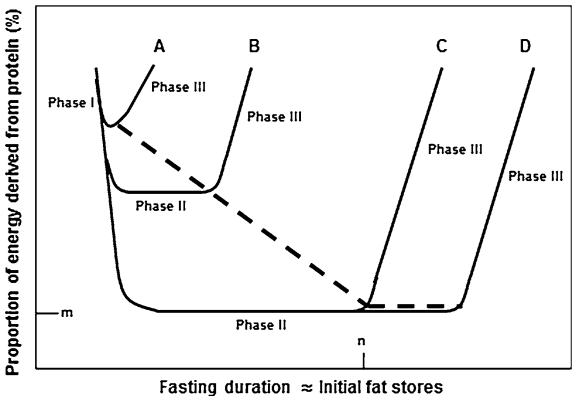

Fig. 11.1 The proportion of energy derived from protein (RPC, in %) as a function of fasting duration (after Cherel and Groscolas 1999). Four hypothetical animals with different initial fat content are shown. Animal A has fat stores below the threshold adiposity and fasts with increasing RPC (phase III only). Animal B has medium initial fat stores, shows the three phases of fasting I, II and III, and an intermediate level of RPC during phase II. Animal C with high initial fat stores (n) is able to fast longer and has reached the minimum RPC (m). Animal D with even higher fat stores fasts even longer, but cannot reduce RPC further. For animals with even higher fat stores which miss phase III, see Cherel and Groscolas (1999). The broken line, connecting the ends of phase II, shows the dependence of RPC on initial fat stores which primarily determine fasting duration. (From Jenni-Eiermann and Jenni 2003)

fatty acids and β-hydroxybutyrate are high, while nitrogen excretion and plasma uric acid levels are low. The proportions of energy derived from protein and lipids depend on the initial amount of lipids (similarly as in mammals). Initially fat birds can lower the proportion of protein used down to 4% (LeMaho et al. 1981; Groscolas 1982; Groscolas 1990), while initially moderately fat birds have a higher proportion of energy derived from protein (Figs. 11.1, 11.2). The duration of phase II depends primarily on the amount of stored lipids, and obviously phase II does not exist in fasting lean birds.

Phase II ends spontaneously when lipid stores drop to a certain level (threshold adiposity), but are not exhausted (Groscolas 1990; Robin et al. 1998). The fasting bird enters phase III of fasting which is characterised by a metabolic and endocrine shift and an increase in body mass loss. Protein catabolism increases dramatically (with consequently increasing plasma uric acid levels) and bone marrow fat starts to be catabolised (Thouzeau et al. 1997; see also Ullrey, Chap. 18). Often, birds change behaviour. Resting fasting birds usually restart foraging activity and may desert their brood (Cherel et al. 1988a). The length of phase III varies between species: about 1 week in chicken (Cherel et al. 1988a), but 2–3 weeks in emperor and king penguins and can be used to walk up to 200 km across the ice to the ocean. Birds starving to death finally die from the loss of protein.

As a side effect, the distinction of the three phases also serves to distinguish between body energy stores (which are accumulated in advance of periods of

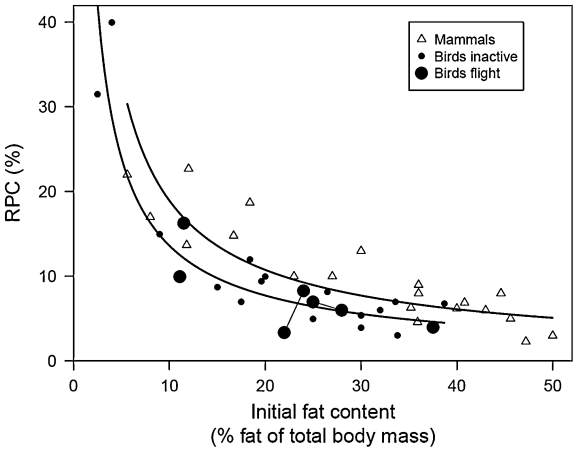

Fig. 11.2 Contribution of energy derived from protein as a percentage of total energy expenditure (RPC) in relation to initial fat content in fasting birds and mammals according to literature data (in long-term fasting birds the values given for phase II of fasting were used). *Open triangles*: inactive fasting mammals ($n = 20$). *Small dots*: inactive fasting birds ($n = 17$). *Large dots*: birds during endurance flight ($n = 7$; lines connect values of male and female bar-tailed godwits and greater snow geese, respectively). Based on data compilation in Jenni and Jenni-Eiermann (1998); Battley et al. (2001), and Klaassen et al. (2000). *Regression lines*: In a general linear model, mammals have a significantly higher intercept (by 0.33 (± 0.10 SE) on the ln-scale; $P = 0.002$) than the two groups of birds which are statistically indistinguishable ($P = 0.5$). The regression line for all birds is ln (RPC) = 4.49 (± 0.24) – 0.82 (± 0.08) ln(fat) and for mammals ln (RPC) = 4.82 (± 0.34) – 0.82 (± 0.08) ln(fat)

shortage and used up during phase I and II of fasting), and body energy reserves (which constitute tissues necessary to support daily functions and can be used in emergencies, i.e. during phase III of fasting) (van der Meer and Piersma 1994).

During long-term fasts lipolysis seems to be regulated by glucagon which induces lipolysis in avian adipocytes (Bernard et al. 2003). In king penguins glucagon increased continuously during phase II and even more sharply during phase III (Cherel et al. 1988b). Protein catabolism in fasting king penguins seems to be mainly regulated by corticosterone. Corticosterone with its catabolic effect on protein is low during the protein sparing phase II but it increases sharply during phase III (Cherel et al. 1988b). Insulin remains low during all three phases of fasting. This indicates that low insulin levels may be a prerequisite for net protein breakdown during starvation, but have no modulating effect on the level of proteolysis in phase II and III. The thyroid hormones T_3 and T_4, known for catabolic actions in hyperthyroid states in mammals, also seem not to play a major role in fasting king penguins. Their concentrations are maintained at low levels after 10 days of fasting before they decrease during phase II (T_3) and phase III (T_4) (Cherel et al. 1988b and literature therein). The thyroid hormones have also been implicated in the regulation of the metabolic rate, i.e. they reduce the metabolic rate thereby preserving fuel stores. However, whether T_3 (chicken) or T_4 (king penguin) is the main hormone controlling

Table 11.1 Energy and water yield of the three main fuel types in birds

	Glycogen	Lipids in adipose tissue	Protein in skeletal muscle or fat-free mass
Energy density (kJ g^{-1}) dry matter	17.5	39.6	17.8
Water content (%)	75–80	5	70
Energy density (kJ g^{-1}) wet mass	3.5–4.4	37.6	5.3
Moles high-energy phosphate produced per g dry mass	0.235	0.51	0.204
Moles high-energy phosphate produced per g wet mass	0.047–0.059	0.485	0.061
Metabolic water production (g H_2O per g dry matter)	0.556	1.05	0.39
Total water production (g H_2O per g wet tissue)	0.89–0.91	1.10	0.82
Water produced (g H_2O per kJ expended from wet mass)	0.21–0.25	0.029	0.155
Water produced (g H_2O per Moles high-energy phosphate expended from wet mass)	15.1–19.4	2.27	13.4

High-energy phosphate (ATP) yields were calculated using fatty acid and amino acid composition of birds (not taking into account transport across cells membranes)
For details and sources see (Jenni and Jenni-Eiermann 1999).
From (Jenni and Jenni-Eiermann 1998)

basal metabolic rate during fasting is not clear and might be species dependent (Decuypere et al. 1985; Williamson et al. 1985; Cherel et al. 1988b).

In summary, a long fasting duration is achieved in resting birds by (1) a reduction of energy expenditure and (2) a high proportion of energy derived from stored lipids, depending on initial adiposity. Energy expenditure during phase II and III is reduced by a reduction of basal metabolic rate during phase I (e.g. LeMaho et al. 1981; Cherel et al. 1988a) and by reducing locomotor activity, searching for protection against wind and cold (e.g. by huddling or staying at protected places) and, in small birds, by reducing the core temperature, entering nocturnal hypothermia (e.g. *Parus major*) or getting even torpid (e.g. *P. montanus*) (Cherel et al. 1988a; Reinertsen 1996) (see also Hohtola, Chap. 10). The main fuel during long-term fasting is fat stored in anticipation, although protein may also be important in anticipation of a moult fast (Cherel et al. 1993). Fat is advantageous over protein and carbohydrates because of its caloric density (Table 11.1) and because it can be catabolised—in contrast to protein—without the loss of function or structures (see 11.3.1). A minimum amount of protein, however, needs to be catabolised and is drawn from various organs in various proportions (e.g. Groscolas 1990). Remarkably, birds obtain a significantly greater proportion of energy from lipids during phase II of fasting than mammals and, consequently spare protein more efficiently (Fig. 11.2).

11.3 Fasting During Migratory Flight

11.3.1 Types of Fuel and Optimal Fuel Composition

Here we evaluate the three main types of oxidative fuels (i.e. carbohydrates, lipids, and proteins) with respect to the particular demands of avian endurance flight. The power needed to carry additional weight in flight is much higher than when walking or swimming (Schmidt-Nielsen 1984). Hence, it is of paramount importance that the energy density of stored fuel is high. Therefore, lipids should certainly be the optimal fuel during flight. The energy density of stored lipids is more than seven times higher than that of glycogen and protein. In terms of high energy phosphate (e.g. ATP), fat from adipose tissue yields eight times more chemical energy than wet protein, and 8.2–10.3 times more than glycogen (Table 11.1). This is chiefly because fat stored in adipose tissue contains only about 5% water (Piersma and Lindström 1997), compared with 70% or more for muscle tissue or stored glycogen. Another advantage of adipose tissue is its comparatively low maintenance costs (e.g. Scott and Evans 1992).

However, there are a number of shortcomings of lipids as a fuel during flight. Lipids are not readily available at the onset of flight, but need some time to be mobilised from adipose tissue (muscular lipids are low). Free fatty acids with their low aqueous solubility need to be transported by soluble protein carriers at every step from adipose tissue to the mitochondria of the flight muscles, a fact which appears rate limiting to fatty acid oxidation in mammals (Weber 1992; McWilliams et al. 2004). Finally, fatty acids cannot cover all of the needs (e.g. glucose for the central nervous system) and therefore a minimum amount of requirements need to be met by glucose (either derived from glycogen stores or via gluconeogenesis from amino acids or glycerol) and protein. Also intermediates of the citric acid cycle, needed to oxidise fatty acids, are constantly drained away and need to be replaced from carbohydrates or certain amino acids (anaplerotic flux; Dohm 1986; Sahlin et al. 1990). Therefore, the energy supplied by lipids needs to be complemented by an inevitable minimum amount of protein or carbohydrates.

Carbohydrates, stored mainly in the form of glycogen in the liver and skeletal muscles, can be readily mobilised and may contribute substantially to energy expenditure at the onset of flight before lipids are fully available (Rothe et al. 1987; Schwilch et al. 1996; Gannes et al. 2001; Jenni-Eiermann et al. 2002a). However, the amount of glycogen stored by birds preparing for migration is very small (e.g. Marsh 1983) and can quantitatively not serve as a fuel complementing lipids during endurance flight.

Proteins have no special storage form; apart from a small free amino acid pool, all proteins serve specific functions in the body. Therefore, an appreciable amount of protein catabolism inevitably results in functional loss. Proteins are catabolised as a complement to lipids for various reasons (Jenni and Jenni-Eiermann 1998): to supplement endogenous protein turnover and repair (there is moderate flight-induced muscle damage; Guglielmo et al. 2001), to provide gluconeogenic

precursors, to refill citric acid cycle intermediates (anaplerotic flux) for oxidation of fatty acids, and to provide water (see also Bauchinger and McWilliams, Chap. 12). Finally by catabolizing flight muscles during flight, they are constantly adapted to the decreasing body mass and energy needs as fuel stores are depleted (Pennycuick 1998).

The optimal composition of fuel types for migratory endurance flight appears therefore to be a maximum of lipids and a minimum of protein; glycogen does not play a critical role. The question is how well can birds, during their strenuous endurance flight, maximise lipid use and minimise protein catabolism. Mammals (including humans) during strenuous endurance exercise cannot rely on lipids to a large extent. During endurance exercise, the relative contribution of energy derived from fat to total energy expenditure is much lower than when fasting and decreases when energy expenditure approaches its maximum (VO_2max) (Roberts et al. 1996; Guglielmo 2010), for example, 40–50% in marathon runners (Callow et al. 1986); only when walking do exogenous fatty acids provide most of the energy (Roberts et al. 1996). Moreover, mammals run on carbohydrates and fat as major fuels, with negligible amounts of protein (Roberts et al. 1996). Surprisingly, endurance flight of birds is fuelled with the same low contribution of energy derived from protein (i.e. about 5% in fat birds) and the same high contribution of energy derived from lipids (about 95%) as during fasting at rest (Fig. 11.2; see also Vaillancourt et al. 2005 for running birds). This pattern is particularly interesting since endurance flight is performed at 60–85% VO_2max, or more when birds are loaded with fuel (Guglielmo et al. 2002). Therefore, endurance flight of birds is fuelled very differently from mammalian running.

There are two potential reasons for not maximizing fat utilisation in birds. The first is that maximizing the contribution of energy derived from fat entails initial costs when setting up the machinery of fatty acid transport and oxidation (see 11.3.2.1). Therefore, for short flights there is probably no advantage to tune the metabolism to a maximum of lipid utilisation. A substantial contribution of protein or glycogen might be the cheaper solution. Second, compared with lipids the catabolism of isocaloric amounts of protein yields about 5.9 times more water (Table 11.1). Therefore, birds flying in conditions of excessive water loss may alleviate water stress by increasing the contribution of energy derived from protein (see 11.3.4). Indeed, fasting birds at rest have been shown to burn more protein when water stressed (Gerson and Guglielmo 2010).

In summary, it is expected that the optimum ratio of fat to protein stored before, and used during, migratory flight primarily depends on the migration strategy (in particular on the length of non-stop flights, i.e. initial fat stores) and the risk of dehydration during flight. The higher the need to carry large fuel stores and the lower the risk of dehydration, the better should the organism be tuned for high lipid utilisation.

11.3.2 Preparation for Fasting During Endurance Flight

11.3.2.1 Fuelling and Fuel Types

Preparations for migratory flights obviously include the storage of an appropriate mixture of fuels. This may occur at the onset of migration and *en route* at stopover sites. According to what is expected from the optimal ratio of fat and protein (see 11.3.1), fat is the main fuel accumulated. Lipids are sequestered mainly as triglycerides in adipose tissues located in several regions of the body and only in small quantities in muscles and other organs (Weber et al. 1996a, b). To support the minimum proportion of energy derived from protein of about 5%, the proportions of fuel deposited in anticipation in terms of wet mass must be 29.5% protein and 70.5% fat (calculated from Table 11.1). Indeed, birds also increase in lean mass and body mass gain consists of about 65–80% lipids (Lindström and Piersma 1993; Klaassen et al. 1997; Battley et al. 2000). Lipids and protein may be deposited in different proportions during early and late phases of fuelling (e.g. Jenni and Jenni-Eiermann 1987; Carpenter et al. 1993; Guglielmo and Williams 2003; Atkinson et al. 2007).

Fuel stores increase not only the power needed for flight, but also maintenance costs and predation risk through impaired flight capabilities (Alerstam and Lindström 1990; Kullberg et al. 1996). Therefore most birds accumulate only small fat deposits (<25% of lean body mass) and refuel at several successive stopover sites (Alerstam and Lindström 1990; Schaub and Jenni 2000). However, when they have to cross extended ecological barriers (sea or deserts), migrants deposit extensive fuel loads and may even double their body mass (Alerstam and Lindström 1990; Fransson et al. 2008). The amount of fat stored may equal or slightly exceed lean wet body mass (e.g. Piersma and Gill 1998; Bairlein 2002).

Fuel deposition is achieved by hyperphagia (an up to 40% increase in food intake, Bairlein 2002), an improved food assimilation efficiency (possibly due to an increase of the intestine mass; Bairlein 2002), and a decrease in basal metabolic rate through hypothermia (Butler and Woakes 2001; Wojciechowski and Pinshow 2009).

Fat deposition may be further optimised by selecting an appropriate diet. Insectivorous birds would have to synthesise fat from protein. However, as shown in chickens and Japanese quail, a diet with a high proportion of protein hampers fat deposition due to a decrease in lipogenic enzyme activity. In contrast a low-protein diet (but still meeting minimum protein requirements) results in fat birds (Kirkpinar and Oğuz 1995; Rosebrough and McMurtry 1993; Bairlein 2002; Jenni-Eiermann and Jenni 2003). Hence insectivorous migrants would benefit from switching to a low-protein diet to facilitate lipogenesis, particularly if this prey is abundant and easy to catch. Indeed many insectivores switch to a fruit diet or to aphids which have comparatively low protein content, are immobile and abundant in autumn in many places (Jenni-Eiermann and Jenni 2003). A change in diet inevitably would also require a change in the digestive system (Starck 1999). Moreover, fruits are selected on their fat content and secondary plant compounds

and migrants prefer diets with certain fatty acids (reviewed in Bairlein 2002; McWilliams et al. 2004) which may maximise performance (see 11.3.3.1).

Preparations for endurance flight also include an upgrading of the transport system of fatty acids to the flight muscles and their oxidative capacity (Lundgren and Kiessling 1985; Driedzic et al. 1993; Guglielmo et al. 2002; McFarlan et al. 2009). In species with exceptionally long nonstop flights the gut is reduced shortly before departure, apparently to reduce the organs that are not required to sustain flight (Piersma and Gill 1998; Piersma et al. 1999; Landys-Ciannelli et al. 2003).

11.3.2.2 Hormonal Regulation During the Migratory Season

The hormonal regulation of preparation for migration is still poorly known. Corticosterone is most probably the best studied metabolic hormone in birds during migration. It is the most prevalent glucocorticoid in birds and is thought to be involved in the regulation of bird migration at the metabolic and behavioural level (Wingfield 2003). We need to distinguish between three different possible actions of circulating corticosterone during migration: (a) the effect on transitions between life-cycle stages (e.g. between breeding, moult, and migration), (b) the effect during refuelling, and (c) the effect during endurance flight (for (c) see 11.3.3.3). Regarding (a), elevated levels of baseline corticosterone have been found in several bird species during the migration period (Holberton et al. 1996; Romero et al. 1997; Holberton 1999; Piersma et al. 2000; Landys et al. 2004a, b; Long and Holberton 2004) and during breeding (reviewed in Romero 2002), suggesting that this hormone may facilitate activities of high energy demand. However, corticosterone levels have not been found to be consistently elevated in both migratory seasons (Romero et al. 1997). Regarding the effect of corticosterone during refuelling (b), when birds replenish their energy stores en route, moderately elevated baseline corticosterone levels have been linked with fat deposition during stopovers (Piersma et al. 2000; Long and Holberton 2004), because corticosterone has a permissive effect on food intake and might alter the responsiveness of other hormones or neurotransmitters, that stimulate food intake (e.g. Landys et al. 2004c). When corticosterone receptors are blocked centrally or peripherally, birds are not able to increase food intake (Landys et al. 2004c), and when circulating endogenous corticosterone levels are experimentally kept low in the periphery, birds are unable to fatten (Holberton et al. 2008). It is therefore suggested that corticosterone promotes hyperphagia and lipogenesis (Holberton et al. 2008). The effect of corticosterone on fat deposition might occur by directly regulating the major enzymes involved with lipid synthesis and fat storage or indirectly via metabolic hormones (reviewed in Holberton et al. 2008).

Other hormones possibly implicated in the regulation of food intake and lipogenesis in migratory birds are less well studied. Prolactin was shown to increase in photo-stimulated dark-eyed juncos together with corticosterone and body mass, but a direct link with the preparations for migration is lacking (Holberton et al. 2008 and literature therein). Thyroid hormones correlated with

body mass of migratory birds in some studies. However, the results are equivocal, showing either a correlation with T_3 (Pant and Chandola-Saklani 1993) or T_4 (Jenni-Eiermann et al. 2002b) or no correlation of T_4 with body mass (Silverin et al. 1989).

11.3.3 Fasting During Endurance Flight

11.3.3.1 Adequate Energy Supply During Flight

During endurance flapping flight, the metabolism is greatly increased to meet the high energetic costs of flight which are 8–18 times higher than basal metabolic rate for flapping flyers (lower in aerial feeders and seabirds; Videler 2005). On the other hand, a small reduction of flight costs may be obtained by hypothermia (Battley et al. 2001a; Butler and Woakes 2001).

In birds, negligible amounts of fuel are stored within the working muscles, in contrast to mammals which derive most of lipids and glycogen from stores within muscle cells (Weber et al. 1996a, b). Therefore, bird flight is fuelled via the circulatory system from stores outside the flight muscles. However, at the onset of flight, when extramuscular lipids and protein are not yet available, intramuscular and hepatic carbohydrates are mainly used, while fatty acids from adipose tissues reach their steady state contribution after about 1–2 h of flight and proteins after 4–5 h (Rothe et al. 1987; Schwilch et al. 1996; Jenni-Eiermann et al. 2002a).

Fatty acid supply may be constrained by the enzymatic system of mobilisation, proteins transporting fatty acids in the blood stream and cytoplasm, and translocation across cell membranes (Weber 1992). Many aspects of fuel supply to the working muscles have not yet been studied in birds. However, several avian adaptations are known. In general, the supply of fatty acids is not constrained by adipocyte mobilisation or by mitochondrial oxidation capacity, but rather by perfusion limitations in adipose tissue, circulatory transport, and sarcolemmal uptake (Vock et al. 1996; McWilliams et al. 2004). As these constraints limit lipid use in mammals, birds seem to have evolved special mechanisms to overcome these limitations.

Triglycerides stored in adipocytes need to be hydrolysed into free fatty acids and glycerol, to be released into the blood. The rate of lipolysis in adipose tissue apparently does not limit the supply of fatty acids, since a large fraction of the fatty acids does not even leave the adipocyte and is reesterified (Wolfe et al. 1990). Circulatory transport capacity seems to be elevated in birds during endurance flight by several means. Whether an increase in heart size (as shown in several species; Piersma et al. 1996, 1999; Guglielmo and Williams 2003), which may increase cardiac output, facilitates fatty acid transport remains to be shown. The flight muscles of long-distance migrants have a higher capillary density than those of partial migrants and sedentary species and are thus well prepared for maximal supply of oxygen and fuel (Lundgren and Kiessling 1988; Maillet and Weber 2007).

Free fatty acids are insoluble in the blood and are thus transported bound to albumin. It is unlikely that albumin concentration can be increased, because of limitations set by blood viscosity and plasma osmotic pressure. Indeed, plasma albumin decreased during flight in pigeons (George and John 1993).

In small passerines, we were surprised to observe elevated concentrations of triglycerides bound in very low density lipoproteins VLDL (Jenni-Eiermann and Jenni 1992). We suggested that apart from flight muscles, fatty acids would also be taken up by the liver with its high lipid processing capacity. This would enable plasma albumin to transport more free fatty acids per unit time. Fatty acids taken up by the liver would be reesterified and released into the plasma in VLDL, a metabolic pathway known from other physiological contexts. This conversion would allow fluxes of large amounts of fatty acids to the flight muscles without a large burden on the plasma and would circumvent the limitations set by albumin to fatty acid transport. However, elevated triglycerides and VLDL have not been observed in other species, sampled under experimental flight conditions (Schwilch et al. 1996; Jenni-Eiermann et al. 2002a; Pierce et al. 2005). It would be important to measure flux, rather than plasma concentrations, and to investigate birds under natural conditions.

The supply of fatty acids may also be constrained by other steps in the transport system, for example, into the cell and across the cell membranes (Weber 1992). In birds, high concentrations of heart-type fatty acid binding protein on the membranes of the muscles (FAT/CD36 and FABPpm) and in the cytosol (H-FABP) were found and upregulated during migratory seasons (Guglielmo et al. 1998, 2002; Pelsers et al. 1999; McFarlan et al. 2009). In the flight muscle of a typical migrant (*Calidris mauri*) the H-FABP concentration was about 10-fold greater than in mammalian muscles and 70% more abundant during the migratory season (Guglielmo et al. 1998, 2002; Guglielmo 2010).

During flight, fatty acids are oxidised by the upregulated enzyme system (CAT, β-oxidation enzymes such as hydroxyacyl-CoA-dehydrogenase HOAD, citrate synthase, cytochrome oxidase; McWilliams et al. 2004; Maillet and Weber 2007). Fatty acid chain length, degree of unsaturation, and placement of double bonds can affect the rate of utilisation of fatty acids from adipose tissue, utilisation by muscles and possibly performance (Price 2010, see also Price and Valencak, Chap. 15).

11.3.3.2 Three Phases of Fasting During Flight?

The question is whether fasting during endurance flight, although metabolically and energetically very demanding (see 11.3.3.1), is governed by the same metabolic shifts as in resting birds and mammals, i.e. whether the three phases of fasting (see 11.2) can also be discerned during endurance flight. Many findings are in favour of such a view.

During an initial phase, birds change from a glycogen-based take-off phase to a steady state fuelled mainly by lipids (Rothe et al. 1987; Schwilch et al. 1996; Gannes et al. 2001; Jenni-Eiermann et al. 2002a). This transition period is shorter when birds have been fasted before the onset of flight (Gannes et al. 2001; Rothe et al. 1987).

Using the plasma concentration of uric acid as an indicator of body protein breakdown, it appears that protein catabolism is comparatively low after the initial phase of transition (Jenni et al. 2000). However, while resting birds reduce protein catabolism, flying birds increase body protein catabolism well over levels before flight or during fasting at rest. Although flying birds have a metabolic rate 8–18 times higher than fasting birds at rest, the proportion of energy derived from protein remains unchanged (Fig. 11.2); it follows that the absolute amount of protein catabolised must increase roughly in proportion to metabolic rate (Jenni and Jenni-Eiermann 1998). This indicates that the main reason for protein catabolism during flight is linked with metabolic rate and is probably the refill of citric acid cycle intermediates, which probably is proportional to metabolic rate (Jenni and Jenni-Eiermann 1998).

As in inactively fasting birds and mammals, the contribution of energy derived from protein of birds during endurance flight also seems to depend on the initial amount of fat stores (Fig. 11.2). It appears that only birds with an initial fat content of about 20% or more can attain a very low protein catabolism of about 5%. Birds during endurance flight apparently also increase protein catabolism at a threshold adiposity, as indicated by plasma uric acid levels (Jenni et al. 2000) and body composition analyses (Schwilch et al. 2002a). Together with increasing circulating corticosterone, this transition resembles the onset of phase III of fasting in inactive fasting birds (but see Battley et al. 2001b). In small passerines, phase III of fasting sets in when fat stores drop below 5–10% of total dry mass (Schwilch et al. 2002a).

Protein is derived in different proportions from the various organs (e.g. Battley et al. 2000; see also Bauchinger and McWilliams, Chap. 12). It appears that in passerines the minimum protein catabolism during phase II of fasting can be met by an adaptive reduction of mainly the flight muscles, while nutritional organs are also reduced, but spared in relative terms. During phase III of fasting, the greatly increased protein catabolism reduces the protein mass of all organs, particularly the nutritional organs, and is probably not adaptive with respect to flight capabilities, but to whole-body metabolism when fat stores are nearing depletion (Schwilch et al. 2002a). The recently advanced hypothesis of tissue-specific protein degradation (Bauchinger and McWilliams 2010) cannot explain the overall increase in protein breakdown during flight and may not explain shifts in the proportions derived from various organs from phase II to III of fasting.

11.3.3.3 Regulation of Fuel Catabolism: The Role of Corticosterone

Apart from effects discussed above (see 11.3.2.2), corticosterone may be involved in the regulation of the metabolism during flight and the composition of fuel types used (Jenni et al. 2000). Elevated levels of corticosterone play a key role in promoting gluconeogenesis from amino acids and, thus, increase breakdown of muscle protein (Kettelhut et al. 1988; Gwinner et al. 1992). Studies in free-living small passerines caught during migratory flight (Falsone et al. 2009), in homing pigeons after a 180 km flight (Haase et al. 1986), and in shorebirds just

after landing from a long spring migratory flight (Reneerkens et al. 2002; Landys-Cianelli et al. 2002) showed slightly increased, intermediate corticosterone levels in comparison with resting birds, supporting the hypothesis of an upregulation of corticosterone as a response to increased energetic demands. However, in laboratory studies results were equivocal showing either slightly increased corticosterone levels (ducks and pigeons exercising in a treadmill; Harvey and Phillips 1982; Rees and Harvey 1987) or unchanged levels (pigeons electrically stimulated for 2 h; John and George 1973; red knots flying in a wind tunnel for 2 and 10 h respectively; Jenni-Eiermann et al. 2009). Whether the latter results might be explained by the experimental situation remains to be shown. In any case corticosterone, if anything, increases only slightly during flight.

When fat depots are nearly depleted and birds during endurance flight enter phase III of fasting, plasma corticosterone rises to very high concentrations (Gwinner et al. 1992; Jenni et al. 2000); similar to those attained as a response to an acute stressor. In this case, corticosterone seems to trigger protein catabolism as suggested by the emaciated breast muscles and the high uric acid levels in those individuals (Gwinner et al. 1992; Jenni et al. 2000).

11.3.4 Limitations of Fasting During Endurance Flight

Fasting during endurance flight may be limited (a) by exhaustion of body stores or (b) by adverse effects incurred during endurance flight. In terms of body stores, flight range may be limited by the exhaustion of fat, or by reaching a fatal threshold in protein or body water. Apart from a very small amount of structural lipids, virtually all lipids can be catabolised. While the amount of lipids largely determines the overall duration of fasting, proteins are the only fuel type at the end of a fasting period, and hence finally limit flight duration. Alternatively, water may be limiting (Carmi et al. 1992; Klaassen 1996). Birds during flight have been estimated to be sensitive to dehydration in ambient temperatures above about 20°C (Giladi and Pinshow 1999; Engel et al. 2006). However, birds flying readily over the Sahara under quite unexpected ambient conditions (mean 30°C, 27% relative humidity) show that current estimates must be refined (Schmaljohann et al. 2008). Birds incurring dehydration during flight may alleviate or compensate water loss by shifting the composition of fuel types from lipids to more protein which produces 5.9 times more water than an isocaloric amount of lipids (Jenni and Jenni-Eiermann 1998). Recently, an increase in protein catabolism has indeed been demonstrated in water-restricted house sparrows (Gerson and Guglielmo 2010). Migrants caught in the Sahara with emaciated flight muscles and ample lipid stores also hint on such a mechanism.

Presently, there is only little information about negative effects incurred during endurance flight such as fatigue (apparently not reported in the literature), sleep deprivation (see Schwilch et al. 2002b), muscle damage (only little; Guglielmo et al. 2001), suppression of immunity (none found; Hasselquist et al. 2007),

excessive stress hormone levels (none found in healthy birds; Hasselquist et al. 2007; Falsone et al. 2009), or elevated oxidative stress (Costantini et al. 2007).

11.3.5 Recovery after an Endurance Flight Fast

After endurance flight the metabolism has to change from a fasting highly active state to a fasting resting state. At the same time the metabolism of a migratory bird has to be prepared for the possibility that the fasting state will proceed if no food is available, or for burst and take-off flights to escape predators or to hunt mobile prey, and the next migratory flight, independent of whether the birds was able to feed.

If there is no food available, migratory birds during postflight recovery should save the remaining body energy stores as much as possible and refill (if necessary) muscle glycogen for emergency burst flights, because these are fuelled via anaerobic catabolism of muscular and hepatic glycogen (George and Berger 1966; Rothe et al. 1987). Birds uniquely seem to have evolved a delayed postexercise ketosis to strongly reduce proteolysis and spare glycogen (Jenni-Eiermann and Jenni 2001).

If food is available, a migrant should be able to forage and to digest food as efficiently as possible to shorten stopover duration. However, while the loss of lipids during endurance flight has no other effect than a decrease in energy store, the degradation of proteins comes along with the loss of functions or structures which may also compromise the digestive system when refuelling (see Lignot, Chap. 14).

11.4 Perspectives

The outstanding feature of long-term fasting of birds is their ability to derive the largest part of the required energy (up to about 95%) from fat and only a minimum amount from protein. This strategy enables them not only to survive several months without structural or functional damage, it even allows birds to fast during the energetically demanding tasks of breeding, moulting, or flying and therewith to exploit the most extreme habitats.

From a physiological point of view the ability of birds to fast on such a high proportion of fat offers an exciting model to study the entire chain from building up to breaking down fat depots in a short and efficient way. There are still many understudied aspects. For instance, the role of glycogen in endurance flight has up to now been widely regarded as negligible, although more detailed studies across several species with different migration strategies are missing to our knowledge. The efficient transport of fatty acids from adipose tissue to mitochondria of the flight muscles, crucial to the high lipid consumption in fasting birds, likely will reveal further exciting adaptations specific to birds. The hormonal regulation of fasting birds needs further studies (Hohtola, Chap. 10), particularly about hormones that regulate metabolism.

Very little is known about effects of endurance flight on fatigue, muscle damage, sleep, immunity, and oxidative stress. Also the peculiar postexercise ketosis after flight needs further investigation. There are a number of very interesting trade offs which we are only beginning to discern, such as the trade-off between maximum fat catabolism and upgrading of the metabolic system, or the trade off between oxygen transport (proportion of red blood cells, haematocrit) and fuel transport (proportion of plasma and albumin concentration) in the blood, both constrained by blood viscosity. The outcome of such trade offs likely are linked to the migration strategy of a bird. Although studies with flying birds in the lab (wind tunnel) or substituting flight with another activity certainly will provide crucial and indispensable results, we think that it is important to verify as many of these aspects in free living and free-flying birds. New noninvasive techniques promise exciting insights from birds in the real life.

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Chapter 12
Tissue-Specific Mass Changes During Fasting: The Protein Turnover Hypothesis

Ulf Bauchinger and Scott R. McWilliams

12.1 Introduction

Facing a period without food intake is common among animals; fasting occurs in a variety of different contexts and it often varies in duration and in the extent to which metabolic and behavioral activity is suppressed during the fasting period (for review see McCue 2010). When the duration of time without food exceeds the time required for digestion and absorption of the previously ingested food, then the animal's biochemical requirements for subsequent activity and physiological processes have to be satisfied from body resources instead of food (McWilliams et al. 2004; McWilliams and Karasov 2005). The availability of resources stored prior to onset of the fasting period determines the tolerated length of the fasting period. Requirements are met by catabolizing body stores (phase II fasting, see Le Maho et al. 1981; Cherel et al. 1988; Robin et al. 1988); once body stores are depleted then animals must catabolize structural tissues to satisfy their continuous biochemical requirements and so avoid death (phase III of fasting). Scaling of metabolic rate and the pace of depletion of body stores adequately explains why heavier species may encounter longer fasting periods compared to lighter species (Kleiber 1932; Calder 1984; Bauchinger and McWilliams 2009), but cannot explain differential mass loss among similar sized animals.

Fasting periods may occur periodically and/or stochastically. Reoccurring patterns of fasting of rather similar length occurs in response to various kinds of rhythms (e.g., circadian, circannual, and tidal) and may often limit the availability of, or access to, nutritional resources (for reviews see Körtner and Geiser 2000;

U. Bauchinger (✉) · S. R. McWilliams
Program in Wildlife and Conservation Biology,
Department of Natural Resources Science, University of Rhode Island,
105 Coastal Institute in Kingston, Kingston, RI 02881, USA
e-mail: ulf@etal.uri.edu

M. D. McCue (ed.), *Comparative Physiology of Fasting, Starvation, and Food Limitation*, DOI: 10.1007/978-3-642-29056-5_12,

Piersma and Drent 2003; Piersma and van Gils 2010) (see also Bar and Volkoff, Chap. 6). Daily and seasonal movements of the earth and the moon determine sleep-awake rhythms of many animals as well as the timing, duration, and extent of seasonal hibernation and seasonal migration, each of which is associated with periodic fasting and refeeding periods. Less predictable, stochastic events, such as sand-, rain-, or snowstorms can make foraging virtually impossible for many animals and so result in fasting periods of less predictable durations (Secor and Diamond 1998, for review see Piersma and van Gils 2010) (see also Jenni-Eiermann and Jenni, Chap. 11).

Seasonal environmental changes often trigger animal movements including migrations to avoid unfavorable conditions at one area and/or anticipate favorable conditions at another (for reviews see Berthold 2001; Newton 2008). Animal movements over great distances may require sustained periods of locomotion that may limit foraging, especially if inhospitable areas have to be overcome (Biebach 1992; Butler et al. 1998; Biebach et al. 2000; Klaassen and Biebach 1994; Gill et al. 2009; Schmaljohann et al. 2007). These fasting periods may last for hours or as long as several days depending on the ecological barrier, and may be repeated several times with only relatively short rest periods at stopover sites before the next long-distance excursion (Butler et al. 1998; Biebach et al. 2000; Gill et al. 2009; Schmaljohann et al. 2007).

Extended fasting periods associated with migration require body stores that must be deposited before the onset of active migration. Large amounts of fat are deposited prior to take off, but protein storage also increases during the premigratory preparations (Fry et al. 1972; Marsh 1984; Piersma 1990; Biebach 1996; Bauchinger and Biebach 2005). Both, fat and protein tissues are subsequently catabolized during the fasting period with fat accounting for about 2/3 to 3/4 of the mass change of the migrating bird and proteinaceous tissue accounting for the remainder (Klaassen and Biebach 1994).

Protein catabolism during fasting causes reduction in mass of internal organs. Much early research focused on the mass reduction of digestive organs (e.g., intestine, liver, and gizzard), which are obviously not in use during fasting (see also Lignot, Chap. 14). Other tissues including kidney and muscle were also shown to atrophy during fasting like that which occurs during migration (Hume and Biebach 1996; Bauchinger and Biebach 1998; Biebach 1998; Thouzeau et al. 1999) as well as during actual long-duration migrations (Biebach 1998; Bauchinger and Biebach 1998; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Karasov et al. 2004; Bauchinger et al. 2005).We have shown that the extent of reduction in these organs seems to be organ-specific when considered for a single species (Bauchinger and McWilliams 2009) and across many species (Bauchinger and McWilliams 2010). The extent of mass reduction for a given tissue was consistent across all species studied to date and was in the following rank order from most to least reduced: small intestine, liver, kidney, heart, flight muscle, and leg muscle (Fig. 12.1, adapted from Bauchinger and McWilliams 2010).

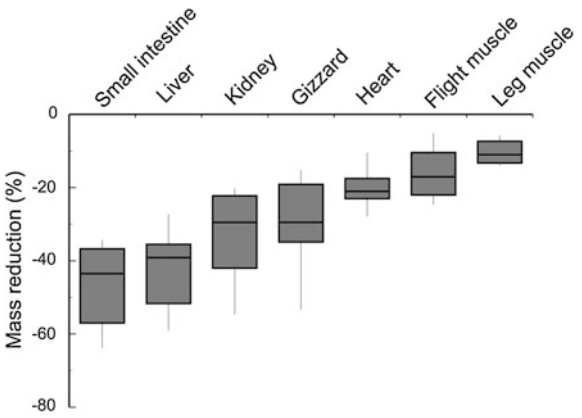

Fig. 12.1 Mass loss (as % of prefast condition) for seven organs in migratory birds sampled before and after a sustained period of flight, or sampled before and after fasting during a simulated migration (Figure adapted from Bauchinger and McWilliams 2010). Median mass change (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles) for small intestine, liver, kidney, gizzard, heart, flight muscle, and leg muscle for great knot *Calidris tenuirostris* (Battley et al. 2000, 2001), blackcaps *Sylvia atricapilla* (Karasov et al. 2004), garden warblers *Sylvia borin* (Hume and Biebach 1996; Schwilch et al. 2002; Bauchinger et al. 2005), pied flycatcher *Ficedula hypoleuca* (Schwilch et al. 2002), and willow warbler *Phylloscopus trochilus* (Schwilch et al. 2002). Only studies that give mass data for at least five of the presented tissues were incorporated (see Bauchinger and McWilliams 2010 for details)

12.2 Hypotheses to Explain Differential Mass Loss

Several functional hypotheses have been proposed to explain why organs are changing in mass during migration, sustained flights, and other long-duration fasting periods (for reviews see Evans 1992; Biebach 1996; Piersma and Lindström 1997; Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998; Bauchinger and McWilliams 2009, 2010). Here we outline five functional hypotheses that have been proposed to explain phenotypic flexibility in organ size, in general, and the loss/gain of tissue protein during migration.

- Use-disuse hypothesis
- Functional allometry hypothesis
- Protein pool hypothesis
- Energy conservation hypothesis
- Protein turnover rate hypothesis

12.2.1 The Use-Disuse Hypothesis

The use-disuse hypothesis proposes that organs in use will hypertrophy whereas organs not in use will atrophy (Alexander and Goldspink 1977). Such changes are well documented for various animal models and humans: inactivity or

immobilization causes quick atrophy of muscles, whereas training increases mass of exercised muscle (Boonyarom and Inui 2006). Typically the 'training effect' requires considerable time to manifest itself. Studies of migratory waterfowl and waterbirds (e.g., geese, ducks, and grebes) provide support for this hypothesis (for review see Portugal et al. 2009). For example, eared grebes (*Podicepsnigricollis)* reduced flight muscle size and increased leg muscle size after arrival at a staging area and commencement of wing molt, flightlessness, and the increased necessity of swimming (Jehl 1997). Flight muscle size of grebes increased toward the end of wing molt when birds increased wing flapping activity (Piersma 1988; Jehl 1997); however, similar muscle mass changes occurred among waterfowl that did not increase their frequency of wing flapping (see Portugal et al. 2009 for review). Other results are inconsistent with the use-disuse hypothesis. For example, pre-migratory flight muscle mass increased without increased flight activity in a wader species suggesting endogenous control of muscle hypertrophy (Dietz et al. 1999). We can use the evidence portrayed in Fig. 12.1 to further test the use-disuse hypothesis.

Two lines of evidence can be used to evaluate the use-disuse hypothesis. First, a comparison of tissue reduction during fasting in birds during natural migration versus during simulated migration should reveal differences between tissues. Specifically, tissues such as flight muscle and heart that must be heavily used during flight should reduce less in mass than during simulated migration (i.e., when birds in cages were exposed to periods with and without food under light: dark cycles appropriate for stimulating migration state). In contrast to these predictions, Fig. 12.2 reveals quite consistent relative reductions in flight muscle, heart, and kidney during fasting for birds during natural and simulated migration. Second, the use-disuse hypothesis predicts that small intestine, gizzard, and leg muscle should reduce more in mass than flight muscle, heart, and kidney in birds during natural migration that are actively flying. Again, Fig. 12.2 provides little support for this prediction—in fact, small intestine was reduced the most and leg muscle reduced the least in birds during flight. Thus, despite the limited data available to date, the use-disuse hypothesis cannot explain the consistent pattern of organ mass loss observed during fasting in migratory birds (Figs. 12.1 and 12.2). This conclusion is consistent with others who have also found weak support for the use-disuse hypothesis in studies of geese (Portugal et al. 2009) and waders (Dietz et al. 2007) as outlined above.

12.2.2 The Functional Allometry Hypothesis

The functional allometry hypothesis proposes that flight muscle mass is adjusted to optimize the power requirements of flying given changes in whole-body mass during migration of birds. Fat stores provide more than 90% of the energy required for sustained flapping flight, and there is a corresponding need to increase flight muscle mass to power flight of the fat-loaded bird at take off (Pennycuick 1978, 2008).

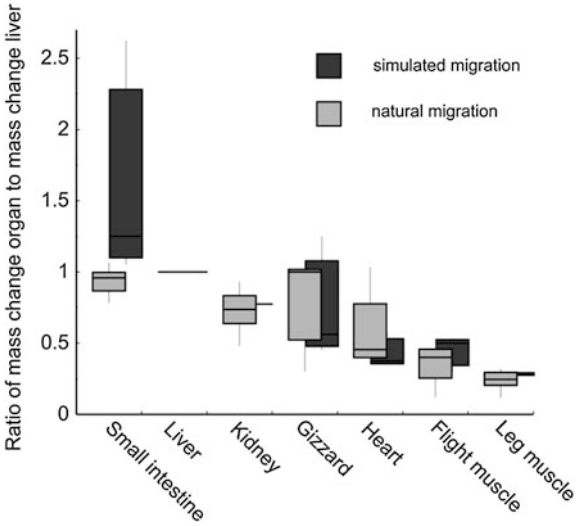

Fig. 12.2 Ratio of organ mass change to liver mass change for seven tissues. For each of the data sets introduced in Fig. 12.1 all organ mass changes were related to that of the liver, and the median ratio for each organ and all data sets was plotted for the same tissues as in Fig. 12.1 (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles). Data for natural migrating birds are depicted in *light gray*, data for birds subjected to simulated migration by food deprivation in the laboratory (for method see Gwinner et al. 1985; Battley et al. 2001) in *dark gray*. Liver data must equal 1.0 by definition for both natural and simulated migration

During long-duration flights, these fat stores are depleted, whole-body mass decreases, and there is a corresponding decrease in power requirements and hence flight muscle mass over the course of the flight (Bauchinger and Biebach 2001; Pennycuick and Battley 2003). The functional allometry hypothesis was originally formulated to explain the increase in flight muscle during premigratory fattening (Evans 1969; Fry et al. 1972; Marsh 1984); increased fat deposition increased power requirements and this was provided by increased flight muscle mass. The tight link between the power requirements of flight and the mass of flight muscle has been confirmed on many occasions (e.g., Lindtsröm et al. 2000; Bauchinger and Biebach 2001). However, the aforementioned example of eared grebes poses challenges to such proposed relationships: recall that birds reduced flight muscle mass while gaining body mass upon arrival at their staging ground (Jehl 1997). Similarly, flight muscle mass of warblers during migration was not always tightly related to their body mass especially when their stage of migration was considered (Bauchinger and Biebach 2005; Barboutis et al. 2011).

The functional allometry hypothesis was postulated to explain mass changes of the flight muscle relative to body mass, but it can be expanded to apply also to other skeletal muscles (e.g., leg muscle) that provide posture and mobility (Bauchinger and Biebach 2005). In fact analysis of tissue mass in warblers during migration suggests a more uniform relationship between leg muscle and body mass

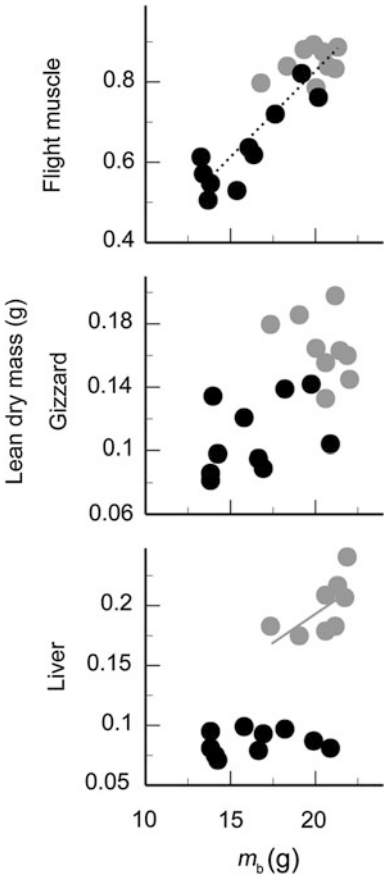

Fig. 12.3 Relationship between lean dry mass of three tissues (flight muscle, gizzard, and liver) and body mass (m_b). Body mass always represents the mass of the body minus the mass of the respective organ (i.e., corrected body mass). *Top graph* shows a significant relationship between flight muscle and body mass ($p < 0.001$); small intestine ($p = 0.041$), and heart ($p = 0.008$) also reveal a significant relationship, but are not shown. *Middle graph* shows that gizzard mass is not significantly related to body mass ($p = 0.54$); leg muscle ($p = 0.11$), and kidney ($p = 0.79$) also revealed a nonsignificant relationship, but are not presented here. *Bottom graph* shows liver mass was significantly related to body mass ($p = 0.046$) for birds in Ethiopia (*gray circles*) but not so for birds in Egypt (*black circles*). We fitted a model with the factors body mass, sampling site, and the interaction of both; nonsignificant interactions were then removed and the final model was recalculated

than for the flight muscle and body mass. We used data for garden warblers sampled before and after the migration across the Sahara desert in spring 1998 (Bauchinger et al. 2005) to test for such a causal relationship. We confirmed a significant, positive allometric relationship for flight muscle, small intestine, and heart. However, three tissues, gizzard, kidney, and leg muscle were not positively

related to body mass (Fig. 12.3). Liver mass of birds sampled before the migration across the Sahara was significantly related to body mass, whereas liver mass of birds sampled immediately after completion of the migration across the Sahara was not related to body mass (Fig. 12.3). We conclude that the functional allometry hypothesis does not fully explain the observed organ mass changes in migratory songbirds.

12.2.3 The Protein Pool Hypothesis

The protein pool hypothesis has been proposed in several different but related forms. In general, the protein pool hypothesis assumes that protein catabolism simultaneously uses amino acids from a variety of different tissues either irrespective of protein pool size (so equal use of amino acids from all tissues) or in rough proportion to the mass of the tissue and hence the size of the protein pool.

Tissue protein serves as a pool of amino acids for repair mechanisms (Piersma 1990), gluconeogenesis to meet the energy requirements of the brain (Jenni-Eiermann and Jenni 1991), and the perpetuation of the the β-oxidation pathway (Jenni-Eiermann and Jenni 1991). Protein stores may also serve as an energy source when fat stores are depleted. Estimation of the contribution of energy derived from protein catabolism to the overall energy requirements ranges 'only' around 5% (Klaassen and Biebach 1994; Jenni and Jenni-Eiermann 1998). Protein is not considered to be the main energy store (Dohm 1986) and its catabolism for energy production is not efficient (Evans 1992), although protein can be the final energy source when birds run out of fat stores during flight (Jenni et al. 2000). Protein catabolism also provides metabolic water during periods without food or free water (Klaassen 1996, see as well Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998; Gerson and Guglielmo 2011) (see also Jenni-Eierman and Jenni, Chap. 11). Finally, protein catabolism may produce antioxidants that counter balance free radical production (Klaassen et al. 2000) (see also Champagne et al., Chap. 19), which is, for example, increased due to lipid peroxidation during flight or heavy muscular workload (McWilliams et al. 2011). Thus, tissue protein has many important functions and organ reduction may either liberate specific proteins or amino acids for these many uses (but see McCue et al., Chap. 8).

We can test the protein pool hypothesis by determining whether fasting results in a uniform decrease in tissue protein across all tissues, or a decrease in tissue protein in proportion to the size of the protein pool in that tissue. In fact, not all tissues were reduced to a similar extent during fasting (Fig. 12.1) or relative to the size of the tissue (Bauchinger and McWilliams 2009, 2010). Thus, the protein pool hypothesis cannot explain the observed pattern of reduction in tissue mass of fasted birds.

12.2.4 The Energy Conservation Hypothesis

The energy conservation hypothesis proposes energy savings due to organ reduction. Small intestine is considered to be the most costly tissue in the vertebrate body to be maintained (Stevens and Hume 2004). The observed reduction of small intestine during simulated migration (Hume and Biebach 1996) was therefore considered to reduce the costs required for maintaining an organ that is of no use during in-flight starvation during migration. The reduction in organ mass that reduces maintenance costs also saves weight and thus reduces flight costs (Hume and Biebach 1996; Biebach and Bauchinger 2003).

Testing this hypothesis requires knowing the organ-specific metabolic costs and the size of the respective organ within the birds body. Unfortunately, specific metabolic rates for bird organs are largely unexplored. We are aware of only one investigation that reports organ-specific metabolic rates for liver and flight muscle in two species, Dunlins (*Calidrisalpina*) and European starlings (*Sturnus vulgaris*, Scott and Evans 1992). It appears that oxygen uptake for liver tissue is consistently 4 times higher compared to flight muscle.

In terms of relative organ mass, we collected data on liver and flight muscle mass for three passerine bird species (Schwilch et al. 2002; Bauchinger et al. 2005) and one wader species (Battley et al. 2001). Flight muscle was on average 5.7 times heavier than liver (range 4.1–7.0), small intestine was similar in mass to liver (the ratio was on average, 0.9, range 0.7–1.1), leg muscle was on average 1.4 times heavier than liver (range 0.8–1.9), gizzard was slightly lighter than liver (the ratio was on average 0.7, range 0.6–0.8), and kidney and heart were much lighter than liver (the ratios were 0.3 and 0.2, respectively; range 0.2–0.4 for kidney, 0.2–0.3 for heart). Thus the relative size of the organs within a bird ranks from highest to lowest: flight muscle, leg muscle, liver, small intestine, gizzard, kidney to heart. And this rank order is not the same as the relative reduction in tissue mass during fasting (Fig. 12.1). We conclude that the energy conservation hypothesis does not fully explain the observed organ mass changes in migratory songbirds, although more information about tissue-specific metabolic rates are needed to more adequately test this hypothesis.

12.2.5 The Protein Turnover Rate Hypothesis

The protein turnover rate hypothesis proposes that organs with the fastest rates of protein turnover will reduce the most during fasting and that organs with the slowest rates of protein turnover will reduce the least during fasting (Bauchinger and McWilliams 2009, 2010). This hypothesis capitalizes on the now available measurements for carbon turnover in specific tissues that can be used as proxy for protein turnover. Rate of isotopic incorporation differs between organs, both in mammals (Tieszen et al. 1983; Arneson et al. 2006; Sponheimer et al. 2006) and birds (Hobson and Clark 1992; Carleton et al. 2008; Bauchinger and

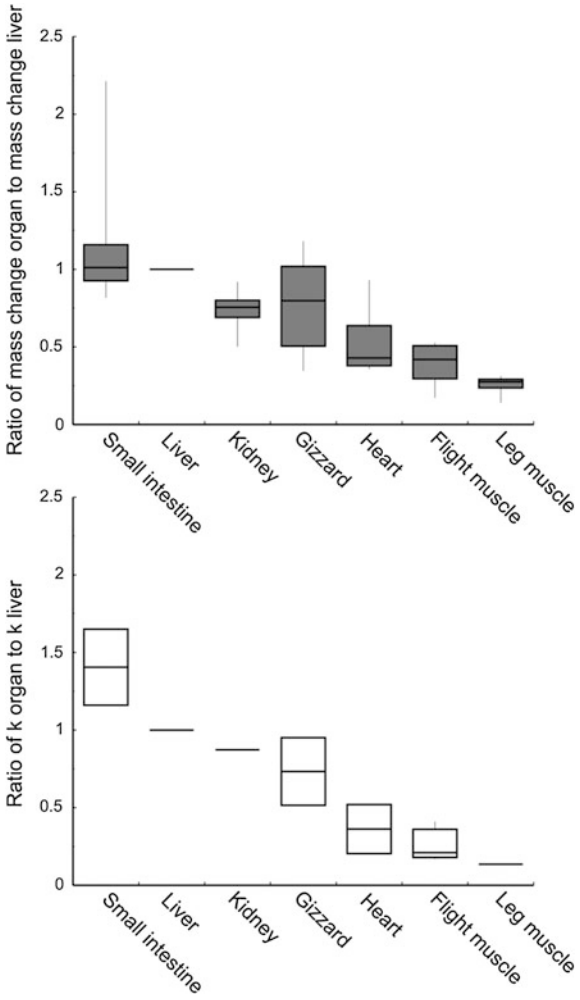

Fig. 12.4 Ratio of organ mass change to liver mass change (*upper graph*) and the ratio of organ rate of isotopic incorporation (k_{organ}) to liver rate of isotopic incorporation ($k_{liver;}$ *lower graph*) for seven tissues. Liver values equal 1.0 by definition. Median ratio of mass change (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles) is shown for small intestine, liver, kidney, gizzard, heart, flight muscle, and leg muscle. Data for ratio of organ mass change to liver mass change (*upper graph*) from Hume and Biebach 1996; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Karasov et al. 2004 and Bauchinger et al. 2005; data for ratio of organ k to liver k (*lower graph*) from Hobson and Clark 1992; Carleton et al. 2008 and Bauchinger and McWilliams 2009, 2010)

McWilliams 2009), with fast turnover rates for small intestine and liver and slow turnover rates for skeletal muscles. We can test the protein turnover rate hypothesis by determining the extent to which differences in reduction of mass between tissues during fasting corresponds with that of tissue turnover rate.

Although rate of isotopic incorporation for organs are only available for three bird species, namely zebra finch, house sparrow, and Japanese quail (see Bauchinger and McWilliams 2010 for review), the rate of isotopic incorporation is allometrically related to body mass. This allows us to standardize the isotopic incorporation for a given tissue by dividing with that of liver for the same species (Bauchinger and McWilliams 2010). Figure 12.4 summarizes the ratio of mass change for an organ to that of the liver (upper panel), and the ratio of isotopic incorporation for a given tissue to that of the liver (lower panel). As predicted by

the protein turnover rate hypothesis, small intestine and liver were reduced the most and had the highest rate of isotopic incorporation, flight muscle and leg muscle were reduced the least and had the lowest rate of isotopic incorporation, while kidney, gizzard, and heart were intermediate (see as well Bauchinger and McWilliams 2010). The clear match between the two measurements is even more remarkable considering the limited data for organ reductions (nine data sets out of six studies: Hume and Biebach 1996; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Bauchinger et al. 2005) and the limited data for isotopic incorporation (three studies: Hobson and Clark 1992; Carleton et al. 2008; Bauchinger and McWilliams 2009). Furthermore, the tight relationship between isotopic rate of incorporation and organ mass reduction remains highly significant if only data for organ mass are analyzed that were either collected from naturally migrating birds, or from simulated migration experiments that were conducted in the laboratory (Bauchinger and McWilliams 2010).

12.3 Conclusion

Analysis of the available data on organ mass changes during natural or simulated migration revealed that some organs were more reduced than others and that the observed pattern was statistically significant (Bauchinger and McWilliams 2010). Small intestine and liver were the organs that reduced the most, flight muscle and leg muscle reduced the least, and organs like kidney, gizzard, and heart ranged intermediate (Fig. 12.1). We reviewed five hypotheses proposed to explain the observed patterns. We rejected three of the five hypotheses (the use-disuse hypothesis, the functional allometry hypothesis, and the protein pool hypothesis). At present, adequately testing the energy conservation hypothesis requires better data on organ-specific metabolic rates. The best available data (though still limited) support the protein turnover rate hypothesis: the rate of protein turnover determines the rate of organ mass reduction observed in birds during migration.

It seems very plausible that the energy conservation hypothesis, at least part of it, namely the reduction of organ maintenance costs may actually be linked to the protein turnover rate hypothesis. The continuous process of protein turnover requires energy for both protein synthesis and protein degradation, and thus the rate of protein turnover of an organ is likely positively related to the potential metabolic costs (Krebs 1950; Martin and Fuhrman 1955; Scott and Evans 1992) that can be saved if organs are reduced. It is therefore not surprising that the most costly organ to maintain, the small intestine (Stevens and Hume 2004), is the organ with the highest rate of isotopic incorporation and therefore protein turnover.

We would like to point out that the support for the protein turnover rate hypothesis can help to explain the rate of organ reductions between organs, but does not address what the degraded protein from the respective organs is used for. Birds may very well use the protein to liberate water bound to the protein (Gerson and Guglielmo 2011), to supply necessary intermediates for fatty acid metabolism

(Jenni-Eiermann and Jenni 1991), or to supply protein for repair mechanisms (Piersma 1990) or as antioxidants (Klaassen et al. 2000).

We have shown that the magnitude of organ flexibility among several species of migratory birds is directly related to tissue-specific protein turnover. We propose that tissue-specific rate of protein turnover determines the pace of organ mass reduction in migrating birds and hence the magnitude of phenotypic organ mass changes observed in migratory birds. As such, no further functional explanation is needed to explain phenotypic flexibility in organ size of migratory birds unless there are detectable differences in organ mass change beyond that predicted by the protein turnover hypothesis.

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Chapter 13
Seasonal Changes in Body Mass and Energy Balance in Wild Small Mammals

Xueying Zhang, Xinyu Liu and Dehua Wang

13.1 Introduction

Limitation of food availability caused by seasonal reduction in vegetation or other food resources is a commonly encountered environmental threat for seasonal small mammals in the wild. In response to this adversity, small mammals inhabiting temperate regions and higher latitudes exhibit seasonal fluctuations in physiology, behavior, and morphology, which may ensure survival in these challenging environments.

Small mammals living in Qinghai–Tibet Plateau (average elevation above 4,500 m), routinely face harsh conditions that include extremely low temperatures (occasionally reaching −37°C) and severe altitudinal hypoxia. Surviving these conditions would be a challenge to most mammals, especially nonhibernating species that do not store food for winter. Small mammals living in the Inner Mongolian grassland must also cope with reduced food availability amidst increased energy requirements. Its climate is characterized by warm summers (mean temperatures of 17.8°C) but cold winters (mean temperature of −18°C) (Wang et al. 2003).

13.2 Seasonal Changes in Body Mass and Thermogenesis

Brandt's voles (*Lasiopodomys brandtii*) and Mongolian gerbils (*Meriones unguiculatus*) are sympatric rodents and primarily distributed in the Inner Mongolian grasslands of China. Brandt's voles are strictly herbivorous and feed mainly on grass leaves,

X. Zhang · X. Liu · D. Wang (✉)
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, China
e-mail: wangdh@ioz.ac.cn

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whereas Mongolian gerbils are granivorous and feed mainly on plant seeds. Both of them are nonhibernating animals (Li et al. 2010; Wang et al. 2003). Plateau pikas (*Ochtona curzoniae*) and root voles (*Microtus oeconomus*) are also strict herbivores eating mainly grass leaves. Despite the extreme winter conditions of the Qinghai–Tibetan Plateau, these two species do not hibernate nor do they cache food for winter survival like other rodents (reviewed in Smith and Reichman 1984).

13.2.1 Small Mammals in Inner Mongolian Grasslands

Body mass, body fat content, energy intake, basal metabolic rate (BMR), nonshivering thermogenesis (NST), and serum leptin levels were measured in freshly captured or seminatural acclimated individuals of Brandt's voles and Mongolian gerbils to understand their survival strategies (Li and Wang 2005a, b; Wang et al. 2003; Zhang and Wang 2007a, b). The body weight of both Brandt's voles and Mongolian gerbils showed significant seasonal changes, with body weight decreasing in winter and increasing in spring and summer (Fig. 13.1a, b). This seasonal variation in body weight was also associated with changes in other physiological measures. For instance, the winter decrease in body weight was accompanied by increased digestible energy intake and enhanced NST, but decreased body fat mass and reduced circulating levels of leptin, which is primarily secreted from adipose tissue (Zhang et al. 1994). These data suggest that leptin may act as a "starvation signal" to permit the increase in energy intake and thermogenesis for winter adaptation.

13.2.2 Small Mammals in Qinghai–Tibetan Plateau

Plateau pikas and root voles were trapped in the wild at different times of the year. Plateau pikas maintained constant body mass and fat mass throughout the year (Fig. 13.2a) and showed no seasonal change in circulating levels of serum leptin. However, NST, cytochrome C oxidase (COX) activity, and mitochondrial uncoupling protein-1 (UCP1) levels in brown adipose tissues (BAT) were significantly enhanced in winter (Liu and Li 1996a; Wang and Wang 1990; Wang et al. 1993, 2006c). Root voles decreased body mass (Fig. 13.2b) and fat mass coupled with enhancement in NST, COX activity, and UCP1 levels in BAT in winter (Wang and Wang 1989, 1990). Consistent with changes in body mass and fat mass, circulating leptin levels declined significantly in winter and increased in summer (Wang et al. 2006b).

Both plateau pikas and root voles increased the wet and dry mass of total digestive tracts in winter, and root voles also increased the length and content mass of total digestive tracts, suggesting voles increase their digestive efficiency during

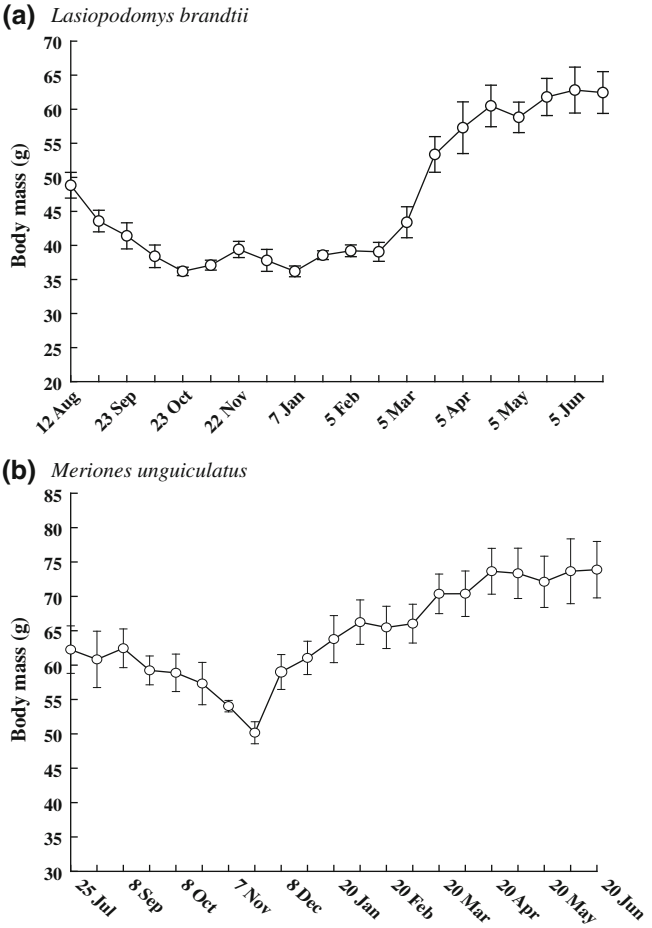

Fig. 13.1 Seasonal patterns of body mass in Brandt's voles (**a**) and Mongolian gerbils (**b**) from the Inner Mongolian grassland [**a** based on data from Li and Wang (2005a); **b** based on data from Li and Wang (2005b)]

this period (Starck 1999; Wang and Wang 2001; Wang et al. 1995). Plateau pikas mainly depend on increasing thermogenic capacities, rather than decreasing body mass, to cope with cold winter, and root voles mainly depend on elevated adaptive thermogenesis coupled with body mass reduction to enhance winter survival. The reduction in body mass in winter is considered to be an adaptive mechanism for the decline in energy requirements when food availability is limited and temperature drops (Wunder et al. 1977). Furthermore, root voles increase fat mobilization in winter and leptin is likely involved in the regulation in body mass and thermogenesis in root voles. Therefore, different species inhabiting the same regions may adopt diverse adaptive strategies to seasonal environments.

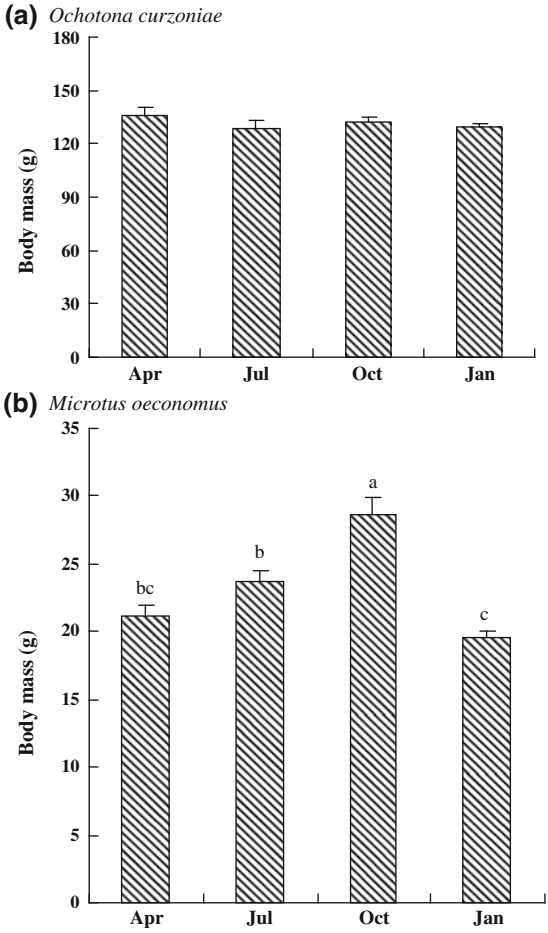

Fig. 13.2 Seasonal patterns of body mass in plateau pikas and root voles from the Qinghai–Tibetan plateau [**a** based on data from Wang et al. (2006c); **b** based on data from Wang et al. (2006b)]

13.3 Environmental Factors Trigger Seasonal Adaptations

13.3.1 Photoperiod

Changes in photoperiod are the most predictable of the seasonal environmental changes including temperature, food quality, and food quantity. Many rodent species use the annual photoperiodic cycle as an environmental zeitgeber for seasonal physiological changes, especially for energy balance and thermogenesis.

We examined the effects of short photoperiod (SD) on body weight and body composition, as well as on several physiological, hormonal, and biochemical

measures indicative of thermogenic capacity in captive Brandt's voles (Zhao and Wang 2005, 2006b). Voles exposed to SD showed higher digestible energy intake and increases in BMR and NST during the 4 weeks photoperiod acclimation. By the end of the experiments, SD voles had lower body weights, body fat mass, and serum leptin levels, but higher COX activity and UCP1 levels in BAT and higher concentrations of serum triiodothyronine (T_3) and thyroxine (T_4) compared to long day (LD) voles. Taken together, these data suggest that Brandt's voles employ a strategy of minimizing body weight, mobilizing fat deposition, increasing energy intake, and enhanced thermogenic capacity in response to single short photoperiod exposure.

In contrast to Brandt's voles, Mongolian gerbils respond differently to short photoperiod (Li et al. 2004; Zhao and Wang 2006a). We did not detect any changes in body mass, body fat mass, or serum leptin levels in Mongolian gerbils during the 4 weeks SD acclimation, and these results are consistent with the findings in adult Mongolian gerbils by Karakas and Gunduz (2002). The SD gerbils showed higher BMR, liver COX activity, and T_4 concentrations than LD controls, indicating that photoperiodic changes may provide cues altering energy budget, but not body mass in Mongolian gerbils (see also Ullrey, Chap. 18).

For pikas, our data showed that 4 weeks SD acclimation did not affect body mass, body fat mass, BMR, NST, or serum leptin levels; however, it did increase energy intake compared to LD (Wang 2006). In contrast, SD root voles showed lower body mass and body fat coupled with higher energy intake than LD voles at the end of 4 weeks acclimation. Moreover, SD greatly enhanced thermogenic capacities in root voles, as indicated by elevated BMR, NST, mitochondrial protein content, and UCP1 levels in BAT (Wang and Wang, 2000; Wang et al. 2006a). Although no variations in serum leptin levels were found between SD and LD both in pikas and voles, serum leptin levels were positively correlated with body mass and fat mass, and negatively correlated with energy intake and UCP1 levels in BAT, respectively (Wang et al. 2006a, b).

Unlike pikas, root voles are sensitive to simple photoperiod changes. Nevertheless, leptin is potentially involved in the photoperiod-induced regulation in energy intake and thermogenesis both in pikas and root voles. Both species differ from Siberian hamsters (*Phodopus sungorus*) in seasonal body mass regulation. In response to SD, Siberian hamsters spontaneously reduced food intake, and body mass declined by approximately 40% over 12 weeks of exposure (Steinlechner et al. 1983; Ebling 1994). Thus, although photoperiod may induce changes body mass and thermoregulation in some rodents, the direction these changes take can differ.

13.3.2 Temperature

Ambient temperature also plays an important role in regulating physiological and behavioral responses in small mammals inhabiting temperate regions. They usually exhibit extraordinary physiological adaptations to extremely low temperatures in winter to ensure their survival (McNab 2002).

During cold exposure, Brandt's voles and Mongolian gerbils met most of their energy demand not only by increasing food intake, but also by mobilizing visceral and subcutaneous fat depots (Li et al. 2004; Zhang and Wang 2006, 2007a, b). The increase in food intake and decrease in body fat are closely related with levels of circulating leptin, which decreased significantly under cold acclimation. Our results also showed a critical role of hypoleptinemia in regulation of body weight and energy balance in cold-exposed voles (Tang et al. 2009). Cold acclimation was accompanied by a decrease in serum leptin levels, and hypothalamic agouti-related protein (AgRP) mRNA levels were significantly increased. Leptin administration in cold-exposed voles decreased food intake as well as hypothalamic AgRP mRNA levels. When cold-exposed voles were returned to warm temperatures for 4 weeks, body mass, food intake, serum leptin, and AgRP mRNA were restored to control levels. These results suggest that hypoleptinemia partially contributes to cold-induced hyperphagia, which might involve the elevation of hypothalamic AgRP gene expression.

Similar to Brandt's voles and Mongolian gerbils, both plateau pikas and root voles can maintain stable body temperature in response to 3 weeks cold (5°C) exposure by increasing NST together with increased mitochondrial protein content and COX activity in BAT (Liu and Li 1996a, b; Wang et al. 1996, 1999a, b).

Temperature can accentuate the effect of photoperiod. For example, adaptive thermogenic capacity is further enhanced when the animals were exposed to cold and short photoperiod in voles, gerbils, and pikas (Li and Wang 2005b; Wang et al. 1999a, b; Wang and Wang 2000). Taken together, these data suggest an interaction between short photoperiod and cold exposure plays a role in body mass regulation in the small mammals rather than photoperiod or temperature alone.

13.3.3 Food Availability

Food availability fluctuates with seasons in the wild, thus it may be one of environmental factors contributing to seasonal adaptations of small mammals. In the laboratory, we manipulated food availability by food deprivation, food restriction, and high-fat or high-fiber diets to simulate dietary changes that occur in nature. We found that food restriction (60% of control food) caused significant decreases in body mass, fat mass, and serum leptin levels. Plateau pikas and root voles exhibited new set-point in body mass that were 26 and 35% lower than control values, respectively (Wang 2006). Food restriction also resulted in significant decreases in BMR, NST, mitochondrial protein content, and UCP1 levels in BAT. These outcomes suggest that plateau pikas and root voles were not merely passively responding to food restriction by reducing body and organ sizes; they could also adaptively conserve energy by decreasing thermogenesis during periods of energy limitation (see also Ben-Hamo et al., Chap. 16). We speculate that decreased leptin levels caused by food limitation may indirectly trigger compensatory responses to modulate food intake following food shortage.

13.4 Fasting

Restricted food intake or even periods of complete fasting are frequent situations in wild animals (reviewed in McCue 2010). During periods of fasting or starvation, small rodents typically reduce their body masses and energy expenditure. In response to 24–36 h fasting, Brandt's voles decreased body mass, BMR, NST, and serum leptin levels, and after refeeding, the aforementioned parameters were restored to the control levels except for body fat mass and serum leptin (Zhan et al. 2009). Surprisingly, unlike rats (Evans et al. 2005), no postfast hyperphagia in Brandt's voles was observed (Zhan et al. 2009). Zhang (2007) demonstrated that 3 days fasting significantly reduced body mass, body fat mass, serum leptin levels, and thermogenic capacity in Mongolian gerbils. Xu and Wang (2010) confirmed the responses above and further found that gerbils fasted for 66 h had lower wet and dry thymus and dry spleen mass as well as reduced white blood cell counts. T cell-mediated immunity, assessed by PHA response, was also suppressed in the fasted gerbils, suggesting acute fasting leads to immunosuppression (see also Champagne et al. Chap. 19; Jenni-Eiermann and Jenni Chap. 11). These results suggest that both Brandt's voles and Mongolian gerbils can adjust the status of physiology integratively to cope with the lack of food, such as reduction in body mass, energy expenditure, and immunologic function. It may also suggest that there is tradeoff between body energy reserve and immunity. The changes in immunologic function would influence animals' ability against environmental pathogens, and hence affect the survival and fitness on the wild populations (Sheldon and Verhulst 1996).

13.5 Future Directions

Environmental cues play important roles in mediating seasonal variations in body mass, thermogenesis, and energy intake in wild small mammals. Small mammals usually take species-specific strategies to adapt to the seasonal environments (see Fig. 13.3 which summarizes the adaptive responses to photoperiod and temperature in the four wild species studied in our laboratory). Serum leptin as the peripheral signal reflecting body energy status acts on the receptors (OB-Rb) in the hypothalamus and thus regulates body mass and energy intake. However, the exact neuroendocrinological mechanisms of energy homeostasis regulation in wild small mammals still need to be further investigated. Regulation in body mass, energy balance, and thermogenesis is apparently complex and progress on this front must include studies of behavior, physiology, biochemistry, cell biology, endocrinology, neuroscience, genetics, and genomics. Thus an integrative approach spanning molecular, cellular, tissue, organ, and organismal perspectives will be required to fully understand these mechanisms. Information for energy balance regulation in wild small mammals will broaden our understanding in biomedical science, particularly in areas such as obesity and aging.

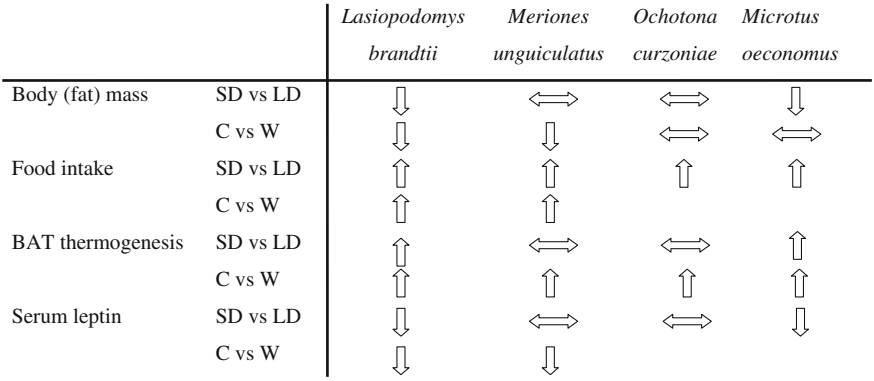

Fig. 13.3 Summary of the responses to photoperiod and temperature of body (fat) mass, food intake, BAT thermogenesis, and serum leptin levels in Brandt's voles, Mongolian gerbils, plateau pikas, and root voles. *Arrows* indicate changes which have been reported (Tang et al. 2009; Wang et al. 1996, 1999a, b; Wang and Wang 2000; Zhang and Wang 2006; Zhao and Wang 2005, 2006a, b). SD, short day; LD, long day; C, cold; W, warm

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Chapter 14
Changes in Form and Function of the Gastrointestinal Tract During Starvation: From Pythons to Rats

Jehan-Hervé Lignot

14.1 Introduction

Over the last 40 years, different authors have studied the successive physiological phases of prolonged fasting corresponding to the differential mobilisation of lipids versus protein reserves as body fuels (Goodman et al. 1980; Le Maho et al. 1981; Robin et al. 1988). However, very few studies in vertebrates to date have examined the morphological and functional changes of the gastrointestinal tract (GIT) according to these metabolic phases. The majority of them have focused on rodent models (Dunel-Erb et al. 2001; Habold et al. 2004, 2006, 2007). In some cases, extremely long fasting periods have also been considered but did not take these phases into account (Sohma 1983).

In terrestrial vertebrates, endurance during fasting condition depends on several parameters: age, sex and energetic requirements, as well as the availability and quantity of energy reserves, which tissues they are preferentially stored in, and which distinct routes of mobilisation are used. As a consequence, survival with little or no food availability over long periods is better achieved by ectotherms compared to endotherms, mainly due to their comparatively very low metabolic rate (McCue 2010). Thus, fasting in some ectotherms can be achieved over several years. As an example, the fasting endurance of the desert frog *Cyclorana platycephala* has been estimated at more than 5 years (van Beurden 1980). However, some mammals such as the male northern elephant seal (*Mirounga angustirostris*), which weighs around 1,500 kg, can also endure prolonged fasting for about 60 days (Coltman et al. 1998). Similarly, birds can achieve prolonged

J.-H. Lignot (✉)
UMR 5119 ECOSYM, Université de Montpellier II, CNRS, IFREMER,
Adaptation Ecophysiologique et Ontogénie (AOE), cc 092, Place E. Bataillon,
34095 Montpellier Cedex 05, France
e-mail: Jehan-Herve.Lignot@univ-montp2.fr

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fasting either at rest or during exercise (i.e. during migration, molting or reproduction). Even humans can last extended periods without food (Stewart and Fleming 1973). Therefore, in all vertebrates, a depression of the standard metabolic rate occurs in response to prolonged fasting resulting from a suite of biological strategies ('starvation syndrome') using fat, carbohydrates and proteins as body fuels to carry out key physiological processes (McCue 2006, 2010). During this response, body mass can be severely decreased due to the atrophy of some tissues and organs while others are preferentially spared (see Bauchinger and McWilliams, Chap. 12). For example, inactive tissues such as skeletal muscles and those of the gut regress during fasting, and thus provide a mechanism to reduce the metabolic rate. Indeed, the digestive tract represents one of the most costly tissues to maintain (Cant et al. 1996). On the other hand, the brain, the cardiovascular system, the kidney and the gonads are usually preserved and can even increase in their relative mass. Nevertheless, the digestive tract represents the functional link between energy intake and the energy used to fulfill all the vital functions and as such must be, and is rapidly reactivated at refeeding, even after prolonged fasting (Bozinovic 1993; Dunel-Erb et al. 2001; Habold et al. 2004, 2006, 2007; Karasov 1997; Secor 2001; see also Campen and Starck, Chap. 9). Therefore, a flexible digestive tract adjusted to functional demands is an important energy-saving mechanism (Dietz et al. 1999). However, although gut atrophy has been repeatedly observed during fasting, the mechanisms underlying this phenotypic flexibility has received comparatively less attention.

Since the intestine is the principal site for digestion and absorption, this chapter will primarily detail the morphological and functional changes of this segment due to prolonged fasting. Other segments of the GIT such as the stomach will also be briefly discussed.

14.2 Morphology of the Midgut in Terrestrial Ectotherms and Endotherms

The digestive system of vertebrates is subdivided into four topographical regions: a headgut, foregut, midgut and hindgut (Stevens and Hume 1995). The main role of the headgut (oral cavity and pharynx) is to acquire food and to mechanically process it. The foregut follows, and is composed of the esophagus and stomach, where chemical digestion of food begins. The midgut or intestine accounts for the longest portion of the gut. In this segment, chemical digestion continues and the majority of absorption occurs. Radially, the midgut wall possesses four concentric layers: (1) the inner *tunica mucosa* consisting of a mucosal epithelium lying on the *lamina propria* (a vascularised and innervated connective tissue), (2) the *submucosa*, an additional connective tissue layer, (3) the *tunica muscularis* possessing circular and longitudinal layers and (4) the outer *tunica serosa*. The *muscularis mucosa* is found between the tunica mucosa and the *submucosa*, and is composed of several thin

layers of differently oriented smooth muscle fibres. The gentle, constant contractions of this muscular layer constantly move the mucosal surface and therefore enhance contacts between the epithelial layer and the luminal content. In all vertebrates, the mucosal epithelium of the inner *tunica mucosa* is composed of absorbing enterocytes that have a characteristic luminal apical surface with numerous microvilli forming a dense brush border. These luminal projections of the apical membrane of the enterocytes are covered by a thin mucous layer produced by goblet cells, the second most numerous cell type forming the intestinal epithelium.

Despite these common characteristics, individual parts of the vertebrate midgut can demonstrate a wide range of structural and functional variation among the taxonomic classes. For example, the length of the intestine can vary according to diet and can also change within an individual during ontogeny. The surface area of the midgut can also be expanded by circular concentric folds (*plicae circulares* or *valvulae conniventes*, present in primates but not in laboratory rodents), and also by villi and intricate folds and ridges (Parsons and Cameron 1977; Stevens and Hume 1995). When present, the folds of the small intestine are formed by all the layers of the mucosa, including the *muscularis mucosae*; their core is the *submucosa*. The villi, however, are outgrowths of the mucosa; their core is the *lamina propria* and each of them contains lacteals and a capillary network. These intestinal villi, when present, have a leaf like or a finger-like structure depending on the species. They create a larger surface area than a midgut with just folds.

The crypts of Lieberkühn found in mammals at the base of the villi are not found in all vertebrates and can also show morphological and functional differences. For example, the intestinal 'crypts' described at the base of the villi in salamanders and in some reptiles (Reeder 1964; Luppa 1977) differ from those found in birds and mammals; they are less developed and their epithelium is similar to that of the surface. In birds, the crypts also vary among species; some contain cells with basophilic granules while others only possess absorptive and goblet cells. Finally, metamorphosis in amphibians induces rapid and profound changes of the midgut (Reeder 1964; McAvoy and Dixon 1977; Stevens and Hume 1995): in anurans, the intestine shortens by 58–90% depending on the species, and its diameter narrows due to longitudinal and radial muscle fibre contraction (Schreiber et al. 2005; Secor 2005). Removal and regeneration of the intestinal epithelium also occur along with the appearance of a distinct hindgut that is lined with columnar epithelium and goblet cells. This epithelial transformation is mainly caused by programmed cell death (apoptosis) in the primary larval epithelium (Ishizuya-Oka and Shimozawa 1992), and proliferation and differentiation of the secondary adult epithelial cells (Hourdry and Dauca 1977; Yoshizato 1989; Shi and Ishizuya-Oka 1996). After metamorphosis, the epithelium folds again and is monolayered. Throughout this larval-to-adult remodelling, DNA replication occurs uniformly throughout the epithelium, as do changes in gene expression. Therefore, larval epithelial cells as a whole, rather than a subpopulation of stem cells, are the progenitors of the adult epithelial cells. After metamorphosis, a trough-to-crest axis of the epithelial folds is reestablished. Epithelial cells at the crest of the folds present the greatest degree of specilisation (well-developed inter and intracellular

membrane systems), while cells in the trough regions between the folds are much less specilaised. This is structurally analogous to the mammalian villous-to-crypt axis, although cells are not segregated to the same degree. Similarly, cell proliferation in the intestinal epithelium of various reptiles with no crypts (as seen with the Burmese python and the painted turtle *Chrysemys picta*) occurs preferentially in the mid and basal segments and less in the apical part of a villus fold (Wurth and Musacchia 1964; Helmstetter et al. 2009a). New cells are produced throughout the epithelium in the layer of cells adjacent to the basement membrane of the epithelium as for *C. picta*, while dying cells and cell fragments are expelled into the lumen. Finally, the time required to renew the intestinal epithelium of the painted turtle was estimated at approximately 8 weeks in winter turtles maintained at 20–24°C (Wurth and Musacchia 1964). In mammals, intestinal epithelial cell renewal rate is well understood and depends on a tightly regulated balance between cell proliferation in the crypts and apoptosis at the tip of the villi (Grossmann et al. 1998; Luciano et al. 1997; Potten and Allen 1977).

14.3 Effects of Starvation and Refeeding on the Midgut

Fasting usually results in an overall decrease in mucosal mass and the atrophy of the intestinal epithelium as already observed for amphibians (Cramp and Franklin 2003, 2005), reptiles (Secor and Diamond 2000; Secor and Lignot 2010), birds (Hume and Biebach 1996) and mammals (Dunel-Erb et al. 2001; Habold et al. 2007). There are two potential mechanisms responsible for this mucosal reduction: cell hypotrophy and cell hypoplasia. Hypotrophy via the decrease in cell size has been identified to be an instrumental factor in the postabsorptive reduction in intestinal mucosa (Lignot et al. 2005). Hypoplasia, or the wasting away of existing cells, can also occur and is achieved by an imbalance between cell proliferation and apoptosis. It results in a reduction in the length of the intestinal villi and thus in a decreased surface area of the absorbing epithelium. This is usually due to the retraction of the *lamina propria* and of its major functional constituents such as the lacteals and blood capillaries. Feeding rapidly reverses the fasting response; intestinal mass and luminal surface area increases and enterocytes swell. However, the magnitude by which the intestine modulates its mass with short-term/long-term fasting and feeding varies among species.

14.3.1 Ectotherms

Some terrestrial amphibians can survive without food for periods ranging from several months to several years. For example, aestivating frogs such as the Australian frog *Cyclorana alboguttata*, the Argentinian horned frog *Ceratophrys*

ornata or the African bullfrog *Pyxicephalus adspersus* burrow into the substrate at the beginning of the dry season, form a cocoon and remain dormant for several months (Cogger 1992; Loveridge and Withers 1981; McClanahan et al. 1976).

Different studies have investigated GIT morphological responses to fasting and aestivation in these species and in others (*Scaphiopus holbrookii*, *Bufo marinus*) in publications by Franklin and collaborators and by Secor (Cramp et al. 2009; Cramp and Franklin 2005; Secor 2005 and for a review, Secor and Lignot 2010). When compared with periods of active digestion, fasting and aestivation results in a significant decrease in the wet mass of the stomach and intestine (small and large). Furthermore, decreased masses are observed in other organs such as the pancreas and the kidneys, while the lungs, heart and liver are usually not affected. In the small intestine, the thickness of the serosa/muscularis layer of is not affected, but the thickness of the mucosa is severely decreased by fasting and aestivation. An example of this is found in the study of Cramp et al. (2009). In *C. alboguttata*, aestivation led to significant decreases in the wet mass of the stomach, small intestine, and large intestine, each declining by 50, 77, and 50%, respectively. Intestinal length and width of fasted frogs had also declined significantly (by 39 and 43%, respectively). Interestingly, even after 3 and 9 months of aestivation, *C. alboguttata* experiences no loss in skeletal muscle mass, long bone structure or capillary or neuromuscular junction architecture (Hudson and Franklin 2002, 2003; Hudson et al. 2004, 2005; Symonds 2007). The enterocytes of these aestivating frogs possess shorter microvilli and mitochondria. These mitochondria were largely restricted to the supranuclear region underneath the terminal web, were more spherical in shape and often displayed a disrupted inner membrane. Additional cellular changes with aestivation include a decrease in the Golgi body and smooth and rough endoplasmic reticulum. Moreover, these cells exhibited more deeply invaginated nuclei.

Refeeding after 3 months of aestivation induced rapid restoration of the GIT of frogs (Cramp and Franklin 2005). Within 2 days of their post-aestivation meal, small intestinal length, as well as the wet mass of the stomach, small intestine and large intestine had been restored to levels of fed control frogs. Similarly, villus height, enterocyte cross-sectional area, length and density of microvilli in fed frogs had returned to fed control levels within 2–6 days of feeding. Feeding not only restored the appearance of enterocyte organelles, but also resulted in a large accumulation of lipid droplets within the cytoplasm. For the Argentinian horned frog *C. ornata*, the intestinal enterocyte area decreased by 48% after a 2-week fast but was not observed to decrease any further after 1 month of aestivation, which resulted in a 45% decrease in the length of the intestinal microvilli. For the African bullfrog, *P. adspersus*, the length of intestinal microvilli was reduced by 40% after 2 weeks of fasting, and by an additional 35% after 1 month of aestivation.

GIT responses to short-term fasting (1 month) on organ mass and intestinal morphology have also been compared between fasted and fed turtles (*Chelydra serpentina*, *Sternotherus odoratus*, *Trachemys scripta*), but no significant changes were observed for the intestine, most likely due to an insufficient fasting period (Secor and Diamond 1999). For lizards, the morphological study of the intestine

for fasted and fed *Heloderma suspectum* and *Sauromalus obesus* revealed a decrease in the mass of digestive organs with fasting and a 50% decrease in enterocyte volume for the Gila monster (*H. suspectum*) (Christel et al. 2007).

In snakes, GIT responses to fasting have been studied in detail (Starck and Beese 2001, 2002; Lignot et al. 2005; Helmstetter et al. 2009a, b). Although different species have been studied, most results concern fasted and fed Burmese pythons. For this species, the fasting condition was analysed 45 days after the ingestion of a meal. Fed snakes were analysed during their postprandial period while digesting a prey representing 25% of the snake body mass. Rapid up- and down-regulations of the intestinal wall were seen to occur during the postprandial period (Secor and Diamond 1995). In both post-absorptive (short-term fast) and prolonged fasting conditions, gastric pH is high ($\sim$7.5) but drops ($\sim$2) rapidly once a prey is ingested (Secor 2003). The reduction of the small intestinal mass, intestinal mucosa width and enterocyte volume also occurs during fasting, although the thickness of the serosa/muscularis layer is barely affected (Secor et al. 2000). Similarly, in response to one month of fasting, the semi-aquatic diamondback watersnake (*Nerodia rhombifer*) experiences 38–44% reduction in small intestinal mass, intestinal mucosa width and enterocyte volume (Secor and Lignot 2010). Therefore, while enterocytes of fed snakes contain large amounts of lipid droplets, in fasting snakes these cells possess very short microvilli, are tightly packed, have no lipid droplets and are arranged in a pseudo-stratified fashion (Lignot et al. 2005; Starck and Beese 2001). No pinocytotic pits are present between the microvilli, the terminal web of the enterocytes is difficult to recognise, and paracellular spaces are not widened. These characteristics were also observed in garter snakes (Starck and Beese 2002). Furthermore, the mucosa of fasting Burmese pythons exhibits low levels of cellular replication and very low levels of expression for the sodium pump (Helmstetter et al. 2009a, b). Compared to previously fed animals, enterocyte width and volume are 23 and 80% lower, respectively (Lignot et al. 2005). Furthermore, the numerous enlarged secondary lysosomes observed in the enterocytes of fasting snakes indicate drastic intracellular digestion, while these lysosomal autophagous vacuoles are not seen in fed animals. Some of these elements are directly phagocytised by intraepithelial macrophages, while others can be present in the epithelial intercellular spaces. Furthermore, most of the lymphocytes and mast cells lined up below the basal membrane of the epithelium recycle these elements. In the Calabar ground boa (*Charina reinhardtii*), these autophagous lysosomes are huge and numerous and are still present, although reduced in size, in fed individuals (Lignot J.-H., unpublished data, Fig. 14.1).

During refeeding, intestinal enterocytes of Burmese pythons increase in width by 30% and is partly due to the absorption of luminal amino acids and lipids originating from the meal. This is most evident for enterocytes along the tips and edges of villi, many of which possess lipid droplets for several days after feeding (Starck and Beese 2001; Lignot et al. 2005). Intestinal hyperplasia through cell proliferation also occurs (Starck and Beese 2001) but is rapidly counterbalanced by an increase in apoptosis occurring at the end of the postprandial period at a

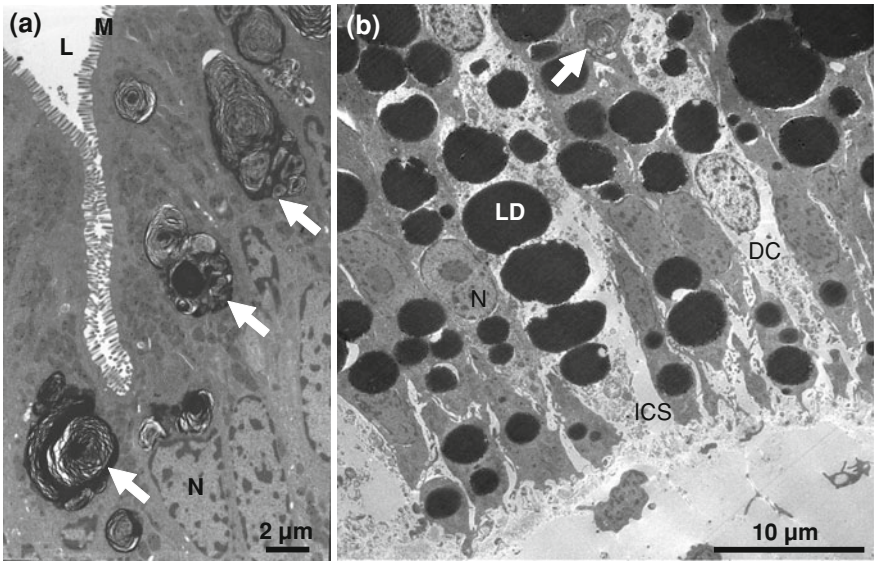

Fig. 14.1 Transmission electron micrography of the intestinal epithelium of the Calabar ground boa (*Charina reinhardtii*) after a 1 month fasting period (**a**) and 2 days after refeeding a whole prey equivalent to 25% of the snake body mass (**b**). *DC* degenerating cell, *ICS* intercellular space, *L* lumen, *LD* lipid droplet, *M* microvilli, *N* nucleus. *Arrows*: autophagous elements. Scale bars: **a** 10 µm, **b** 20 µm

higher rate than in fasting snakes (Starck and Beese 2001). This indicates that a significant portion of the mucosal cells are replaced during the postprandial period while the non-replicating cells must respond morphologically and functionally to fasting and refeeding.

The GIT responses to fasting on organ mass and intestinal morphology have also been studied with the comparison of fasted and fed American alligators and broad-nosed caiman (*Alligator mississippiensis*; *Caiman latirostris*) (Starck et al. 2007; Secor and Lignot 2010). Here, neonate and juvenile *A. mississippiensis* a relatively short fasting period (1 month) resulted in a significant decrease of the gastric mass by 10–30%, but no significant impact was observed on intestinal mass, enterocyte dimension or microvillus length (Secor and Lignot 2010). In contrast, a 3-month fast reduced the wet mass of the liver and small intestine of the caiman (*C. latirostris*) by 44 and 47%, respectively, although the thickness of the muscle layer of the small intestine remained unchanged in digesting and fasting caimans (Starck et al. 2007). Furthermore, the effects of fasting appeared similar to those found along the ophidian intestinal mucosa, i.e., the presence of a typical pseudostratified epithelium, numerous folds between neighbouring epithelial cells, reduced length of the microvilli and the absence of lipid droplets and pinocytotic pits between the microvilli. The microvilli of many enterocytes were observed to have enlarged tips, clear signs of lysis and no actin filaments. Numerous

membrane vesicles formed at the tip of the villi were seen to be dissociated from the microvilli and were obviously discharged into the lumen. Finally, lymphocytes and mast cells were lined up below the basal membrane of the epithelium in a condensed *lamina propria* possessing smaller capillaries and lymphatic vessels than in digesting caimans.

14.3.2 Endotherms

14.3.2.1 Birds

In white leghorn hens, fasting up to 20 days induced an atrophy of the midgut with a significant reduction in villus height. This decrease was gradual from the anterior to posterior intestine and was more pronounced within the first hours of fasting (Yamauchi et al. 1996). Whatever the duration of the fasting period, refeeding induced a rapid lengthening of the villi. Cell area and cell proliferation also decreased due to fasting but were rapidly activated at refeeding; significant increases in villus height were already apparent after just 3 h of feeding (Yamauchi et al. 1996; Shamoto and Yamauchi 2000). This recovery is greatly influenced by the type of realimentation diet (Shamoto and Yamauchi 2000) normal feeding allowed more rapid recovery than other protocols (e.g. enteral, parenteral refeeding; Tarachai and Yamauchi 2000). It also indicates that compensatory growth occurs at the whole body level and that the highly reduced cell proliferation occurring within the intestinal crypts during short-term fasting (3–5 days) is then increased beyond the values obtained in normally fed Leghorn chicks (Tarachai and Yamauchi 2000). Finally, in chicken farms, newly hatched chicks typically endure 2 days of fasting or more at the transition from the endogenous nutrient supply of the yolk to dependence on exogenous food intake. At this critical time, the small intestine mucosa is immature, undergoes rapid developmental changes and exhibit proliferating enterocytes along the villi (Uni et al. 1998). Fasting was seen to decrease development in the duodenum and jejunum, but was less apparent in the ileum (Geyra et al. 2001). The consequent effects at this critical time of development (e.g. number of crypts per villus, crypt cell proliferation, villus area and the rate of enterocyte migration) were more severe than a similar fasting period at later times.

These results suggest that the higher the intestinal absorptive ability is under normal feeding conditions, the more rapidly villus height will be influenced by nutritional conditions. It also demonstrates that villus height mainly varied with the numbers of epithelial cells. Finally, intracellular digestion occurs during fasting. This was demonstrated by the presence of large lysosomal autophagous vacuoles including mitochondria and dense bodies inside the enterocytes of chickens following a 20-day fast. These elements are rapidly reduced in size by refeeding (Yamauchi et al. 1996). Finally, it is worth considering that villus size, RNA and DNA concentrations within the intestinal wall are dependent on the

weight of the animals (Uni et al. 1995; Yamaushi and Isshiki 1991; Yamauchi et al. 1992).

For migrating birds, the reduction in the size and functionality of digestive organs (Bauchinger 2002; Karasov et al. 2004; Piersma 1998; Piersma and Lindström 1997) must maximise their flight performance by reducing maintenance and transport cost and resting metabolic rate (Battley et al. 2000; see also Jenni-Eiermann and Jenni, Chap. 11). For example, gizzard mass in the migratory red knot (*Calidris canutus*) is finely monitored and reduced before takeoff in the course of just 1 week, but is rapidly rebuilt through refeeding along the migratory route. Furthermore, the stopover sites seem to be selected on the basis of prey quality (van Gils et al. 2005). By stopping over at such 'hotspot' stopover sites, refuelling at maximal rate is feasible with relatively small gizzards (~8 g). This saves precious time in adjusting the gizzard size (Dekinga et al. 2001). As knots fly with ultra-light gizzards (~6 g usually), the selection of a hotspot will enable them to fuel at full speed after relatively little 'gizzard-adjustment-time'. And, on departure from the hotspot, they therefore lose little time shifting back to a 'cheap-flight gizzard'. As studied in the Japanese quail (*Coturnix japonica*) the flexible response of the gizzard mass when food quality is reduced (switch from standard to high-fibre diet) appeared to be due to hypertrophy and hypotrophy of the smooth muscle cells; yet, while under similar experimental conditions, the phenotypic plasticity of the small intestine results from cell proliferation (Starck and Rahmaan 2003).

In the blackcap (*Sylvia atricapilla*), another long-distance migratory bird species, fasting for 2.5–3 days can reduce by 52% the mass of the small intestine, pancreas, and liver (Karasov and Pinshow 1998). However, the stomach mass is not markedly reduced. A similar result was observed in chickens following a 4-day fast (Yokota et al. 1992). Although the muscle layer underlying the intestinal mucosa is not affected by fasting in blackcaps, the length of the villi is reduced and disintegration of the distal part of the villi also occurs (Karasov et al. 2004). These alterations must induce a delay for regaining body mass at refeeding, most probably due to the restoration processes of the digestive organs (McWilliams and Karasov 2001; Karasov et al. 2004).

In common barn owls (*Tyto alba alba*), winter-induced fasting may be an important cause of mortality when deep snow cover leads to a scarcity of prey (Glue 1973; Marti and Wagner 1985). The effects of prolonged fasting on the intestinal morphology and function have therefore been studied according to the different successive physiological phases occurring in this species like for other fasting birds and mammals (Raul F., unpublished data). In the common barn owl, phase I lasts less than a day, phase II up to 6 days and phase III occurs within 7–10 days of starvation (Handrich et al. 1993). For this species, intestinal mucosal mass and protein content of the anterior part of the midgut decreased by 40% in phase II fasting individuals while a further decrease was observed in phase III (50% in the duodenum and 60% in the jejunum). Protein content of the ileal mucosa was further pronounced (70%) compared to nourished controls (Raul et al., unpublished data, Table 14.1). The length of the intestinal villi in the

Table 14.1 Mucosal mass and protein content of the small intestine (duodenum, jejunum and ileum) in the nourished and food-deprived barn owl

Groups	Mucosal mass (g/cm)			Mucosal protein content (mg/cm)		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Nourished ($n = 4$)	0.17 $\pm 0.02^a$	0.16 $\pm 0.01^a$	0.05 $\pm 0.003^a$	27.25 $\pm 0.85^a$	23.00 $\pm 2.35^a$	6.75 $\pm 0.85^a$
Phase II ($n = 6$)	0.11 $\pm 0.002^b$	0.08 $\pm 0.003^b$	0.02 $\pm 0.002^b$	16.38 $\pm 0.32^b$	13.00 $\pm 0.53^b$	3.00 $\pm 0.016^b$
Phase III ($n = 5$)	0.09 $\pm 0.005^c$	0.06 $\pm 0.005^c$	0.02 $\pm 0.002^b$	14.00 $\pm 0.73^c$	9.20 $\pm 0.73^c$	2.20 $\pm 0.20^c$

Values are mean ± SEM. The number of animals per group is in brackets. For each intestinal segment a $\neq$ b $\neq$ c, $p < 0.05$

duodenum, jejunum and ileum was also reduced by 30, 40 and 70%, respectively. In the distal segment of the midgut, numerous villi also appeared to be broken, with a disorganised mucosa. Therefore, the anterior and mid segments seem to be better preserved during prolonged fasting and this might allow better functional maintenance during food deprivation. Also, enzyme activities expressed as specific activities in isolated brush border membranes along the brush border of the enterocytes are higher than in nourished individuals (Fig. 14.2). This increase in hydrolase activities might be related to a lower breakdown of these proteins in the membrane during fasting (and thus a preservation of the absorbing capacities of the cells), but could also be explained by the fact that the enterocytes lining the villi exhibit a higher degree of maturation due to lower cell renewal during fasting. This would be consistent with data obtained from other bird species (Yamauchi et al. 1996).

Changes in intestinal morphology have also been studied in female mallards (*Anas platyrhynchos*) that have reached their second and third metabolic phases during fasting (Geiger et al. 2009). Ducks can experience this type of energetic challenge during the wintering period and may even starve to death (Dobinson and Richards 1964; Owen and Cook 1977; Suter and Van Eerden 1992; Pawlina et al. 1993). Experimentally, villus atrophy proved to rapidly occur through fasting (33% in phase III), along with profound epithelial damages and disintegration at the tip of the intestinal villi. However, cell proliferation within the intestinal crypts is not modified. Despite profound epithelial disintegration, refeeding is successful whatever the fasting phase reached by each individual and/or its duration. The absorption of nutrients in the jejunum seemed to be restored rapidly as the length of the villi increased by one-third and the proportion of cells in DNA synthesis increased by 70% within 24 h of refeeding.

Finally, another adaptation worth mentioning is the modulation of the gastric pH and motility, allowing the long-term preservation of stomach contents as seen in incubating king penguins (*Aptenodytes patagonicus*) (Gauthier-Clerc et al. 2000; Thouzeau et al. 2003). During the final part of egg incubation in king penguins, these birds are able to store undigested food in their stomach for up to

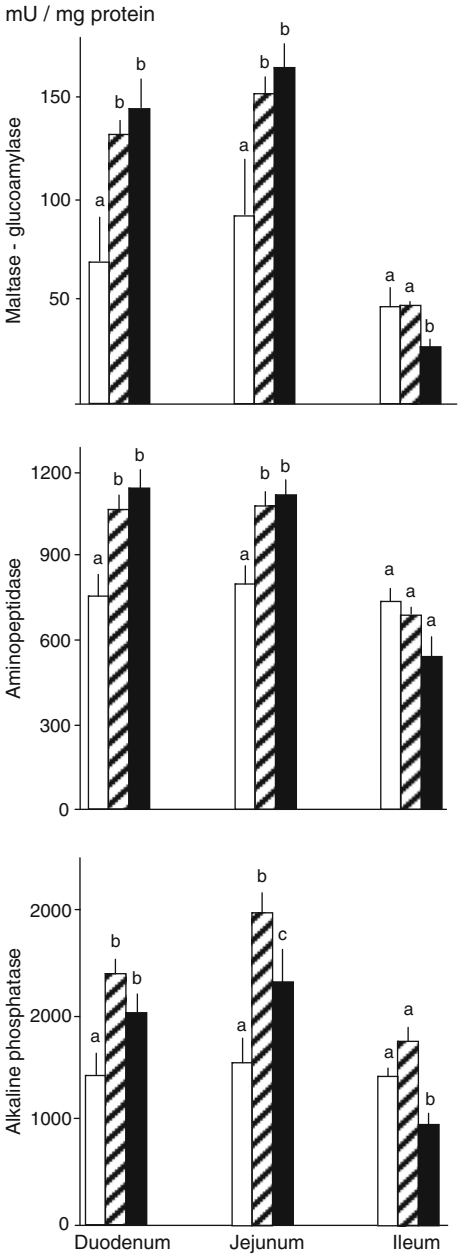

Fig. 14.2 Maltase-glucoamylase, aminopeptidase and alkaline phosphatase expressed as specific activities (mU/mg protein) isolated brush border membranes in the duodenum, jejunum and ileum of barn owls. Nourished controls (open column, n = 4), after a fasting period of 4–6 days corresponding to phase II (hatched column, n = 6) or after a fasting period of 7–10 days corresponding to phase III (black column, $n = 5$). Results are mean ± SE. For a given hydrolase and for a given intestinal segment, columns that do not share a common superscript letter differ significantly; a ≠ b ≠ c, $p < 0.05$

3 weeks during their incubation fast. This adaptation ensures the survival of the hatchling if the mate's return is delayed. Among the main underlying mechanisms are: a relatively high luminal pH value (∼6) unfavourable for avian gastric

proteinase activity, a markedly reduced gastric motility and the increased luminal concentration of spheniscin, an antimicrobial peptide (AMP) belonging to the beta-defensin subfamily. These characteristics allow conservation of the stomach content despite a gastric temperature maintained at 38°C.

14.3.2.2 Mammals

In the small intestine, previous studies have shown that short and long-term fasting induces a drastic resorption of the intestinal lining, with the atrophy of the intestinal villi worsening over time (Aldewachi et al. 1975; Altmann 1972; Brown et al. 1963; Goodlad et al. 1988; Hopper et al. 1968; Sohma 1983). More recent data are also available concerning the effects of prolonged fasting on the intestine according to the different metabolic fasting phases endured by the animals (Dunel-Erb et al. 2001; Galluser et al. 1991; Habold et al. 2004, 2005, 2006, 2007). Mucosal mass can be decreased by 50% in phase II fasting rats and by more than 60% in phase III fasting rats (Dunel-Erb et al. 2001; Galluser et al. 1991). Villus length, thickness, and volume density (percentage of the absorptive mucosa versus the mucosal generative tissue containing the crypts) as well as the dimensions of the crypts of Lieberkühn are reduced during the different metabolic fasting phases, villi being reduced by 37 and 55% in phase II and phase III fasting animals respectively in comparison to normally fed rats (Dunel-Erb et al. 2001). The smooth muscle cells present in the *lamina propria* are contracted, the lacteals inside this layer are drastically retracted towards the base of the villi and the tip of the *lamina propria* is retracted leaving numerous vacuoles between this layer and the epithelium (Dunel-Erb et al. 2001; Habold et al. 2007). Within the epithelium, enterocyte width is only slightly reduced at the tip of the villi and unaltered at the base of the villi in phase III fasting animals compared to normally fed rats. Prolonged fasting also induces subcellular changes in the basal part of the enterocytes such as the presence of small vesicles and many autolysosomes of various sizes and shapes, atrophied mitochondrion-like bodies, short and sparse rough-surfaced endoplasmic reticulum and reduced density of the cytoplasm (Sohma 1983). However, there is no disintegration of the entire epithelium (as seen in birds and metamorphic anurans) and the apical brush border of the intestinal enterocytes is far better preserved than in fasting species that drastically down-regulate their intestinal epithelium (as seen in snakes and anurans). For instance, microvilli are slimmer and longer (although often crooked) in fasting rats compared to those observed in normally fed animals inducing, thus, an increased surface area despite their largely reduced density (Dunel-Erb et al. 2001).

The morphological preservation of the microvilli has been considered as a compensatory response to maximise absorptive functions during fasting and immediately after refeeding. In this view, a study of the expression of some key nutrient transporters showed that while some are down-regulated during fasting, others are maintained or even upregulated. For example, FATP4 (fatty acid transport protein), GLUT2 (hexose transport by facilitated diffusion) and GLUT5

(fructose transport by facilitated transport) all decreased due to fasting, but rapidly increased again within hours of refeeding, with GLUT2 translocated to the apical membrane (Habold et al. 2006). However, the expression of other brush border transporters such as PepT1 increased not only during short fasting periods (Ogihara et al. 1999; Ihara et al. 2000) but also after prolonged phase III fasting during both metabolic fasting phases, or down-regulated in phase II and over-expressed in phase III fasting rats (e.g. the sodium-glucose cotransporter SGLT1). This over-expression is explained by intestinal glucose uptake rates that are higher in phase III fasted rats than in phase II fasted and fed rats (Habold et al. 2006). Similarly, a 72 h-fast causes an overall increase in glucose absorption in rats (Das et al. 2001). Fasting also induces an increase in gluconeogenesis in the small intestine, producing up to one-third of total endogenous glucose after 72 h of fasting (Mithieux et al. 2004). This is concordant with the increased mRNA, protein levels and activities of key gluconeogenic enzymes (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) in phase III fasting rats (Habold et al. 2006). During this metabolic phase, gluconeogenic precursors may be amino acids resulting from protein catabolism, whereas glycerol may be the main gluconeogenic precursor in the phase II fasting period. Finally, in the absence of intestinal GLUT2 during fasting, glucose-6-phosphatase could be involved in glucose release to the blood stream via membrane trafficking.

The effects of prolonged fasting on other enzyme activities (sucrase, lactase and aminopeptidase) located along the brush border of the intestinal enterocytes have also been studied in sham-operated or thyroidectomised phase II and phase III fasting rats (Galluser et al. 1991). In the jejunum of sham-operated animals, starvation led to an increase in lactase activity and a decrease of the aminopeptidase activity in the brush border membrane (measured as mU/mg protein^{-1}). Sucrase activity decreased during starvation but was restored in the thyroidectomised phase III fasting rats to the values found in normally fed rats. Thyroid hormones are involved in the regulation of the brush border hydrolase activities (Koldovsky 1981) and also activate protein breakdown (Goldberg et al. 1980). However, thyroidectomy reduced protein loss of the brush border membrane to only 18% in phase III fasting rats while this was reduced to 47% in sham-operated phase III fasting rats, indicating that the drop in plasmatic thyroid hormones during fasting allows a better maintenance of hydrolase activities in the apical brush border of the enterocytes.

It has also been demonstrated that proliferation rate and cell mitosis in the intestinal crypts are modulated by the metabolic status reached by fasting animals, these cellular events being depressed during short-term fasting (phase II of fasting) (Aldewachi et al. 1975; Altmann 1972; Brown et al. 1963; Clarke 1975), whereas crypt cell proliferation and enterocyte migration rates along the crypt-villus axis are increased in phase III fasting animals (Habold et al. 2004). This intestinal mucosa renewal in phase III fasting animals has been considered as an anticipatory process prior to refeeding. This cellular restoration could explain why jejunal repairs are complete after 3 days of refeeding following either phase II or phase III (Habold et al. 2004). It occurs even in the absence of food ingestion or the exertion

of other external stimuli and is concomitant with the induction of the 'refeeding signal' which has been suggested to operate below a critical metabolic status in fasting birds (Groscolas and Robin 2001; Robin et al. 1998) and with increased resting energy expenditure in *anorexia nervosa* patients near death (Rigaud et al. 2000). The levels of energy depletion reached by fasting animals also affect apoptosis at the tip of the intestinal villi (Habold et al. 2004, 2006). While apoptosis increases during short-term fasting (phase II of fasting) (Iwakiri et al. 2001; Kakimoto et al. 2008), it is absent during phase III, contrary to the case in normally fed and phase II fasting animals. This is strongly correlated with the decreased expression of both the pro-apoptotic cytokines TNFα and TGFβ1 and the intestinal specific transcription factor Cdx2 (Habold et al. 2006). Finally, these events (preservation of the absorption capabilities of the villi by the suppression of apoptosis and the initiation of cell proliferation during phase III fasting) coincide with a peak of locomotor activity already observed in phase III fasting animals. This is induced by a rise in plasma corticosterone and reflects the search for food (Challet et al. 1995; Koubi et al. 1991).

More recent data indicates that the initial intestinal increase in apoptosis in fasted rats (short-term fasting equivalent to phase II fasting) is mainly regulated by the type I pathway and that endogenous production of nitric oxide and reactive oxygen species (ROS) is induced (Fujise et al. 2006; Iwakiri et al. 2001; Ito et al. 2010). Also, indigestible material (e.g. expanded polystyrene) can attenuate this enhanced apoptosis without having any effect on cell proliferation, most probably by inducing mechanical stimuli (Kakimoto et al. 2008). This may explain why geophagy occurs in wild animals and humans during fasting conditions or when food supplies are limited (Krishnamani and Mahaney 2000; Vermeer and Frate 1979) although this behaviour is also linked to other benefits (Klein et al. 2008) (see also McCue et al., Chap. 8). Finally, complementation with clay, another indigestible material, does not optimise the functionality of the GIT during prolonged fasting but does favour intestinal nutrient absorption during refeeding despite reduced food uptake (Reichardt et al. 2011).

14.4 Conclusion

The overall GIT response to fasting and starvation in terrestrial vertebrates presents shared characteristics that most probably result from evolutionary traits. These have been adapted according to the species and the specificity of the fasting episodes. Transient atrophy of the digestive system through fasting is the most evident of these shared traits. It mainly occurs in the mucosal layer, while the thickness of the serosa/muscularis layer is not affected. In species undergoing long episodes of fasting in a repetitive manner (e.g. snakes and anurans) this atrophy can lead to a 60% loss of the intestinal wet weight. Fasting and refeeding implies a reorganisation of the mucosal layer, with rapid movement of the lacteals, hyper/hypotrophy and hyper/hypoplasia of the absorbing enterocytes. One extreme of the

adaptive range to fasting is the drastic epithelial transformation occurring in some species (e.g. snakes and anurans) with repeated feeding and fasting cycles. This up- and down-regulation of the GIT is advantageous but demanding on the enterocytes, which not only have to absorb large quantities of nutrients during feeding (e.g. bulk quantities of lipids) but also recycle numerous proteins and cell elements (e.g. cell membranes) at the end of the postprandial period. The timing for cell renewal is also finely tuned. In other species, despite the intestinal atrophy, a ready-to-use intestinal epithelium is maintained and subcellular changes are modest but decisive (i.e. preservation of the brush border membrane functionality). However, increased apoptosis (perhaps as potential energy source for the other enterocytes) and reduced cell renewal (allowing villus shortening and also acting as an economical means to reduce the maintenance cost of the intestine) may lead to the partial or severe disintegration of the epithelium (as seen in some birds). Strikingly, laboratory rats can switch their intestinal morphology and functionality in relation to the threshold of body fuel depletion reached during prolonged fasting. However, other examples of this anticipatory process prior to refeeding which allows intestinal mucosa renewal are still lacking in literature to date. Nevertheless, all these examples highlight the variety of responses that the GIT can encompass, although using common subcellular and histological tools. However, it is still unclear how the GIT can be so finely tuned. Potential hypotheses to be explored include luminal signals, mechanical stimuli, intestinal flora, brain gut peptides, other humoral factors and neuronal control. The precise mechanisms at play are only just beginning to be unraveled in snakes and rodents (i.e. Costello et al. 2010; Ito et al. 2010), and future studies will hopefully describe these mechanisms in other species successively enduring the different metabolic fasting phases.

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Chapter 15
Changes in Fatty Acid Composition During Starvation in Vertebrates: Mechanisms and Questions

Edwin R. Price and Teresa G. Valencak

15.1 Introduction

Fatty acids have many important functions in animals as energy, structural, and signaling molecules. During starvation, vertebrates rely heavily on stored fat to meet energy demand, may remodel membranes to control metabolism, and must control various physiological processes with eicosanoids (fatty acid-derived hormones). Many different fatty acids are commonly found in vertebrates, and the composition of these fatty acids (i.e., the molar proportions of the fatty acids as part of total fatty acids) can affect these various functions. Animals often undergo changes in fatty acid composition during starvation, and this has led to various adaptationist explanations for these alterations. However, changes in fatty acid composition that are observed during starvation may simply be a result of ongoing physiological processes, and might not themselves represent an acclimation response to starvation.

In this chapter, we will review the changes in fatty acid composition that occur during starvation in vertebrates, including periods in which animals do not feed such as hibernation. We will examine many of these changes in light of two important processes that should occur even in the absence of an acclimation

E. R. Price (✉)
Department of Forest and Wildlife Ecology,
University of Wisconsin-Madison, Madison, WI 53706, USA
e-mail: eprice2@wisc.edu

T. G. Valencak
Research Institute of Wildlife Ecology, University of Veterinary Medicine,
Savoyenstrasse 1, 1160 Vienna, Austria

T. G. Valencak
Institute of Biological and Environmental Sciences, University of Aberdeen,
Aberdeen, AB24 2TZ, Scotland, UK

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response, and thus we seek to provide a baseline against which to measure the physiological importance of fatty acid compositional changes.

15.1.1 Definitions and Notation

Here we treat 'starvation' and 'fasting' synonymously, referring to any extended period in which an animal does not feed. While 'lipid' can refer to many lipid-soluble compounds (e.g., cholesterol), in this chapter we will focus on fatty acids and some compounds built from fatty acids: triacylglycerols and phospholipids. Fatty acids can have zero double bonds joining their carbon atoms ('saturated'), or have one ('monounsaturated') or more ('polyunsaturated') double bonds in the carbon chain. Fatty acids are denoted by the number of carbon atoms and the number of double bonds followed by the position of the most terminal double bond (e.g., 18:2ω6 has 18 carbons and 2 double bonds, the first of which is located at the 6th carbon from the methyl end). The common polyunsaturated fatty acids (those of the ω6 and ω3 families, e.g., 18:2ω6 and 18:3ω3) are essential for vertebrates as they cannot be synthesized de novo but must be obtained from the diet, although they can be modified to longer chain lengths and more double bonds.

15.2 Two Important Processes in Fatty Acid Utilization During Starvation

15.2.1 Utilization of Fuel Lipids Versus Structural Lipids During Starvation

Triacylglycerols are composed of three fatty acids esterified to a glycerol moiety, and are usually considered energy storage molecules. The composition of triacylglycerols can be readily modified by dietary fatty acid composition (Simandle et al. 2001; Thil et al. 2003; McCue et al. 2009; Price and Guglielmo 2009), indicating that some dietary fatty acids can be directly deposited into triacylglycerols intact (Klasing 1998). Dietary fatty acids may also undergo some modification in the liver (e.g. elongation, desaturation) before deposition in triacylglycerols. Dietary fat content can also affect the composition of triacylglycerol stores; low fat/high carbohydrate diets can increase de novo synthesis of fatty acids (Griminger 1986; Nelson 1992; Hudgins 2000). De novo synthesized fatty acids are generally limited to 16:0, 16:1, 18:0, and 18:1 in vertebrates. Triacylglycerols are primarily stored in adipose tissue/fat bodies, and can also be found in intramuscular fat droplets.

Phospholipids are found in every cell as constituents of membranes. They are synthesized from glycerol and two fatty acids, sharing a common anabolic

pathway with triacylglycerol. However, instead of a third fatty acid, these lipids are attached to a phosphate-containing head group. Phospholipids are not as malleable with regard to fatty acid composition as triacylglycerol. Although some degree of manipulation can be achieved by diet, the range of composition is much reduced, and is often primarily found in changes in ω3 and ω6 fatty acids, with the total percent of polyunsaturated fatty acids staying relatively constant (Ayre and Hulbert 1997; Newman et al. 2002; Thil et al. 2003; Turner et al. 2004; Guderley et al. 2008; Nagahuedi et al. 2009; Price and Guglielmo 2009). Phospholipids in some tissues, notably in the brain, are particularly resistant to modification (Zar 1977; Tidwell et al. 1992; Thil et al. 2003; McCue et al. 2009). This refractivity to diet may result from lower turnover of phospholipids relative to triacylglycerol (thus slowing any changes to phospholipid fatty acid composition), as well as homeostatic regulation of the fatty acid composition of membranes. This difference between triacylglycerol and phospholipids in their variability of fatty acid composition presumably reflects their functions. Phospholipids must maintain fluidity within a certain tolerance, interact with membrane-bound proteins, maintain ion gradients, and perform other structural roles; triacylglycerols are simply stored in a droplet for later oxidation. The composition of fatty acids in phospholipids and triacylglycerols is generally distinct; phospholipids usually contain a high proportion of polyunsaturated fatty acids, while triacylglycerol usually contains high proportions of saturated and monounsaturated fatty acids (Nelson 1962; Delgado et al. 1994; Price and Guglielmo 2009).

The compositional difference between triacylglycerols and phospholipids has important implications for the total fatty acid composition changes that occur during starvation. Unfortunately, often only total lipids are measured, which lumps together the triacylglycerol, phospholipid, and non-esterified fatty acid fractions. During a period of food deprivation, an animal is expected to draw on its fuel reserves, by mobilizing and oxidizing fatty acids from triacylglycerols. Because of this, the total amount of stored triacylglycerol decreases during fasting/starvation, sometimes substantially. On the other hand, we should not expect large decreases in total structural lipids (phospholipids) during fasting/starvation. Cells should resist the loss of functional structures (including plasma membrane and intracellular membranes), particularly during short-term fasting. Changes in the composition of total fatty acids may therefore reflect the changing ratio of triacylglycerol to phospholipids. As triacylglycerol is reduced, fatty acids found primarily in phospholipids (e.g. 22:6ω3, 20:4ω6, and other polyunsaturates) can be predicted to become more abundant as a proportion of total lipids (Fig. 15.1). If triacylglycerols represent a substantial fraction of the tissue lipids (e.g., in adipose tissue), the rate of change in the proportion that is polyunsaturated will be small at the beginning of starvation. However, as triacylglycerol stores become depleted, the proportion that is polyunsaturated will rapidly approach the proportion in the phospholipids. If triacylglycerols initially represent a small fraction of the tissue lipids, this curve will be left-shifted, and rapid changes should occur sooner.

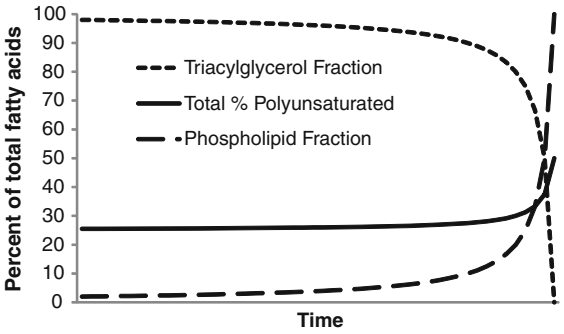

Fig. 15.1 Predicted changes in polyunsaturated fatty acids during starvation based on the differential oxidation of membrane lipids (phospholipids) and storage lipids (triacylglycerol). A simple hypothetical model is illustrated where the % polyunsaturated is constant within the phospholipid fraction (50%) and within the triacylglycerol fraction (25%), and the total triacylglycerol mass is reduced by a constant amount daily. Total phospholipid mass was assumed not to change. When triacylglycerol makes up a large fraction of the total lipids (e.g., at the beginning of a fast), the polyunsaturated proportion in the tissue is similar to that in the triacylglycerol alone (25%). As triacylglycerol is reduced over time (simulating the oxidation of fat), the polyunsaturated proportion in the tissue rises to approach the proportion in the phospholipids alone (50%)

Fig. 15.2 Fatty acids that are polyunsaturated or have shorter carbon chains tend to be mobilized faster than those that are saturated and have longer carbon chains. The order of fatty acids shown here follows Raclot (2003)

15.2.2 Baseline Selective Mobilization of Fatty Acids

While triacylglycerols can be expected to be utilized faster than phospholipids, the individual fatty acid components of those triacylglycerols are not utilized at equal rates. Studies in mammals, birds, and fish demonstrate that at the tissue level, certain fatty acids are selectively mobilized from adipose tissue (mobilized in greater proportion than their proportion in adipocyte triacylglycerol), while other fatty acids are selectively retained (Groscolas 1990; Raclot and Groscolas 1993; Raclot et al. 1995; Connor et al. 1996; Halliwell et al. 1996; Raclot 2003; Hazel and Sidell 2004; Price et al. 2008). Specifically, fatty acids with more double bonds, and those with shorter chain lengths are preferentially mobilized (Fig. 15.2). As this pattern of mobilizing certain fatty acids while retaining

others is common across several vertebrate taxa, we will therefore consider it 'baseline selective mobilization' ('selective' in this sense refers only to the differentiation of the various fatty acids during mobilization, not to natural 'selection').

Shorter and more unsaturated fatty acids tend to be relatively more water soluble and hydrophilic, and a mechanism for selective mobilization may lie in the likelihood of particular triacylglycerol-bound fatty acids to encounter hormone-sensitive lipase at the fat droplet/water interface (Raclot 2003). Therefore, the selective mobilization of these fatty acids may not indicate an adaptive preference for their mobilization, but instead may be merely a consequence of the solubility properties of the individual fatty acids. Selective mobilization/retention is not a property inherent to the fatty acid molecule, but instead is relative to the overall composition of the triacylglycerol in question. Thus we should not expect absolute rates of selective mobilization to be invariant across experiments, but we should expect a similar rank-order of fatty acids with respect to selective mobilization.

Oxidation of fatty acids is also a selective process. In vitro studies in birds (Price et al. 2011) and fish (Sidell et al. 1995), and an in situ study in humans (Hagenfeldt and Wahren 1968) have demonstrated that muscles preferentially oxidize unsaturated fatty acids. The mechanism of this effect is unclear, but could be related to activity of carnitine palmitoyl transferase, a mitochondrial membrane fatty acid transporter. This enzyme is thought to largely control flux through the fatty acid oxidation pathway (Spurway et al. 1997; Eaton et al. 2001), and its activity is generally higher with shorter chain lengths and greater unsaturation of the fatty acid substrate (Gavino and Gavino 1991; Cake et al. 1998; Price et al. 2011), although this pattern may vary somewhat among taxa (Egginton 1996; Stonell et al. 1997; Price et al. 2011). The preferential use of unsaturates could also reflect their greater diffusional mobility in water. Whatever the mechanism, the pattern of preferential oxidation of fatty acids in muscle appears to match fairly well the patterns of mobilization from adipocytes (Hazel and Sidell 2004; Price et al. 2011), with unsaturates, and tentatively, shorter chain fatty acids, being preferentially oxidized. This pattern can be seen at the whole-animal level as well; orally dosed rats (Leyton et al. 1987) and birds (McCue et al. 2010) oxidize unsaturated and shorter chained fatty acids faster than saturated and longer chained fatty acids.

What effect should these tissue level processes have on lipid changes in starving animals? If shorter and more unsaturated fatty acids are preferentially mobilized, then during a period of fasting we should expect adipose triacylglycerol stores to become more enriched (as a proportion) with saturated and longer chain fatty acids (Fig. 15.3). This process is likely to depend on the starting composition of the triacylglycerol, and may approach a limit over time.

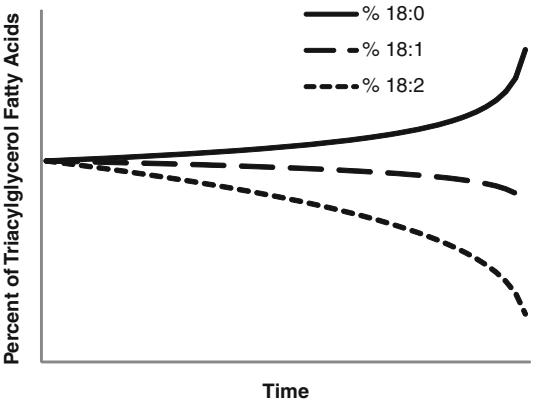

Fig. 15.3 Predicted changes in triacylglycerol fatty acids based on baseline selective mobilization of fatty acids. A hypothetical composition starting with equal amounts of three 18-carbon fatty acids is depicted for ease of comparison. Selective mobilization was assumed to increase with increasing unsaturation, and this pattern was assumed to be invariant over the duration of the starvation period. In this hypothetical example, 18:0 was relatively retained, while 18:1 and 18:2 were relatively mobilized during the fast

15.3 Changes in Fatty Acid Composition Observed During Starvation

While the above processes are predicted to be important during starvation, in this section we will review the literature and examine the changes that are actually observed in starving animals. In many cases we examine the original published data and draw our own interpretations of the data in light of these two processes.

15.3.1 Changes in Whole-Animal Fatty Acid Composition

Given the importance of triacylglycerol oxidation during starvation, one might expect that changes to whole-animal fatty acid composition would be dominated by the changing ratio of triacylglycerol to phospholipids. This is supported by a recent study of squamate reptiles. McCue (2008) studied whole-body fatty acid composition during starvation in five snakes and a monitor lizard. Percent composition of saturated, monounsaturated, and polyunsaturated fatty acids remained relatively constant through much of the fast, but at the end of the period, the saturated proportion rapidly decreased, while the polyunsaturated proportion rapidly increased in nearly all species. McCue (2008) did not separate the phospholipid fraction in this study, but generally membranes are enriched with polyunsaturated fatty acids (for an example in reptiles, see Turner et al. 2005), so these data are consistent with this explanation. This phenomenon may also explain

the substantial increase in the proportion of 20:4 in common eiders (*Somateria mollissima*) that fast during egg-laying and incubation (Parker and Holm 1990); 20:4 is a common component of membranes, and is relatively enriched in phospholipids compared to neutral lipids in other bird species (Maillet and Weber 2006; Klaiman et al. 2009; Price and Guglielmo 2009).

In rats (*Rattus novegicus*) that were alternatively fed for 72 h and fasted for 24 h, carcass lipids (whole-body minus liver and adipose) became relatively enriched with 20:4ω6 and 22:6ω3 after each fast (Chen et al. 1995). Both are important components of membranes in rats (Helge et al. 2001), but are not major components of rat triacylglycerol (Chen et al. 1995), indicating that phospholipids are being retained while triacylglycerols are oxidized.

In northern pike (*Esox lucius*), 22:6 increased 20% as a proportion of total lipids during a 2-month period of starvation (Kluytmans and Zandee 1973a), and this might represent its greater proportion in phospholipids than in triacylglycerol (Kluytmans and Zandee 1973b). However, other fatty acids (notably 20:4, 20:5, and 22:5) increased substantially as a fraction of total body lipids, yet each of these is found in higher proportion in triacylglycerols than phospholipids in pike (Kluytmans and Zandee 1973a, b). Moreover, 20:4 and 20:5 are highly unsaturated and tend to be relatively mobilized fatty acids, making their retention in starving pike more remarkable.

15.3.2 Changes in Adipose Fatty Acid Composition

Although many studies of adipose fatty acid composition do not separate phospholipids from triacylglycerols, the bulk of adipose tissue is likely to be triacylglycerols, and at least as a null hypothesis, one might expect that baseline selective mobilization patterns would dominate any changes during starvation. This is, in fact, observed in several species of mammals and birds. In weight-cycled rats, fatty acids that are typically highly mobilized (20:5ω3, 16:1ω7, 18:3ω3) decreased in percentage of adipose tissue during 24 h fasting periods, while the less mobilized 18:1ω9 was disproportionately retained during these periods (Chen et al. 1995). An exception to this general trend occurred with 18:2ω6, a relatively mobilized fatty acid which was nonetheless retained in adipose tissue of the fasting rats. Interestingly, 20:4ω6 and 22:6ω3 (moderately to highly mobilized fatty acids) were essentially unchanged or decreased in adipose tissue during these fasts, even though both were relatively enriched in the remaining carcass lipids during the 24-h fasts (noted in Sect. 15.3.1). This finding highlights the importance of the differential effects of baseline selective mobilization and membrane lipid retention operating at the adipose tissue and whole-body levels, respectively.

During its 110-day breeding fast, the male emperor penguin (*Aptenodytes forsteri*) exhibits changes in adipose composition that are in accordance with the baseline pattern of selective mobilization (Groscolas 1990). For example, 20:5ω3

is highly mobilized, while long chain monounsaturates are retained during the fast, a finding that mirrored in vitro results with penguin adipocytes (Groscolas 1990). Similarly, the adipose tissues of pheasants (*Phasianus colchicus*) were more enriched with long chain fatty acids and more depleted of short chain and unsaturated fatty acids following 4 days of food deprivation (Mustonen et al. 2009). Interestingly, some polyunsaturated fatty acids that are normally selectively mobilized (e.g. 22:6ω3, 20:4ω6, 20:4ω3) instead increased (as a percentage) in adipose tissue during starvation. The selective retention of these polyunsaturates may indicate that these fatty acids were abundant in phospholipids but not triacylglycerols. The fatty acids in question represented a small fraction of the total lipid mass (<1%; Mustonen et al. 2009), further supporting this conclusion. This illustrates an important point; as triacylglycerol stores near depletion we can expect the effect of baseline selective mobilization to be less important, and the effect of a changing phospholipid to triacylglycerol ratio to become dominant in terms of causing changes to total fatty acid composition, even in adipose tissue. This occurs because, near depletion, the total mass of a given fatty acid in the triacylglycerols decreases enough such that the mass of the same fatty acid in the phospholipids is relatively large.

Raclot and Groscolas (1995) examined the changes in fatty acid composition, as well as the in vitro selective mobilization rates, of white adipose tissue in rats fasted 7 or 10 days (representing losses of 56 and 90% of original adipose mass, respectively). Isolating triacylglycerol (and thus excluding membrane phospholipids), they found that in vivo changes in adipose composition were highly correlated with in vitro selective mobilization rates. Moreover, although nominal rates of selective mobilization differed between 7- and 10-day fasts, the rank-order of mobilization of the various fatty acids was substantially similar, and at both sampling times mobilization was related to structural properties such as chain length and unsaturation in the predicted pattern (Raclot and Groscolas 1995).

Groscolas and Herzberg (1997) studied fasting-induced changes to brown adipose tissue in rats. Changes to triacylglycerol in this tissue after 7 or 10 days fasting was similar to those observed in white adipose tissue, with the notable exception of 18:2ω6 which was paradoxically selectively retained. This retention of 18:2ω6 was also observed in brown adipose tissue following a 3-day fast in rats (Ohno et al. 1994). Phospholipids from rat brown adipose tissue did not decrease as much as triacylglycerols during fasting, and changes in their concentration did not follow similar structural rules. The total mass of some phospholipid fatty acids actually increased during the fast, including 18:2ω6.

Several studies of starving carnivores have examined the changes that occur in fatty acid composition at multiple white adipose depots. These studies in marten (*Martes americana*), mink (*Mustela vison*), raccoon dog (*Nyctereutes procyonoides*), polecats (*Mustela putorius*), and sable (*Martes zibellina*) show that the baseline pattern of selective mobilization is important in nearly all of the adipose locations examined (Nieminen et al. 2006a, b, 2009; Mustonen et al. 2007; Nieminen and Mustonen 2007). In contrast to subcutaneous and intra-abdominal depots, the fatty acid composition of adipose tissues in the extremities (paws and

tail) of marten were relatively unchanged by a 2-day fast (Nieminen et al. 2006b), but it is not clear whether the overall mass of these tissues was decreased during the fast. Nieminen et al. (2006a, b) note that there are some slight differences between the other adipose depots with regard to the mobilization pattern, although this was primarily restricted to polyunsaturated fatty acids that may have been part of membranes.

Overall, baseline selective mobilization appears to dominate changes to white adipose fatty acid composition during starvation and fasting. An important exception may occur when animals lower their core body temperature, both daily and seasonally, in order to save energy. Yellow-bellied marmots (*Marmota flaviventris*), which do not feed during hibernation, also selectively retained 18:2ω6 in adipose tissue during the hibernation period, even though 18:1ω9 was unchanged (Florant et al. 1990). Similarly, the proportion of 18:2ω6 increased in echidnas (*Tachyglossus aculeatus*) and alpine marmots (*Marmota marmota*) during hibernation (Falkenstein et al. 2001; Arnold et al. 2011). In these studies, triacylglycerol was not separated from phospholipids, leaving open the possibility that these changes were caused by an increase in membrane lipids as a proportion of the total. However, incubated adipocytes isolated from marmots selectively retained 18:2ω6 in triacylglycerols while mobilizing saturated fatty acids, corroborating this finding at the tissue level (Cochet et al. 1999). This retention of 18:2ω6 might be achieved by upregulation of monoacylglycerol acyltransferase, an enzyme that re-esterifies fatty acids to monoacylglycerols to make triacylglycerol (Xia et al. 1993). Selective retention of 18:2ω6 at the expense of ω3 polyunsaturates has been observed in white adipose tissue and also in brown adipose tissue total lipids in hibernators (Arnold et al. 2011). In general however, brown adipose tissue total lipid composition resembles more closely the fatty acid composition of the organ phospholipids such as heart and liver than that of white adipose tissue lipids (Arnold et al. 2011). This is well documented, as brown adipose tissue neutral lipids soon get depleted when the tissue is activated (Rafael et al. 1985) so the special thermogenic role of brown adipose tissue can explain its distinct compositional pattern.

Over the past few decades it has been shown that the occurrence of and depth of torpor in hibernators and daily heterotherms are influenced by the proportion of polyunsaturated fatty acids in their body fats (reviewed by Munro and Thomas 2004; Ruf and Arnold 2008). This effect has been attributed to the lowered melting points of the fats and thus the increased fluidity of the membranes and triacylglycerol stores. Yet, the effects on membrane fluidity do not fully explain the beneficial effects of polyunsaturated fatty acids on torpor and minimal metabolic rate, as this could be achieved by incorporation of monounsaturates (which can be synthesized de novo by the animals (Munro and Thomas 2004)). Thus, other mechanisms have been proposed, such as the impact certain polyunsaturated fatty acids (e.g., 18:2ω6) have on transmembrane enzymes involved in calcium handling in myocytes (Ruf and Arnold 2008). As a common observation, hibernators and daily heterotherms increase the amount of ω6 polyunsaturates in their body fats in preparation for torpor (Geiser and Kenagy 1987; Frank 1992; Hill and Florant 1999) and the content of ω6

polyunsaturated fatty acids in white adipose tissue is a good predictor of hibernation body mass loss over winter in alpine marmots (Ruf and Arnold 2008). Notably, hibernators such as marmots do not feed at all during the hibernation season, so during winter, body fats are the only source for polyunsaturated fatty acids (Florant 1998). Recently, it has been demonstrated that 18:3ω3 along with longer chain ω3 polyunsaturates were used from white adipose tissue depots during winter hibernation (Arnold et al. 2011). In parallel, the proportion of 18:2ω6 increased and these effects could be observed in multiple white adipose depots such as subcutaneous, peritoneal, cardial, and renal white adipose tissue stores (Arnold et al. 2011). The selective retention of particular fatty acids (e.g. 18:2ω6) in adipose tissue during the hibernation fast (which contrasts with the baseline mobilization pattern) may be important in guarding them against early oxidation and thereby make them available to the membranes of various other tissues later during the fast (which may protect those tissues against dysfunction in calcium metabolism).

15.3.3 Changes in Liver Fatty Acid Composition

As a central metabolic processing organ, the liver plays a major role in fat metabolism. During feeding, the liver receives ingested fats and can modify them (e.g. with elongases and desaturases) and package them into lipoproteins for transport to other tissues. The liver also produces fatty acids de novo from carbohydrate precursors. Furthermore, the liver may receive free fatty acids from other tissues and process them (Zhou and Nilsson 1999). The wide array of metabolic processes occurring in the liver makes changes in its composition during fasting difficult to predict. During prolonged fasting, dietary input is nil, and one might expect de novo synthesis to greatly decrease as well. Similarly, one might expect a decrease in desaturase activity. Indeed, Δ9, Δ5, and Δ6 desaturase activity decreases during starvation in rodents (de Gomez Dumm et al. 1970; Enser and Roberts 1982).

Overall, changes in total fatty acid composition of liver during starvation might be driven by the loss of triacylglycerol as any fat that was stored in liver is exported or oxidized. In several studies, particularly in fish, liver mass was decreased during starvation, and this included decreased triacylglycerol mass (Dave et al. 1976; Jezierska et al. 1982; Zamal and Ollevier 1995). During starvation in Atlantic salmon (*Salmo salar*), total liver lipids, while decreasing in mass, became relatively enriched with 22:6ω3 and 16:0 at the expense of 20:5ω3 and 18:1ω9 (Einen et al. 1998), a result the authors suggested could represent a shrinking ratio of triacylglycerols to phospholipids. A general result from several other studies in fish is that 22:6ω3 is retained in liver lipids, and this could also represent a changing triacylglycerol to phospholipid ratio, although lipid fractions were not separated in these studies (Dave et al. 1976; Jezierska et al. 1982; Tidwell et al. 1992; Zamal and Ollevier 1995).

Delgado et al. (1994) examined the changes that occur in the triacylglycerol and phospholipid fractions, as well as total lipids, during 2 months of fasting in livers of sea bass (*Dicentrarchus labrax*). Interestingly, triacylglycerol composition changed little during the fast, but total liver fatty acid composition changed both as a result of changes in the triacylglycerol to phospholipid ratio, and to changes in the composition of phospholipids. For example, 16:0 decreased in total liver lipids, while it increased in relative abundance in the phospholipid fraction. Notable phospholipid changes included increased 16:0 and 22:6ω3, and decreased 18:0. The importance of this membrane remodeling is unclear.

The loss of triacylglycerols from liver during starvation is not a universal phenomenon. Some animals exhibit hepatic steatosis during starvation, i.e., unusual retention and/or accumulation of fat in the liver (Nieminen et al. 2009; Rouvinen-Watt et al. 2010). Without dietary input, fatty acids accumulating in the liver must have as their source either de novo synthesis, or fatty acids mobilized from other tissues, particularly adipose depots. The latter seems most likely, as every change in polecat liver fatty acid composition during starvation was in the direction toward the composition of the adipose tissue (Nieminen et al. 2009). Similarly, mink liver fatty acid composition became more similar to that of adipose tissue over a 4- or 7-day fast (Nieminen et al. 2006a; Rouvinen-Watt et al. 2010).

In sable and marten, liver lipids were similarly altered to become more like the composition of adipose tissue during starvation (Nieminen et al. 2006b; Nieminen and Mustonen 2007). However, this is not the case in all animals. During cyclic fasting in rats, total liver fatty acid mass did not change, and liver lipid composition did not tend toward that of adipose tissue (Chen et al. 1995). In pheasant, total liver mass was substantially reduced by fasting, but liver lipid composition was unchanged (Mustonen et al. 2009).

In genetically obese or lean mice, liver composition responded differently to starvation. Total fat content of livers from obese mice declined during a 72-h fast, and this was accompanied by decreases in monounsaturates and increases in 18:0, 18:2, and 20:4 (Enser and Roberts 1982). In contrast, livers from lean mice increased in fat content, and this included decreased proportions of 16:1 and 20:4, with increased 18:1 and 18:2. With the exception of 18:1, fatty acid proportions in both strains of mice did not become more like adipose (Enser 1979). The compositional changes observed may alternatively represent changes in the ratio of triglycerides to phospholipids, and this may further explain the differences between strains, as livers from the lean strain apparently gained triacylglycerols while livers from the obese strain apparently lost triacylglycerols. Although Enser and Roberts (1982) did not separate and measure the different lipid fractions in their study, the phospholipid fatty acid composition of mouse livers from another study (Nelson 1962) supports this explanation.

Strikingly, liver phospholipids of hibernating animals such as alpine marmots, which do not feed during torpor, have been shown to be substantially remodeled over the year, both in preparation for hibernation and during hibernation itself (Arnold et al. 2011). In autumn pre-hibernation, there was a doubling of ω6 fatty

acids in liver phospholipids during a period of only 2 weeks. There was also a detectable increase in long chain ω3 polyunsaturates such as 22:5ω3 and 22:6ω3 in liver over the hibernation season (Arnold et al. 2011), possibly due to selective transfer of these fatty acids into the organs from white adipose tissue. Altogether, liver phospholipids of hibernating marmots became more unsaturated during the course of hibernation with a peak at the end of hibernation. The rapid pre-hibernation changes to liver phospholipids indicate an adaptive preparation for hibernation, likely related to body temperature regulation (Ruf and Arnold 2008; Arnold et al. 2011), and thus they highlight the functional importance of liver membrane composition during hibernation. However, the changes actually occurring during hibernation are of less clear origins; they may occur passively as a result of starvation (due to lack of dietary input of essential fatty acids), or might be part of ongoing functional remodeling in response to low temperatures.

15.3.4 Changes in Plasma Fatty Acid Composition

During fasting, plasma triacylglycerols (transported in lipoproteins) decrease as the supply of exogenous nutrients declines (Chen et al. 1995; Seaman et al. 2005). Simultaneously, non-esterified fatty acids (transported from adipocytes to other tissues bound to albumin) increase, as animals draw on the energy reserves stored in adipose tissue (Ramenofsky 1990). Because adipose tissue is the source of non-esterified fatty acids in the plasma, we could expect the composition of both to be similar (with perhaps slight adjustments for selective mobilization). This is in fact observed during exercise, when hydrolysis of adipose triacylglycerols is high to meet the increased energy demand. Mougios et al. (1995) showed that the composition of plasma non-esterified fatty acids approached the composition of adipose tissue during human exercise. However, it is interesting to note that the resting values for plasma non-esterified fatty acids were different from that of adipose (Mougios et al. 1995), implying that under resting conditions, other processes (e.g., selective utilization of fatty acids) become important in determining plasma non-esterified fatty acids composition. Nonetheless, percent composition of several non-esterified fatty acids in plasma was significantly correlated with their percent composition in adipose tissue in (short-term) fasted humans (Halliwell et al. 1996; Hodson et al. 2008).

As a proportion of total plasma lipids, however, the non-esterified fatty acid fraction can be small and total fatty acid composition is dominated by the influence of triacylglycerol fatty acids (Chen et al. 1995; Hodson et al. 2008). This may in turn be affected by liver composition, as liver is the source of plasma triacylglycerols. The importance of plasma triacylglycerols in determining overall plasma fatty acid composition is compounded by the fact that fasting can sometimes result in increased plasma triacylglycerol composition (Mustonen et al. 2007; Rouvinen-Watt et al. 2010). In marten, plasma fatty acid composition was generally more similar to liver than to adipose composition (Nieminen et al. 2006b). Similarly, in

sable, plasma fatty acid composition appeared more similar to liver than to adipose tissue in both the fed and fasted state, and tended even more toward the liver's composition during a 5-day fast (Nieminen and Mustonen 2007). This pattern is not universal, however. Plasma composition in mink and polecats was rather distinct from both liver and adipose tissue (Nieminen et al. 2006a, 2009). Overall, there does not yet appear to be a strong predictable pattern of fatty acid changes in plasma during starvation; however, most changes are likely to represent changes to the primary sources of plasma lipids—adipocytes and liver.

15.3.5 Changes in Muscle Fatty Acid Composition

Muscle tissue can contain substantial fat (triacylglycerol) droplets, and at least some of the changes in fatty acid composition of muscles during fasting/starvation are likely to reflect usage of intramuscular triacylglycerol. Zar (1977) showed that total lipids in house sparrow (*Passer domesticus*) muscles became enriched with polyunsaturates during starvation, a result that was attributed to a decreasing ratio of triacylglycerol to phospholipids. Herzberg and Farrell (2003) showed that during a 48-h fast in rats, muscle triacylglycerols were mobilized in a pattern substantially similar to the baseline selective mobilization patterns seen in adipose tissue, i.e., unsaturated and shorter fatty acids were most readily mobilized. In contrast, Delgado et al. (1994) observed a selective depletion of saturates (16:0 and 18:0) and retention of polyunsaturates (18:2 and 20:5) in muscle triacylglycerols of fasting sea bass. The mechanistic and/or adaptive reasons for this difference between species are unknown.

Phospholipid composition of muscles during complete fasting is poorly understood, although there are important studies of muscles during caloric restriction. Heart phospholipid composition was reported to change markedly when rats were chronically calorically restricted by 40% (Cephalu et al. 2000). Specifically, dietary restriction significantly increased the proportion of saturated fats as well as that of 22:6ω3, while the amount of 18:2ω6 decreased. Interestingly, these effects were only observed in 29-month-old rats while they were absent in heart tissues from 11- to 17-month-old rats. Clearly, even a 40% reduction in caloric intake is not as dramatic as the energy reduction during starvation, but it should be noted that it distinctly alters membrane composition and it has been suggested that the changes might be linked to longevity (reviewed by Hulbert 2010). Faulks et al. (2006) assessed the impact of different levels of caloric restriction on membrane phospholipid composition in mice and confirmed that, with the extent of the change being dependent on the tissue, low caloric input led to an increase in 18:2ω6 at the expense of ω3 fatty acids. Thus, the membranes become less susceptible to lipid peroxidation and might thereby have lower oxidative damage and extend life span (Faulks et al. 2006; reviewed in Hulbert 2010) (see also, Champagne et al., Chap. 19 and Bauchinger and McWilliams, Chap. 12). Notably, however, dietary energy intake did not impact all tissues measured and

skeletal muscle phospholipid composition did not significantly change (Faulks et al. 2006). Except for a trend toward a significant change in the $\omega 3/\omega 6$ ratio with decreasing caloric intake, muscle phospholipid fatty acid composition was tightly regulated (Faulks et al. 2006), as was expected from interspecific comparisons which reveal that muscle phospholipid composition is a species-specific regulated trait in mammals (Valencak and Ruf 2007). In contrast, a study of fasting sea bass demonstrated increases in 20:4, 20:5, and 22:6 at the expense of 16:0 and 18:2 in muscle phosphatidylcholine (Delgado et al. 1994). In conclusion, phospholipid fatty acid compositional changes during fasting in mammalian skeletal muscles appear to be of minor importance but are poorly understood so far. As pointed out earlier, skeletal muscle phospholipid fatty acid composition is maintained even during prolonged fasting and most likely even during starvation due to the importance of structural lipids on membrane-bound enzymes. The reasons for remodeling of fish muscle phospholipid composition are unclear.

15.4 Conclusions

15.4.1 Is There an Adaptive Fatty Acid Composition Response to Starvation?

Change in lipid content during starvation is undoubtedly an adaptive response; animals oxidize fat during periods of negative energy balance. Changes in fatty acid *composition* are of less clear significance. Overall, the literature we have surveyed indicates little evidence of a 'starvation response' in fatty acid composition—most changes that occur can be ascribed to processes such as baseline selective mobilization or changes in the phospholipid to triacylglycerol ratio. That is to say, the changes in fatty acid composition are resultant from a homeostatic response to starvation, but are not themselves part of that response.

15.4.2 Future Directions

Fasting presents a simplified physiological model; without the confounding effect of dietary intake, changes in tissue lipid composition should be easier to ascribe to particular processes. Additionally, fasting provides a useful model for studying the use of essential fatty acids. Establishing some baseline expectations for the lipid changes that should occur during fasting is a necessary first step for identifying interesting animal models that break the standard paradigm. We have derived these baseline expectations based on only a few principles, and our development of these expectations is theoretical. Further studies should directly test these ideas in a variety of contexts, and we strongly recommend that the future work should separate lipid fractions (e.g., triacylglycerols and phospholipids) for a better

understanding of the importance of any observed changes. Focusing on unusual cases can lead us to an improved knowledge of how fatty acid composition is regulated and the importance of particular fatty acids in various physiological contexts. Exceptional cases we identify here include alterations of muscle and liver phospholipids in sea bass, the selective retention of 18:2ω6 in stored lipids of hibernators, and the membrane remodeling occurring in organ phospholipids during hibernation and daily torpor. These latter phenomena appear to be related to heterothermy and not starvation per se. But they do offer insight into the usage of essential fatty acids during a period of starvation, and should yield fruitful avenues of research.

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Chapter 16
Physiological Responses to Fasting in Bats

Miriam Ben-Hamo, Agustí Muñoz-Garcia and Berry Pinshow

16.1 Introduction

The evolution of powered flight in bats (Order Chiroptera) took place more than 50 million years ago (see summary in Speakman 2001) and afforded them the opportunity to fill in ecological niches void of non-volant mammals, a circumstance that might explain their remarkable diversity in terms of number of species, diet, and habitat types that they occupy. With over 1,000 species, bats are the second most diverse mammalian order, outnumbered only by rodents (Altringham 1996). Generally, bats are considered small mammals, most having body masses (m_b) ranging from less than 2 g in the bumblebee bat, *Craseonycteris thonglongyai*, to 100 g, but some are larger, the largest being the Giant golden-crowned flying fox, *Acerodon jubatus*, with a m_b in excess of 1 kg. Bats eat a wide variety of diets; many species are insectivorous, others are frugivorous, nectarivorous, carnivorous, piscivorous, omnivorous, or hematophagous. Moreover, bats are found in almost all climatic zones, and they are distributed worldwide, except for the polar regions.

Besides its clear ecological advantages, the evolution of powered flight brought about high energetic costs for bats; bats in flight increase metabolic rate (MR) 15 times over MR at rest (Austad and Fischer 1991; Harrison and Roberts 2000; Munshi-South and Wilkinson 2010; Szewczak 1997), but compensate for this enormous expense through a variety of energy-saving thermoregulatory patterns. Many species become torpid on a daily basis and have relatively short periods of activity, almost invariably at night (Speakman and Thomas 2003). Some species that feed on resources not available throughout the year, such as insects, cope with reduced prey availability by hibernating in winter. Thus, bats

M. Ben-Hamo (✉) · A. Muñoz-Garcia · B. Pinshow
Mitrani Department of Desert Ecology, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel
e-mail: miriammi@bgu.ac.il

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need to rely on their endogenous energy reserves on a regular basis, sometimes for very long periods. In this regard, bats are remarkable in that the size of their endogenous energy stores may vary dramatically on seasonal, daily, and even hourly timescales (Kronfeld-Schor et al. 2000; Kunz et al. 1998; Weber and Findley 1970). One might envision that bats have evolved mechanisms that allow them to make efficient use of their endogenous reserves, thus maintaining energy balance in periods of food shortage. However, surprisingly little is known about the responses of bats to fasting. In this chapter, we summarize the known responses of bats to fasting, suggest new directions for future studies, and discuss hypotheses concerning the physiological responses of bat species to fasting.

16.2 Physiological Responses of Bats to Fasting

Vertebrates that consume different diets often differ in the way they store and mobilize body energy reserves, and therefore one might expect different species to have different responses to fasting. Among all mammalian orders, bats have the largest variety of diets (Neuweiler 2000a). Although most bats are insectivorous, there is also a significant number of bat species that feed exclusively on fruit, or nectar and pollen, and others can feed on small vertebrates, and even blood of other mammals and of birds (Neuweiler 2000a). Many species of bats spend relatively short times foraging and drinking during their daily activity cycle (up to 4 h) and spend the reminder of the day in sheltered roosts (Bassett 2004), where they neither eat nor drink. Therefore, short- and long-term fasting are regular occurrences in the life cycles of bats. Accordingly, bats lend themselves to the investigation of how different diets affect physiological mechanisms, such as endurance of fasting.

16.2.1 Frugivorous Bats

Fruit eating bats have specialized gastrointestinal tract structure and function that allow them to digest carbohydrate-rich diets efficiently and rapidly (Keegan 1977; Okon 1977). Both the Egyptian fruit bat, *Rousettus aegyptiacus*, and the great fruit-eating bat, *Artibeus lituratus*, have high rates of paracellular absorption of carbohydrates that apparently compensate for their relatively short intestinal tract (Caviedes-Vidal et al. 2007, 2008; Tracy et al. 2007). This mechanism allows fruit bats to store relatively large amounts of hepatic glycogen (Fig. 16.1; Protzek et al. 2010).

Two species of microchiropteran fruit bat, the above-mentioned *A. lituratus* and the Jamaican fruit bat, *A. jamaicensis*, maintain relatively high blood glucose levels after short-term fasting (2–6 days) (Pinheiro et al. 2006). These species tend to have large hepatic glycogen stores that likely play a role in maintaining blood glucose levels when food is absent (Pinheiro et al. 2006). Because of their large hepatic glycogen stores, *A. lituratus* can endure 2–6 days with no food and still

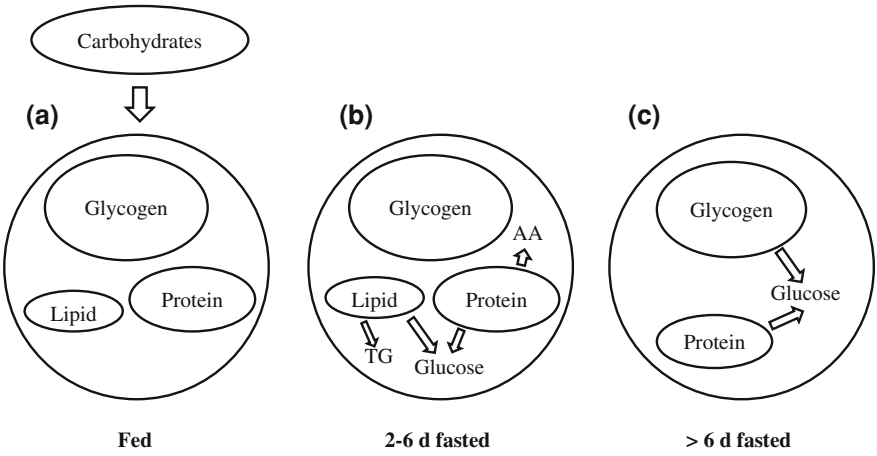

Fig. 16.1 Nutrient use by fasting frugivorous bats. **a** When bats eat their normal carbohydrate-rich diet, they accumulate large glycogen reserves, mainly in the liver. **b** During a short of fast, glycogen reserves remain intact, and bats oxidize body fat and use gluconeogenesis from amino acids to maintain a high blood glucose level. **c** If the fast is longer, frugivorous bats eventually deplete their glycogen stores. *TG* triacylglycerides, *AA* amino acids

maintain normal levels of blood glucose (Pinheiro et al. 2006), an ability rarely seen among mammals. This phenomenon is peculiar given that insulin levels remain similar in both fasted and fed *A. lituratus* (Protzek et al. 2010). Although hyperinsulinemia is characteristic of insulin insensitivity (Modan et al. 1985), *A. lituratus,* which possesses a comparatively high number of pancreatic β-cells (Protzek et al. 2010), also has high insulin sensitivity and high blood glucose tolerance. Consequently, glycogenolysis does not seem to be used as a mechanism of short-term fast resistance in other fruit bat species, as indicated by their limited use of hepatic glycogen during this period (Freitas et al. 2010; Pinheiro et al. 2006). For example, *R. aegyptiacus* has large variations in its blood glucose levels, and its total blood N increases when faced with a shortage of food (Keegan 1977; Korine et al. 1999), suggesting that these bats use triacylglycerides and proteins as fuels while at rest by day (Korine et al. 1999).

Frugivorous bats face a challenge in maintaining water balance while they fast since the bulk of their water intake comes from their diet and since they have relatively high rates of mass-specific evaporative water loss (EWL) (Studier 1970) and poor urine-concentrating abilities (Studier and Wilson 1983); thus frugivorous bats are exposed to a greater risk of dehydrating than similar sized, non-volant mammals (Bakken and Sabat 2007). Bakken et al. (2008) reported that when deprived of food and water for 12 h, glomerular filtration rate (GFR) in Pallas's long-tongued bats, *Glossophaga soricina*, decreased to 90% less than when fed and watered adequately (Bakken et al. 2008), which they interpreted as a water saving mechanism.

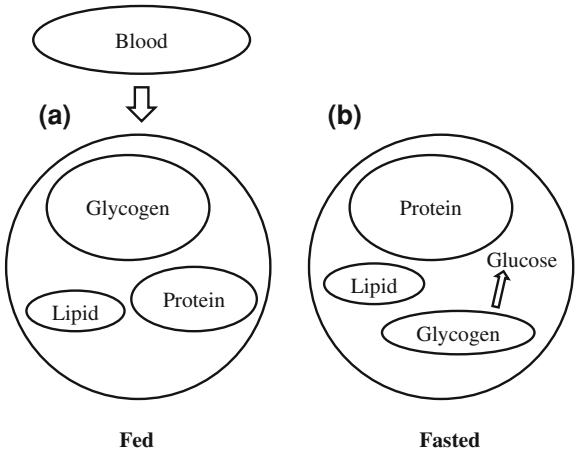

Fig. 16.2 Nutrient use by hematophagous bats. **a** Vampire bats do not accumulate large quantities of glycogen, as opposed to other mammals that eat diet protein-rich diets. **b** Hematophagous bats do not tolerate fasting for long periods. They are unable to mobilize fat or protein efficiently, and they deplete their small glycogen stores quickly

16.2.2 Hematophagous Bats

Although animals that eat foods with high protein content usually store large amounts of hepatic glycogen (see Harlow, Chap. 17), vampire bats are apparently an exception (Fig. 16.2). These bats loose m_b rapidly when they do not eat, and are unable to survive more than 3 days of fasting (McNab 1973). For example, the common vampire bat, *Desmodus rotundus*, fails to maintain adequate blood glucose levels when fasting, and its hepatic glycogen stores are at least 40% smaller than those of other mammal species (Freitas et al. 2003, 2005; Kettelhut et al. 1980). Vampire bats are apparently unable to build up fat stores (Breidenstein 1982) as seen in other species and seem to lack the ability to mobilize fat rapidly. When vampire bats do not eat, the small decrease in their lipid stores is not accompanied by a significant increase in free fatty acid concentration in the blood (Freitas et al. 2003, 2005) as has been reported for other species. Moreover, no measurable change in muscle (heart, limb, and breast) and liver proteins has been found in fasting vampire bats, suggesting that they are unable to catabolize protein when deprived of food (Freitas et al. 2003, 2005). A plausible explanation for *D. rotundus* being able to fast for the reported 3 days is that they have highly elastic stomachs capable of distending and storing large quantities of blood (Freitas et al. 2003; Rouk and Glass 1970). This characteristic could mask the fact that these animals may be postabsorptive and therefore are not truly fasting. Further, *D. rotundus* has a unique behavioral adaptation to cope with fasting; related bats participate in reciprocal food sharing through regurgitation of blood to one another, allowing unsuccessful foragers to survive the night (Wilkinson 1984).

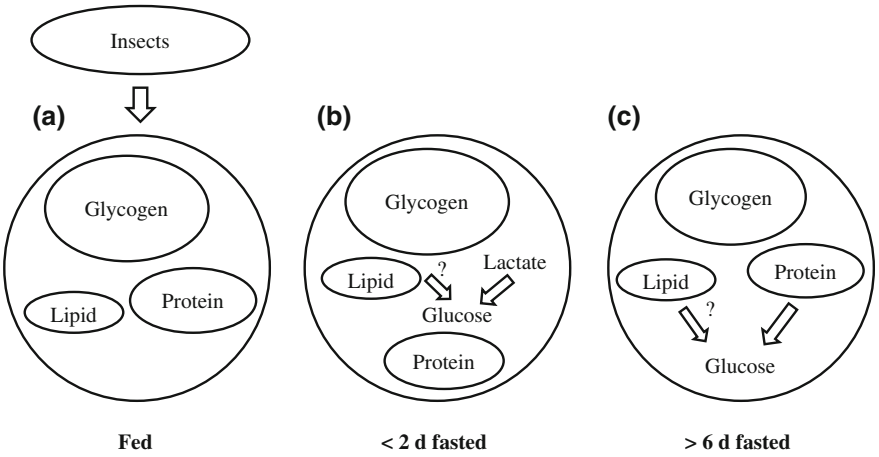

Fig. 16.3 Insectivorous bats can survive the longest periods of fasting among bats. **a** When feeding, they accumulate large quantities of glycogen in the liver and the muscles. **b** During short-term fasting, they make use of body fat reserves and activate gluconeogenesis from skeletal muscle lactate for energy, and to maintain normoglycemia. **c** When the fast is longer, insectivorous bats begin catabolizing protein, but they continue to retain large amounts of hepatic glycogen

16.2.3 Insectivorous Bats

Approximately 70% of all bat species are insectivorous (Neuweiler 2000a), and they probably have the highest resistance to long fasts among chiropterans. Freitas et al. (2010) found that male Pallas's mastiff bat, *Molossus molossus*, maintained blood glucose levels constant during 48 h of fasting, while free fatty acid concentrations did not increase in their blood. These observations suggest that these bats did not shift to phase II of fasting (sensu Cherel et al. 1988) even after 48 h of food deprivation. This response is further supported by the small decrease in their hepatic glycogen stores, indicating that they did not completely exhaust their glycogen stores within this period (Fig. 16.3; Freitas et al. 2010).

It is thought that insectivorous bats maintain normal levels of blood glucose in the initial 48 h of fasting by gluconeogenesis. The concentration of plasma lactate was found to decrease in response to fasting, suggesting that lactate from skeletal muscle might be one of the main substrates for gluconeogenesis in fasting bats (Corssmit et al. 2001; Pinheiro et al. 2006). Following 24 h of fasting, plasma urea levels did not change in the pallid bat, *Antrozous pallidus,* (Bassett 2004), indicating that there is no change in protein catabolism in response to short-term food deprivation. However, after longer periods of fasting (48–72 h), incorporation of amino acids into the liver, particularly alanine, seems to increase; these amino acids are used for gluconeogenesis (Corssmit et al. 2001; Pinheiro et al. 2006).

McGuire et al. (2009) reported that plasma triacylglyceride concentrations are positively correlated with changes in m_b, and can be used to infer differences in short-term m_b changes and feeding activity of insectivorous bats, a relationship analogous to that found in birds (e.g., Jenni-Eiermann and Jenni 1994). However, the concentrations of triacylglycerides are surprisingly low in Mexican free-tailed bat, *Tadarida brasiliensis* (McGuire et al. 2009; Widmaier et al. 1996). It is possible that this species has unique mechanisms for rapid clearance of lipids from the blood, such as secretion in the milk of lactating females (Widmaier et al. 1996). However, because low levels of triacylglycerides were also observed in non-lactating individuals, it is more likely that the mechanism responsible for these levels involves secretion through the skin or a high blood concentration of protein lipases that rapidly catabolize the triacylglycerides (Widmaier et al. 1996). Morris et al. (1994) reported that Gould's long-eared bat, *Nyctophilus gouldi*, store some digesta in the rectum during short fasts (Morris et al. 1994), where little absorption may occur (Warner 1981). Many microchiropteran species cope with long periods of food shortage or deprivation by becoming torpid daily, and all temperate species hibernate during the winter in response to food scarcity and cold temperatures (Speakman and Thomas 2003).

16.2.3.1 Use of Torpor

Many animals face periods of fasting in which they have to rely on stored nutrients (McCue 2010). In endothermic vertebrates, maintenance of a high, constant body temperature (T_b) is the main determinant of depletion of endogenous reserves in periods of fasting (Bennett and Ruben 1979; see also Hohtola, Chap. 10; Ullrey, Chap. 18). Thus, endotherms that decrease costs of thermoregulation while fasting are at an advantage over those that do not (Geiser 2004). Bats are the only mammals that have evolved powered flight, an ability that imposes higher energetic cost than incurred by other mammals; moreover, bats have high surface to volume ratios, which increases rates of heat dissipation (Speakman and Thomas 2003). To minimize energy expenditure when food is restricted or absent, many species of bats make use of torpor, and reduce T_b to values close to ambient temperature (T_a). Torpor allows bats to reduce significantly the cost of thermoregulation and subsequently overall energy expenditure during fasting.

Among bats there is a continuum of thermoregulatory patterns, from species that strictly defend euthermic T_b, to others which regulate T_b at temperatures as low as 20°C, and even 5°C (Speakman and Thomas 2003). Insectivorous bats from temperate regions are probably the most thermolabile species, hibernating during the cold winter when food availability is low, and regularly entering torpor during their daily inactive phase throughout the year (e.g., Turbill et al. 2003; Willis et al. 2005). In insectivorous bats, a temporary decline in food availability can induce torpor even when the T_a is relatively high (Neuweiler 2000b). For example, fasting Gould's Long-eared bat, *Nyctophilus gouldi*, entered torpor even at T_a of 30°C (Neuweiler 2000b). Although the decline in T_b and MR is less pronounced during

short bouts of torpor that are used throughout the year, than during the long bouts that characterize hibernation (Geiser and Ruf 1995; Wojciechowski et al. 2007), even small reductions in T_b and MR lead to significant savings of energy (Heldmaier et al. 2004). For instance, a bout of daily torpor may last up to 12 h during which MR is reduced to about 25% of the active MR (Heldmaier et al. 2004). As a result, total daily energy requirements may decline by 40% (Heldmaier et al. 2004). In the long-term, energy budgets of animals that enter torpor regularly may only be two-thirds of the energy requirements of normothermic individuals (Ortmann et al. 1997; Ruf and Heldmaier 1992; Schmid et al. 2000). Therefore, daily torpor is an important strategy for a substantial reduction of an animal's total energy requirements, thereby saving endogenous reserves in periods of food restriction or fasting.

16.2.3.2 Hibernation and Arousal

Insectivorous bats can endure fasting when food availability is low by their ability to hibernate, a characteristic common among the temperate members of the Vespertilionidae and Rhinolophidae (Altringham 1996). Hibernation in bats is not a constant state of reduced metabolism and T_b, rather it consists of repeated cycles of torpor and arousal. Arousals occur periodically, roughly every 12–15 days, and account for more than 85% of energy used throughout the winter (Wang 1978) and do so to drink, eat or both (Boyles et al. 2006).

During winter both food supply and T_a decline, and while some species of bats escape this energetic bottleneck by migrating, others hibernate (Speakman and Thomas 2003). Prior to both hibernation and migration, bats generally accumulate up to 25% of their m_b in fat (Speakman and Thomas 2003; Widmaier et al. 1996) that serves as metabolic fuel during hibernation and migration (Dark 2005; Guglielmo 2010). Both of these energetically challenging periods are dependent upon a high capacity for fatty acid mobilization, and therefore the bats' survival is affected by adaptive changes in fatty acid transport proteins (Eddy and Storey 2004; Guglielmo et al. 1998). In addition, fatty acid composition is also important in lipid mobilization since fatty acids are preferentially mobilized when they are short, less saturated, and have double bonds located close to the terminal methyl group of the chain (Groscolas and Herzberg 1997; see also Price and Valencak, Chap. 15).

Since hibernating bats decrease their metabolism, enter prolonged fasting, and rely almost solely on lipids as fuel, it is not surprising that hibernation results in modifications in the composition of triglycerides, fatty acids, amino acids, ketones, and other blood metabolites (Arevalo et al. 1990; Dark 2005; Wells et al. 1965). The major changes in metabolic physiology during hibernation are probably the switch from primarily carbohydrate to primarily lipid metabolism, reduction in protein synthesis and protein degradation (Van Breukelen and Carey 2002; Van Breukelen and Martin 2001; Yacoe 1983), and increased gluconeogenesis during periodic arousals (Burlington and Klain 1967).

Lipids fuel-basal metabolism during torpor, non-shivering thermogenesis by oxidation in brown adipose tissue (BAT), and shivering thermogenesis by skeletal muscle that rewarm the animal during arousals (Geiser 1988). Since lipids supply most metabolic fuel during hibernation, hibernating mammals should optimize mobilization and oxidation of lipids (see Harlow, Chap. 17). One factor significantly affecting lipid mobilization is the activity and concentration of fatty acid binding proteins that play an important role in intracellular transport of fatty acid (Stewart 2000). The little brown bat, *Myotis lucifugus*, has been reported to undergo modifications in fatty acid binding proteins during hibernation that may increase the efficiency of lipid mobilization (Eddy and Storey 2004). In addition, the fatty acid composition of adipose tissue in hibernating bats undergoes changes as well (Arevalo et al. 1990; Geiser and McMurchie 1984; Widmaier et al. 1996). Wells et al. (1965) found a greater proportion of unsaturated fatty acids in the tissues of hibernating *M. lucifugus* than in non-hibernating animals. A higher proportion of unsaturated fatty acids might enable longer torpor bouts, lower T_b, and thus reduce MR, because the relatively lower melting point of unsaturated fatty acids will maintain the fluidity of cell membranes in such a way that energy can be produced even when T_b is low (Munro and Thomas 2004; Ruf and Arnold 2008).

During the lengthy fast of hibernation, there is a decrease in both blood glucose and liver glycogen (Burlington and Klain 1967), and thus the capacity to induce carbohydrate catabolism is apparently retained (Hannon and Vaughan 1961). The only sources of glucose available to fasting hibernators are glycogenolysis and gluconeogenesis. Yacoe (1983) found that fasting big brown bats, *Eptesicus fuscus,* undergo significant decreases in lean mass, pectoral muscle mass, and in total pectoral muscle protein, suggesting significant oxidation of tissue protein. Studies of hibernating Syrian hamsters, *Mesocricetus auritus*, and 13-lined ground squirrels (*Citellus tridecemlineatus*) may suggest an explanation for this; during arousal, their respiratory quotient approaches 1.0 (Mokrasch et al. 1960), evidence that protein catabolism is used for gluconeogenesis (Lyman and Leduc 1953).

During arousal, after two months of hibernation with no access to food, the rate of gross protein synthesis in the liver of *E. fuscus* was similar to that found in the liver of active bats after an overnight fast during the summer (Yacoe 1983), suggesting that the source of the amino acids used for synthesis of new proteins must be endogenous, i.e., from breakdown of tissue protein. This contrasts what was found in hibernating13-lined ground squirrels, *Spermophilus tridecemlineatus*, in which hepatic polyribosomes are disaggregated (Whitten et al. 1970), and there was significantly lower protein synthesis in in vitro cultures of liver samples collected in winter than in summer samples from the same species (Klain and Whitten 1968). These observations contradict what one might expect in animals that have fasted for two months, since during prolonged periods of fasting protein metabolism declines due to decreased levels of mRNA (McNurlan et al. 1979), and low protein degradation levels lead to low amino acid availability (Cahill 1976; Gan and Jeffay 1967).

Similar to other fasting-adapted species, urea does not accumulate in the blood of *E. fuscus* during bouts of torpor lasting up to 12 days (Castellini and

Rea 1992; Yacoe 1983). The concentration of plasma urea does not differ between torpid and normothermic bats, perhaps the result of a low accumulation rate, and corresponds to only 0.01% loss of total protein per day (Yacoe 1983). A massive use of protein occurs during arousals when pectoral muscle and liver protein catabolism are similar to the rates in normothermic bats in summer. Since glycogen stores are small (Dodgen and Blood 1956) and the contribution of glycerol is coupled to the rate of fat oxidation, the increased demand for glucose during arousals must be met by gluconeogenesis from tissue protein. The relatively large loss of pectoral muscle and liver protein, together with increased hepatic gluconeogenic capacity (Klain and Whitten 1968) and high production of urea during arousals, suggest that during periodic arousals tissue protein is used to supply gluconeogenic demand. This is in contrast to what happens during torpor, when the rates of tissue protein synthesis and degradation are very low (Yacoe 1983). Fuel use during prolonged fasts is well documented in the literature, and the switch between different metabolic fuels is known to occur in three distinct phases (Cherel et al. 1988), therefore the switch back and forth between metabolic fuels during torpor and arousal in hibernating bats is remarkable.

16.2.3.3 Hormonal Control

Almost all of the endocrinological studies done on fasting bats focused on hibernation, when bats do not feed for several weeks or even months at a time (Roy and Krishna 2010; Srivastava and Krishna 2008, 2010). Therefore, our discussion focuses on hormonal control of prolonged fasting in hibernating bats (Fig. 16.4).

In all mammals, there is a complex system that regulates fuel stores and energy balance (Friedman and Halaas 1998). This system allows the storage of sufficient quantities of energy-dense triglyceride in adipose tissue to survive periods of food deprivation encountered during the lifetime of the animal; tight regulation of fuel stores also helps avert the presence of excess adipose tissue that can be maladaptive (Friedman and Halaas 1998; see also Zhang et al., Chap. 13). Leptin, a hormone secreted by adipose tissue, and its receptors, are integral components of this system, and have been the focus of most studies on hormonal control of fasting during hibernation. Leptin, mainly via the hypothalamus, controls and modulates the nutritional status of the animal by inhibiting appetite (Friedman and Halaas 1998; Kronfeld-Schor et al. 2000). Leptin levels decline during hibernation in the greater Asiatic yellow bat, *Scotophilus heathi*, and in *M. lucifugus* (Kronfeld-Schor et al. 2000; Roy and Krishna 2010; Srivastava and Krishna 2008; Widmaier et al. 1997), which is consistent with the general finding that leptin levels decrease during fasting. Although the functions of leptin are not yet well understood, it is known that low leptin levels induce metabolic and endocrinological changes resulting in frugal use of energy, enhancing the survival of the bats during the long, challenging fast in winter.

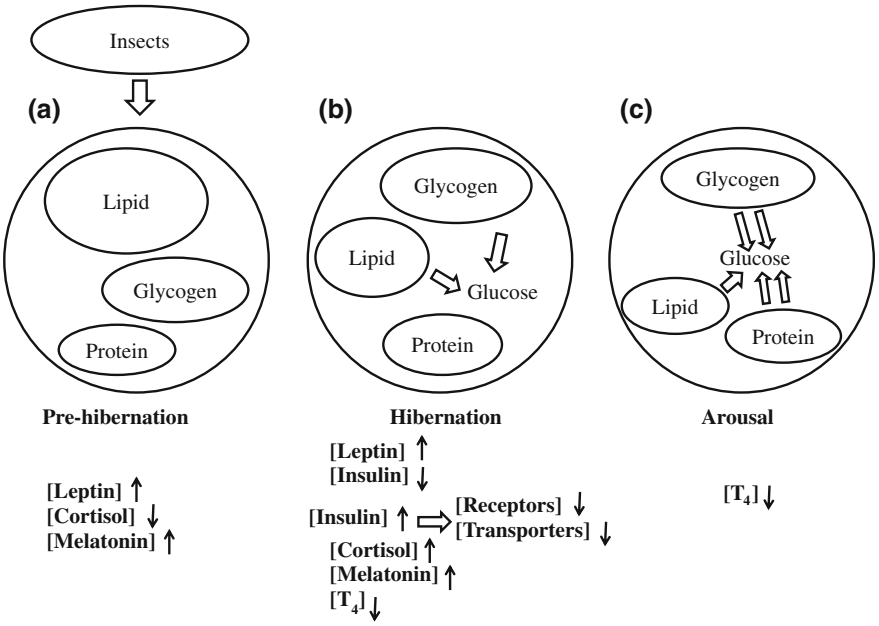

Fig. 16.4 Nutrient use and hormonal changes during the hibernation cycle of insectivorous bats. **a** Prior to hibernation, bats accumulate large amounts of body fat, which will become the main source of metabolic fuel during hibernation. While fattening, bats have high blood levels of leptin and melatonin, and low levels of cortisol, which favors the storage of nutrients. **b** During hibernation, bat blood glucose levels are relatively low, and, for energy, they switch from carbohydrate to fat catabolism. Leptin, insulin, and melatonin all seem to play a crucial role in this switch. In some species, insulin levels are high in the blood, but cells are not responsive since the expression of receptors and glucose transporters is down-regulated. Blood T_4 is high before hibernation, but decreases gradually during this period. **c** Torpor bouts during hibernation are interrupted by periodic arousals, which account for 85% of the energy used in winter. During arousals, bats use fat to rewarm, but after that, they burn hepatic glycogen for energy. Much synthesis and degradation of proteins take place during this period. The levels of plasma T_4 are low during arousals, indicating that the concentration of T_4 in the blood might act as a signal for bats to arouse

Lord et al. (1998) and Srivastava and Krishna (2008) hypothesized that a decline in leptin levels triggers numerous neuroendocrine responses to fasting, such as suppression of reproduction, thermogenesis, and immune defense, and also the stimulation of the stress response. According to this function, leptin levels should be high during the fattening period when bats prepare for migration and/or hibernation. As expected, in hibernating *M. lucifugus*, plasma levels of leptin are high prior to the fattening period, but decrease during the late fattening period (Kronfeld-Schor et al. 2000). However, it was shown that in these bats leptin plasma levels and adiposity are dissociated (Kronfeld-Schor et al. 2000), suggesting a possible state of gradual leptin insensitivity in target organs early in the prehibernatory period (Kronfeld-Schor et al. 2000; Townsend et al. 2008).

Gavrilova et al. (1997) hypothesized that the dissociation is achieved by plasma leptin being sequestered by circulating leptin binding-proteins, as appears to be the case in mice during the hyperleptinemic period of pregnancy. Whether such binding proteins exist in bats is not yet known.

One might expect insulin levels to decrease during winter hibernation, and, indeed, that was found in *Scotophilus heathi* (Srivastava and Krishna 2008), leading to the notion that leptin and insulin may work in synergy to stimulate fat mobilization in winter and to provide the bats with a satiety signal during their seasonal dormancy (Srivastava and Krishna 2008). However, plasma insulin remained high during hibernation in *M. lucifugus* (Bauman 1990; Srivastava and Krishna 2010). The cause of high plasma insulin during hibernation is yet unknown, but it might be a consequence of an increase in blood melatonin (see below). Despite the high concentrations of insulin in the plasma, the down-regulation of insulin receptors and glucose transporter 4 (GLUT-4) ensures reduced insulin sensitivity of cells during hibernation (Srivastava and Krishna 2010).

Cortisol levels were reported to decrease during activity and to increase during hibernation (Gustafson and Belt 1981). These results were interpreted to suggest that the activity of the adrenal cortex increases during hibernation (Gustafson and Belt 1981; Krutzsch and Hess 1961). In addition, levels of corticotrophin releasing hormone (CRH) were reported to decrease significantly in *M. lucifugus* when they fast (Widmaier et al. 1997). Therefore, the mobilization of fatty acid from deposited fat correlates with elevated glucocorticoid levels. However, the opposite was found in *S. heathi*, possibly due to different types of glucocorticoids measured in the different studies (Gustafson and Belt 1981; Srivastava and Krishna 2008; Widmaier et al. 1997).

The insectivorous species *S. heathi* and the long-winged tomb bat, *Taphozous longimanus,* had higher plasma concentrations of T_3 and T_4 in winter than in summer (Singh et al. 2002; Srivastava and Krishna 2008). Fasting may increase the secretion of hypothalamic TRH, although a mechanism is not yet known (Decuypere and Kuhn 1985). However, in *M. lucifugus* plasma T_4 levels were lowest at the beginning of hibernation and reached a maximum at the time of arousal (Damassa et al. 1995). It is possible that the increased T_4 plasma levels result from increased synthesis and release of T_4 during late hibernation and/or reductions in the uptake and clearance of this hormone that signals the time of arousal in this bat (Damassa et al. 1995).

Photoperiod is also a key determinant of the metabolic processes occurring prior to and during winter fasting in hibernating mammals. The pineal gland, through melatonin, the hormone it secretes, is a major regulator of seasonal changes in animals, informing the brain of the need for metabolic changes required to meet the approaching challenges (see also Ulrey, Chap. 18). These observations have led to intensive research of the effects of melatonin on seasonal metabolic changes, and several studies have reported that melatonin influences insulin secretion, lipid, and glucose metabolism (Hoyos et al. 2000; Nishida et al. 2002; Peschke 2008). In hibernating *S. heathi* plasma melatonin concentration increases with fat accumulation and attains a peak level during the period of maximum body

fat deposition. Remarkably, melatonin levels remained high even during hibernation, when blood glucose levels were low (Srivastava and Krishna 2010). The authors found that in bats deprived of food for 36 h, until glycogen stores are depleted, high concentrations of exogenous melatonin were associated with an increase in the level of blood glucose (Srivastava and Krishna 2010). They concluded that the high melatonin level in combination with high insulin concentrations during hibernation may be facilitating gluconeogenesis, as observed in rabbit kidney cultures (Derlacz et al. 2005). Melatonin might inhibit glycogen breakdown, thus sustaining a slow release of glucose during a long fast. Other studies support the role of melatonin in preserving glycogen stores (Mazepa et al. 2000; Mustonen et al. 2002). High melatonin levels during winter also elevate the use of fat as the major metabolic fuel, activating lipolysis, which is a primary source of energy during hibernation (Srivastava and Krishna 2010). In support of this observation, it was found that pinealectomy directly affects lipid metabolism causing a severe alteration in the balance between lipogenesis and lipolysis (Borges-Silva et al. 2005).

16.2.3.4 Migration

Many species of bats migrate, either to avoid food shortage (e.g., *T. brasiliensis,* straw- colored fruit bat, *Eidolon helvum*) or to an area with T_a's more conducive to hibernation (Bisson et al. 2009). During migratory flight, many bats are unable to feed, yet they have very high energy demands. Migration, as documented in the avian literature, requires several physiological adaptations, such as premigratory fat deposition, increased muscle activity, and even changes in the digestive system (McWilliams et al. 2005; Popa-Lisseanu and Voigt 2009). However, while migration was intensively investigated in birds (see Bauchinger and McWiliams, Chap. 12; Jenni-Eiermann and Jenni, Chap. 11), there is little information about physiological adjustments in migratory bat species, and it is not yet known whether they undergo similar physiological adaptations (McGuire and Guglielmo 2009). There is, however, some evidence that Brazilian free-tailed bats, *Tadarida brasiliensis,* increase fat stores prior to migration (O'Shea 1976), but most bats that migrate hibernate first, and it is likely that the fat that is deposited in autumn is completely exploited during hibernation (Kunz et al. 1998).

Because of the very high metabolic cost of flight, while flying, bats must mobilize and oxidize metabolic fuels efficiently. It is still not clear how bats fuel flight. The differences in fuel use between birds and bats during flight are manifested in some aspects of protein catabolism. While small, passerine birds use some protein from the muscle and digestive organs to obtain energy and water, and end their migratory flights with less digestive system muscle mass (Karasov and Pinshow 2000; McWilliams and Karasov 2001), bats stop to feed between relatively short flights and insectivorous species can feed on the wing and are thus expected to have intact digestive systems at feeding stops between flights (McGuire and Guglielmo 2009). However, during hibernation, when the bats face

prolonged fasts, there is a reduction in the mass of the digestive system as well as a decline in gut enzyme concentrations (Carey et al. 2003).

The main factor limiting mobilization of extramuscular lipids in mammals is their transport across muscle membranes (Vock et al. 1996). Bats, unlike their nonflying mammals of similar m_b, seem to overcome this problem by increasing the concentration of fatty acid binding proteins (Eddy and Storey 2004). In addition, bats may have increased muscle oxidation activity due to modifications in muscle fiber morphology and physiology (Foehring and Hermanson 1984; Hermanson and Foehring 1988). In fact, the oxidative enzymes in the pectoral muscle of *M. Lucifugus* have the highest activity among vertebrates (Armstrong et al. 1977).

16.3 Conclusions

Bats are fascinating model for exploring physiological responses to fasting for numerous reasons. For example, bats dedicate a very small portion of their daily activity cycle to forage and drink. Therefore, fasting is a regular part of their life cycle and all bat species are adapted to cope with short-term fasting on a daily basis. In Table 16.1 we summarize what is known. Apparently, there is a great variation in the ability of bats to undergo long periods of fasting, which seem to be associated with their respective diets. At one extreme are hematophagous species that are unable to fast longer than 72 h due to its apparent inability to store and mobilize endogenous fuels. Interestingly, these bats seem to have evolved behavioral responses that allow them to cope with several foodless days. At the other extreme are insectivorous vespertilionid and rhinolophid species that can fast for several months. They are able to endure these long periods of fasting due to several different physiological mechanisms. First, they are thermolabile and are thus able to significantly reduce their T_b and metabolism, save energy and minimize the use of endogenous stores. Second, they have a specialized machinery (e.g., transport proteins) that allows them to mobilize fuels efficiently. Third, they are able to double their m_b and store significant amount of fat in preparation for periods of shortage. Last, some species migrate long distances in search of food and/or an environment less challenging or more conducive to torpor or hibernation.

Despite their ecological importance, far less is known about fasting adaptations in bats than non-volant mammals or birds. Many questions remain open regarding the physiology, endocrinology, biochemistry, and energetics of fasting in bats. For example, substantial differences in the life histories of females and males of the same species may significantly affect their seasonal responses to fasting and their ability to withstand periods of fasting. Another question of importance is how do bats switch between different fuel types compared with other species that fast. While migration is a well-studied phenomenon in birds, very few studies focused on bats. The comparison between these two taxa is highly interesting; because of

Table 16.1 List of studies exploring physiological responses to fasting in bats

Species	Suborderchiroptera	Body mass (g)	Diet	References	Characteristics studied
Desmodus rotundus	Micro-	42	Blood	McNab (1973); Freitas et al. (2003), (2005); Rouk and Glass (1970); Wilkinson (1984)	Energetics; fuel use in fasting; reciprocal food sharing; gastric histology
Diaemus youngi	Micro-	43	Blood	McNab (1973)	Energetics
Diphylla ecaudata	Micro-	34	Blood	McNab (1973)	Energetics
Rousettus aegyptiacus	Mega–	160	Fruits	Keegan (1977); Caviedes-Vidal et al. (2007); Korine et al. (1999)	Carbohydrate assimilation; plasma nitrogen levels
Eidolon helvu	Mega-	280	Fruit	Okon (1977)	Gastric histology
Artibeus lituratus	Micro-	70	Fruits	Caviedes-Vidal et al. (2007, 2008); Pinheiro 2006; Protzek et al. (2010)	Carbohydrate assimilation; fuel use in fasting; hormone levels
Artibeus jamaicensis	Micro-	50	Fruit	Pinheiro et al. (2006)	Fuel use in fasting
Glossophaga soricina	Micro-	11	Fruit	Bakken et al. (2008)	Renal function in fasting
Tadarida nigeriae	Micro-		Insects	Okon (1977)	Gastric histology
Myotis velifer	Micro-	12	Insects	Rouk and Glass (1970)	Gastric histology
Antrozous pallidus	Micro-	21	Arthropods	Rouk and Glass (1970); Bassett (2004)	Gastric histology; renal function in fasting
Molossus molossus	Micro-	11	Insects	Freitas et al. (2010)	Fuel use in fasting
Tadarida brasiliensis	Micro-	12	Insects	Widmaier et al. (1996); Rouk and Glass (1970)	Plasma triacylglycerides; gastric histology
Nyctophilus gouldi	Micro-	9	Insects	Morris et al. (1994)	Reabsorption from rectum
Scotophilus heathi	Micro-	33	Insects	Srivastava and Krishna (2010); Roy and Krishna (2010)	Hormone levels in fasting
Myotis lucifugus	Micro-	10	Insects	Bauman (1990); Kronfeld-Schor et al. (2000); Townsend et al. (2008); Eddy and Storey (2004)	Hormone levels in fasting; fuel mobilization
Eptesicus fuscus	Micro-	20	Insects	Yacoe (1983)	Protein catabolism
Leptonycteris sanborni	Micro-	22	Nectar	Rouk and Glass (1970)	Gastric histology

their flight ability, these two groups have similar selection pressures, but their physiological and biochemical machineries are different. We hope this review will stimulate others to address the many questions and gaps in our knowledge to which we have called attention.

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Chapter 17
Muscle Protein and Strength Retention by Bears During Winter Fasting and Starvation

Hank Harlow

17.1 Introduction

The grizzly (*Ursus arctos*) and black bear (*Ursus americanus*) share similar exposure to extreme food deprivation, cold temperatures, and immobility during the winter. This discussion will focus on the black bear and comparative studies with the grizzly bear to celebrate the uniqueness of their cardiovascular, respiratory, digestive, and urinary systems working in concert to conserve skeletal muscle strength while confined for 5 months within a den completely without food or water. The underlying mechanisms for protein sparing by bears during this time involves biochemical adjustment originally defined by Cahill on humans (Cahill 1970) and applied to other animals (Castellini and Rea 1992; Cherel et al. 1992; Wang et al. 2006) as a time-dependent alteration in the use of carbohydrate, fat, and protein categorized in three progressive phases. Historic reference to these phases have been extremely valuable in understanding an animal's physiological status when not eating; however, they are not discrete entities but overlap as an animal undergoes a transition of behavioral and biochemical alterations to the continued lack of food (McCue 2010). This chapter addresses the events in Fig. 17.1 which is a modification of Cahill's categories to emphasize that long-term adapted winter fasting by bears is closely tied to circannual fall and spring transitions with summer accretion of reserves. A brief overview of this figure is appropriate followed by a more detailed account of how bears adjust through these stages.

Energy is like currency in the bank; it can be deposited and used at a later date. Animals dealing with seasonal food scarcity have a higher hypothalamic set point

H. Harlow (✉)
Department of Zoology and Physiology, University of Wyoming,
1000 E University Ave, Laramie, WY 82071, USA
e-mail: hharlow@uwyo.edu

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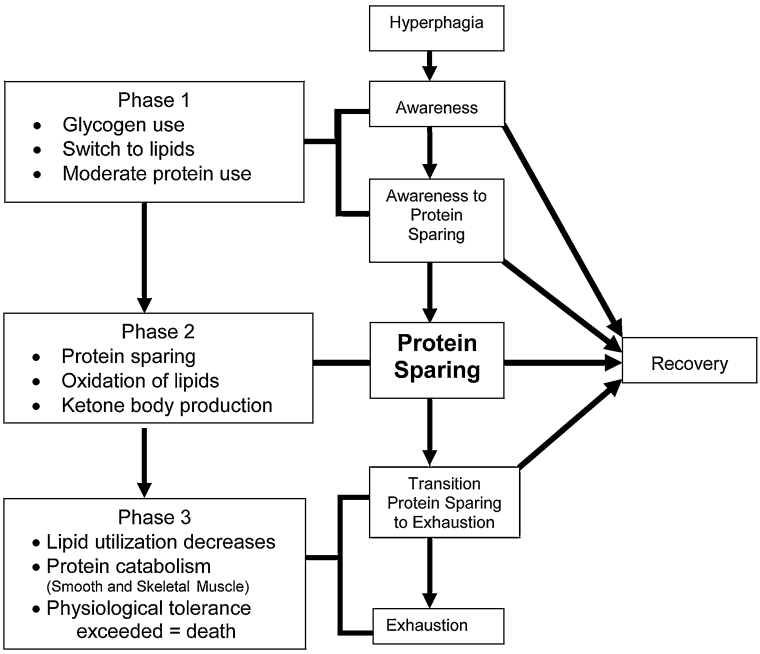

Fig. 17.1 Six stages expressed by black bears and other animals adapted to long-term food deprivation

for appetite and hyperphagy (Mrosovsky and Powley 1977) to maximize deposition of body stores. However, when food becomes scarce, the initial behavioral response is not uniform among animals. This can be represented as an Awareness Response (Fig. 17.1) where some species increase their metabolic rate and foraging behavior in an attempt to locate a dwindling food resource (see examples in Ben Hamo et al., Chap. 16; McCue et al., Chap. 8) while other animals exhibit decreased activity and metabolism as soon as food is in short supply (see examples in Hervant, Chap. 7; Ullrey, Chap. 18). This behavioral 'choice' occurs early during phase I of a fast, when glycogen stores are rapidly depleted (Cahill 1970; Castellini and Rea 1992) and brain cells receive glucose that is newly synthesized by gluconeogenesis (Morris 2005), using carbon from amino acids and glycerol cleaved from fatty acids (Fig. 17.1). The biochemical adjustment to enter phase II is not immediate, but requires a transition period of varying lengths to accommodate changes in lipid metabolism and gut microflora needed to adequately dampen protein catabolic pathways (see also McCue, Chaps. 1 and 24). For black bears, this is referred to as fall 'walking hibernation'. As the fast continues, an animal enters phase II, a state of fat use and protein sparing. Here, breakdown of protein is minimized, fat becomes the predominant source of energy, ketone bodies act as a glucose substitute and inhibit gluconeogenesis as well as cellular glucose transport (Morris 2005) thereby decreasing the body's demand for protein

breakdown (Cahill 1970; Castellini and Rea 1992). The extent to which the bear is adapted to prolonged food deprivation is expressed by the duration and depth of its resistance to protein loss (Fig. 17.1).

As fat becomes severely depleted and more protein is catabolized (Castellini and Rea 1992) an animal enters phase III which is gradual and does not necessarily involve irreversible functional damage to the body. There is a biochemical transition when the animal can use protein but still be capable of a timely recovery when resources become available (Fig. 17.1). This transition is of variable length among fasting animals depending upon the location and amount of labile protein reserves that conserve skeletal and cardiac muscle and other vital cellular protein components. For black bears, this period has been referred to as spring 'walking hibernation'. If the magnitude of protein loss compromises organ and skeletal muscle function without the possibility of a complete and timely recovery, a state of exhausted reserves is reached (Fig. 17.1) resulting in death. Black bears provide a classic model of adaptive long-term fasting. I will present the work we have conducted along with others who investigate the remarkable bear as it progresses through these stages of seasonal nutrient accretion and transition to accommodate 5 months where it does not eat, drink, urinate, or defecate.

17.2 Hyperphagia

During fall, black bears more than double their daily energy intake (Nelson 1980) achieving a total weight gain of 40% (Farley and Robbins 1995; Lundberg et al. 1976) with concomitantly high lipoprotein lipase (LPL) activity (Herminghuysen et al. 1995) and body fat content. The peptide hormone leptin, produced from ob gene expression in adipocytes reaches its peak when bears are at their maximal fat content (70 pg/ml leptin @ 40% body fat vs. 20 pg/ml leptin @ 10% body fat; Spady et al. 2009). The inhibitory effect of leptin on neuropeptide Y (NPY) from the arcuate nucleus signals the hypothalamus appetite centers and may initiate the onset of anorexia as bears get ready to enter their den for the winter (Spady et al. 2009; Hissa et al. 1998). However, the body fat set point (Mrosovsky and Powley 1977) is not the same for all cohorts of bears. Female black bears breed during the summer and harbor the fertilized blastocyst in diapause until midwinter implantation with a short (40 days) gestation and protracted lactation in the winter den. Thus, black bears are unique among vertebrates in that they express high reproductive energy demands while in a state of prolonged and complete food deprivation (see also Champagne et al., Chap. 19). We have found that reproductive bears have a 70% greater rate of mass and energy loss to produce two cubs while in their natural dens during the winter. To compensate, they put on 47% greater fat and 26% greater protein mass during the fall hyperphagic period (Harlow et al. 2002).

What signal stimulates the reproductive female bears to have a greater appetite, eat more, and store more reserves during the hyperphagic periods when

the fertilized ovum is only at a 64 cell stage with virtually no energy demands on the mother? We found elevated progesterone levels at this time (Harlow et al. 1990) that is known to enhance appetite and food intake during menstruation and pregnancy in animal models by increasing Agouti-related peptide (AgRP) and NPY in the arcuate nucleus (Faas et al. 2010). Increased progesterone in diapaused female bears would enhance their hyperphagia by circumventing the effect of leptin, thereby establishing a higher body fat set point to accommodate winter reproductive demands. But, what possible advantage is delayed implantation with protracted lactation during a period without food? This may be important for protein conservation by bears.

The fetal black bear has an obligatory requirement of glucose as the primary metabolic fuel that is provided by the mother through gluconeogesis of protein while lipids are the major substrate for suckling bear cubs during development (Oftedal et al. 1993). Indeed, we found that reproductive females expended an additional 1,216 kJ/day energy from fat and only 214 kJ/day more energy from protein to produce two cubs than non-reproductive females during denning. Therefore, a short *in utero* gestation would conserve protein and draw upon the enhanced fat stores for lactation.

17.3 Awareness-Transition to Protein Sparing, Phase I

When bears experience a decline in food availability during the fall, their response is not to increase foraging effort as seen in some animals but it is one of reduced movement and a metabolic adjustment to prepare for the long winter fast. LPL activity that was high during hyperphagia declines during this transition in bears (Herminghuysen et al. 1995) along with appetite and a shift from the accretion of fat to its catabolism as a result of elevated leptin signaling to the hypothalamus (Herminghuysen et al. 1995). This transition is further influenced by alteration in the gastrointestinal tract (GIT) microflora when black bears have a higher efficiency of energy assimilation that offsets their reduced food intake (Nelson et al. 1983). There is a decrease in thyroxin (Tomasi et al. 1998) and altered cortisol (Harlow et al. 1990) that influences how fat and protein are used during this time because bears show a reduced propensity to develop ketosis or ketonuria (Nelson et al. 1973) and urea:creatinine ratios begin to decline (Nelson et al. 1975). All together, this transition in GIT assimilation and mechanism of protein and fat use in bears has been referred to as fall 'walking hibernation' that is an extremely important interval to consider when evaluating how cardiac and skeletal muscle of bears adjust to winter fasting conditions. As discussed later, studies that compare bears only during mid-summer and mid-winter overlook this transition phase, and thereby make it difficult to interpret seasonal alterations of summer protein accretion and early to late winter protein sparing by bears.

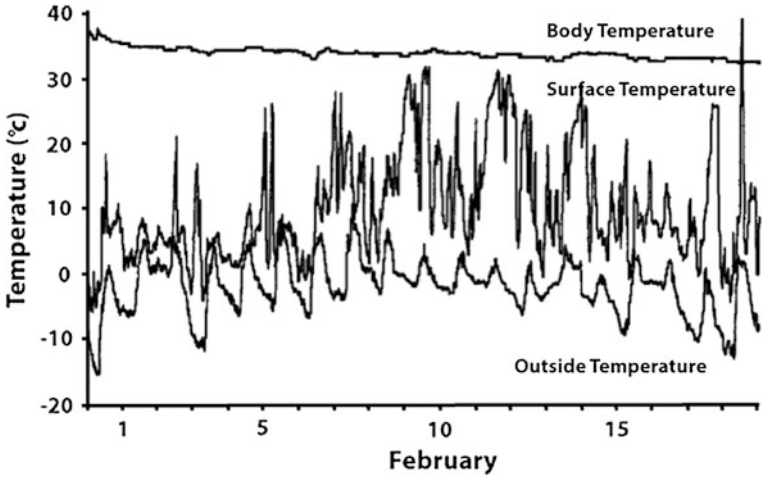

Fig. 17.2 Deep core, skin, and den temperature of black bears during the winter (Harlow et al. 2004)

17.4 Protein Sparing, Phase II

17.4.1 Torpor Cycle

Reduced food intake in many animals results in a state of mild hypothermia and hypometabolism (Wang et al. 2006; Bicego et al. 2007). Small mammal hibernators take this to extreme with a drop in body temperature close to freezing, punctuated by rhythmic arousals to normothermia every 5–15 days (Geiser 1998). We have implanted intraperitoneal temperature loggers into bears and found that free ranging individuals in their natural dens have a relatively mild ($T_b \approx 35°C$) hypothermia (Fig. 17.2) but with an associated metabolic depression of about 50% (Christopher et al. 2010) that is considerably greater than the temperature Q_{10} prediction (Toien et al. 2011; see also Hohtola, Chap. 10). Also, we observed no arousal bouts throughout the winter (Fig. 17.2) a very important factor because the dozens of arousals by small mammals account for the majority of their winter energy expenditure and it primarily uses protein as a substrate. The lack of arousal by bears for 5 months therefore conserves not only energy but also body protein stores.

17.4.2 Fat Composition and Use

All vertebrates can synthesize saturated fatty acids (SFA) or fatty acids with one double bond (MUFA); however, polyunsaturated fatty acids (PUFA), particularly linoleic and linolenic acids must be obtained from their diet that bears obtain from

plants such as cow parsnip (*Heracleum maximum*). Some animals when food deprived, like the raccoon dogs (*Nyctereutes procyonoides*) and emperor penguins (*Aptenodytes forsteri*), mobilize fatty acids with shorter carbon chains and more unsaturated bonds (MUFA and PUFA) at a higher rate than longer, saturated fatty acids (Raclot and Groscolas 1993) (see also Price and Valencak, Chap. 15). In contrast, fasting reptiles (McCue 2008) and hibernating marmots (*Marmota flaviventris*) selectively mobilize SFAs (Florant et al. 1990) and retain MUFA and PUFA that may offer a homeoviscous adaptation to maintain membrane fluidity while the tissue is cool during torpor (Florant et al. 1990). It is also thought that superficial subcutaneous fats are more unsaturated than deeper reserves due to a thermal gradient between core and skin temperatures (Ben-Hamo et al. 2011) (see also Hohtola, Chap. 10). While we found the three main fatty acids in subcutaneous white adipose tissue of bears are palmitic (16:0), oleic (18:1n9), and linoleic (18:2n6) similar to that reported for small mammal hibernators and captive winter black bears (Hissa et al. 1998; LeBlanc et al. 2001), we did not identify a difference in individual FA composition or unsaturation index ($\sum$weight % UFA $\times$ number of double bonds) of superficial and deep fat deposits or of samples taken from bears during late winter and early winter. These findings do not support a preferential retention of PUFA as seen in winter hibernation fasted small mammals (Florant et al. 1990) nor a preferential use of PUFA as reported on euthermic fasted birds and mammals that may be due to the mild hypothermia and a more random arrangement of SFA, MUFA, and PUFA within adipocytes of bears and consequent uniform mobilization while food deprived (see also Price and Valencak, Chap. 15).

17.4.3 Cardiac Tissue Conservation

Long term fasting in obese humans with body fat content similar to prehibernation bears cannot be met by fat alone and results in cardiac muscle protein loss and dysfunction (Yuan et al. 2008; see Hall, Chap. 22; Varaday, Chap. 23). This is compounded by left ventricular resculpturing and reduced thickness during inactivity and mild hypotension (Pehonen et al. 2001). We implanted high sensitivity EKG and ventilatory rate loggers (Laske et al. 2005) in free ranging bears, and documented dramatic respiratory sinus arrhythmia (RSA) with long sinus pauses between breaths (up to 13 s) and profoundly depressed heart rate as low as 4.5 bpm followed by rapid elevations that corresponded with inspiration (Laske et al. 2010). Although humans also exhibit RSAs, the extent in hibernating bears is more than an order of magnitude greater. Echocardiographic images of bears during early and late winter (Laske et al. 2010) showed no reduction in left ventricle thickness (Fig. 17.3). Moreover, we observed no altered primary cardiac electrophysiological parameters using 12-pin lead EKG electrodes or elevated blood taurine (the primary amino acid in cardiac tissue) levels (Lohuis et al. 2005).

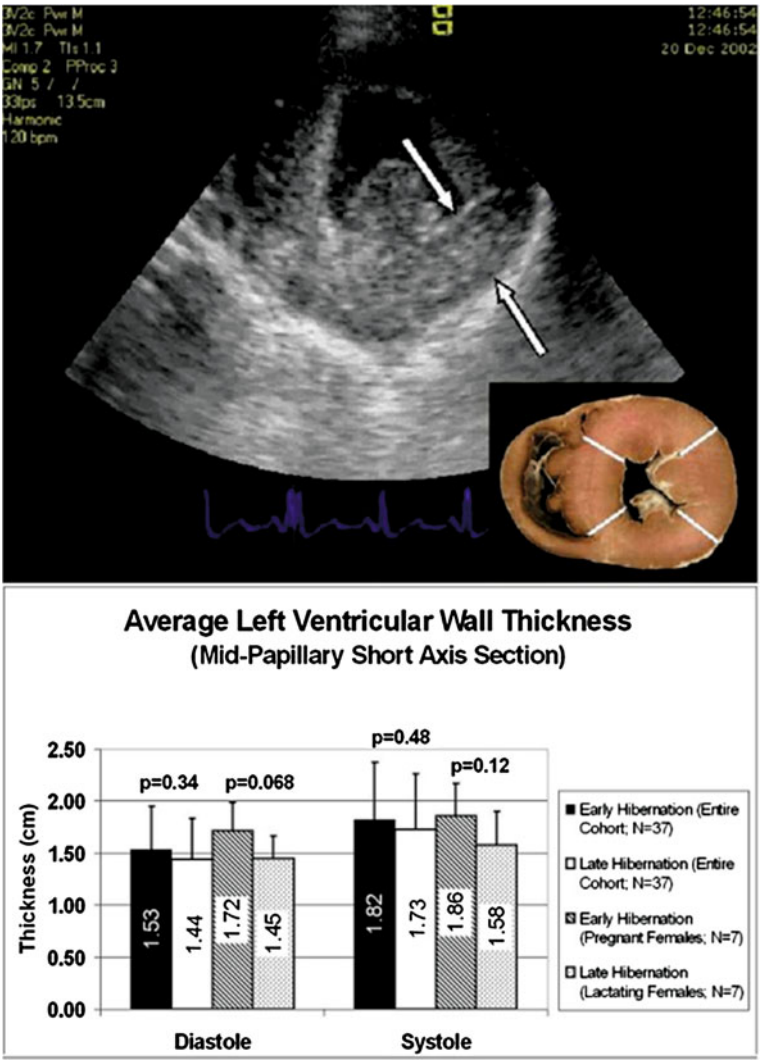

Fig. 17.3 *Top panel* -Cross-sectional echocardiographic image showing left ventricular wall thickness of black bears. *Bottom panel* -Plot of wall thickness in early and late winter (Laske et al. 2010)

We believe the accelerated heart rate during RSA enhances oxygen transport within the heart tissue while the period of dramatically reduced heart rate acts to minimize energy expenditure and conserves cardiac protein. Similar results were recently reported on captive black bears (Toien et al. 2011) that, together with our study, infer an adaptive consequence of RSA to bears by maintaining sufficient perfusion of blood throughout cardiac and skeletal muscle tissue during

winter to enable a quick response if disturbed. However, research on captive grizzly bears showed reduced cardiac mass and performance when compared between midsummer and mid winter (Nelson et al. 2003). We propose that the loss of cardiac muscle observed in captive bears (Nelson et al. 2003) may have occurred during the 'walking hibernation' transition phase from summer to fall before biochemical processes became fully implemented as opposed to our study showing no alteration while fasting and in mild hypotension during 5 months of denning.

17.4.4 Skeletal Muscle Conservation

17.4.4.1 Muscle Fiber Composition

Skeletal muscle, as cardiac tissue, is at risk of functional decay due to prolonged food deprivation and disuse atrophy. Models of muscle atrophy consistently predict a profound decrease in number and mass, fiber size, conversion of slow oxidative (SO) to fast glycolytic (FG) fibers with associated loss of myosin heavy chain slow isoforms (MHC_1) with concomitant fast twitch mechanical contraction properties. To test these predictions we took muscle biopsies from the biceps femoris, gastrocnemius, and vastus lateralis of bears during the early and late denning season that were sectioned and stained for muscle fiber characteristics and assayed for MHC isoform expression. We found no loss of number or cross-sectional area of fibers in any muscle tested that is similar to findings on overwintering captive grizzly bears (Hershey et al. 2008). In addition, bears exhibited no marked transition of SO to FG fibers in muscles except for a marginal drop in SO fiber ratio in the biceps femoris of reproductive females that was not observed in overwintering non-lactating bears and showed an increase in the slow MHC_1 compared to fast MHC_{2x} isoform (Rourke et al. 2006). This increased MHC_{2X} isoform is also characteristic of small mammal hibernators, but not evident in captive overwintering grizzly bears (Hershey et al. 2008). Moreover, we did not observe altered contraction and relaxation times by the tibialis anterior muscle tested in vivo (Lohuis et al. 2007a) or the vastus lateralis muscle tested in vitro (Lohuis et al. 2012) that would be expected if atrophy induced SO to FG conversion had occurred. However, Hershey et al. (2008) did observe increased twitch contraction times of captive grizzly bears between midsummer and midwinter the authors attribute to potentially altered calcium kinetics needed to maintain slow oxidative fibers. Although these studies show subtle differences between muscles of bears, the general profile is one of relatively unaltered fiber composition during the winter that is atypical of starvation or disuse-induced skeletal muscle atrophy models (Hortobagyi et al. 2000; Haddad et al. 2003) (see also Bauchinger and McWilliams, Chap. 12).

17.4.4.2 Protein Content

Food deprivation and atrophy causes a profound decrease in myofibrillar and sarcoplasmic protein (Lecker and Goldberg 2002); however, the p-ratio (proportion of energy expended from protein oxidation) is vastly lower in fasting obese bears which show high ketone body inhibition of gluconeogenesis (Nelson et al. 1973), low thyroid activation of proteolysis (Azizi et al. 1979; Hissa et al. 1994; Lundberg et al. 1976; Tomasi et al. 1998), and concomitantly low urea:creatine ratio. In spite of this, a basal level of protein catabolism in all animals is needed to sustain a source of citric acid cycle intermediates and oxidative water production (Bintz et al. 1979). Some protein may be provided by skeletal muscle catabolism, but it is important to note that not all muscles behave identically during periods of disuse and fasting. Tinker et al. (1998) report a 4 and 11% loss of protein from the gastrocnemius and biceps femoris muscles, respectively, by lactating, free ranging black bears between early and late hibernation. Similarly, an 8.2% loss of protein in the biceps femoris was reported in captive brown bears between midsummer and midwinter (Hershey et al. 2008) but no loss from the gastrocnemius and extensor hallucis longus (Koebel et al. 1991) between mid-hibernation and post denning, or the vastus lateralis (Lohuis et al. 2007b) over the same period. Therefore, either marginal loss or complete protein preservation occurs in most, if not all, hind limb muscles of bears during winter denning.

Barboza et al. (1997) proposed that small losses of protein from several muscle groups in bears would be sufficient to meet the denning bears' metabolic demand for protein without severely compromising muscle function. We originally extrapolated the marginal protein reduction from the biceps femoris and gastrocnemius, to estimate total skeletal muscle loss as representing 13.3% of the overwinter weight loss (Tinker et al. 1998). However, if one now considers that the whole body skeletal muscle performance in response to disuse and fasting is a sum of its individual muscles, the total lack of protein loss by some muscle suggests that the overwintering black bears may be conserving even more skeletal muscle protein than previously estimated (Barboza et al. 1997; Harlow et al. 2002; Lohuis et al. 2007a, b). Indeed, we found that nonreproductive female bears lose about 0.03% protein/day, a value that is an order of magnitude below that reported for rats and humans (Berg et al. 1997). Interestingly, while we saw a marginal loss of protein by some muscles, we did not see a change in total nitrogen content of any muscle tested (Lohuis et al. 2007a, b; Tinker et al. 1998) that suggests variable protein turnover by specific muscles during the winter.

17.4.4.3 Protein Turnover (Synthesis and Breakdown)

Protein turnover is defined as the amount of synthesis and degradation at any point in time (Waterlow 2006). If anabolism is greater than catabolism, protein accretion is the outcome; however, when deposition does not adequately match depletion, then protein loss or atrophy will result. The evidence from previous research on

skeletal muscle turnover at different seasons by bears is contradictory. Lundberg et al. (1976) reported that rates of protein synthesis and breakdown were elevated 3- to 5-fold in hibernating bears with a suggested de novo synthesis of essential amino acids (Nelson 1980; Nelson et al. 1973, 1975, 1983). In addition, muscle protein anabolism was believed to be elevated in hibernating bears relative to summer active animals (Koebel et al. 1991). A genomics study conducted by Fedorov et al. (2009) on biopsies taken from the skeletal muscle of captive black bears during late summer and late winter reported that genes for protein synthesis were elevated while genes for protein breakdown were unchanged with the implication of skeletal muscle protein accretion during 5 months of no energy or nitrogen intake.

Because confusion existed as to the sources of nitrogen for protein synthesis, we conducted an in vitro study of turnover on muscle biopsies taken from bears at the den during the summer, early, and late winter periods. Protein synthesis of the vastus lateralis was measured using ^{14}C phenylalanine uptake into biopsied samples under controlled temperature, pH, PO_2, and PCO_2, while protein breakdown was measured as tyrosine release by the tissue sample. A portion of the biopsy was also measured for protein, total nitrogen, RNA and DNA content, and nitrogen stable isotope $\delta^{15}N$ enrichment, all of which are indicators of protein metabolism (see Hatch, Chap. 20). We found that bears underwent a period of net muscle protein accretion with synthesis rates 1.4 times higher than degradation during the summer contrary to previous studies suggesting winter (not summer) protein accretion (Koebel et al. 1991; Lundberg et al. 1976; Nelson et al. 1973, 1975, 1983). We interpret our higher ratio of muscle protein synthesis to catabolism in summer bears as representing the regeneration of protein lost during the previous fall that is similar to a condition in human athletes where turnover is enhanced by ingestion of carbohydrates and amino acids, resulting in sarcoplasmic and myofibrillar protein synthesis to replace earlier utilized tissue, leading to net muscle deposition (Rasmussen et al. 2000). During the transition from summer to fall we saw both rates of synthesis and breakdown to decrease by 60–70% behaving in a fashion similar to fasting and disuse models (Waterlow 2006; Mitch and Goldberg 1996) (see also Bauchinger and McWilliams, Chap. 12). Unlike these models, however, we did not see an increase in muscle degradation. Here altered protein turnover was represented simply as a lower amplitude drop in degradation than the drop in synthesis resulting in matched rates for protein balance and protein conservation during the remainder of the winter (Table 17.1).

Additional evidence for protein sparing is gained from other indices of protein turnover. Between the summer active and early denning period bears showed a 30–60% drop in RNA content (tissue capacity for protein synthesis), a decrease in the ratio of protein synthesis to RNA content (ribosomal efficiency), and a reduced ratio of RNA to DNA (protein synthesis per cell number) (Table 17.1). Fractionation of ^{15}N expressed as the $\delta^{15}N$ value is known to elevate during fasting in bears due to a differential retention of the heavier nitrogen isotope (see also Hatch, Chap. 20). We found enrichment in the biopsied black bear muscle at a similar magnitude (18%) as the decrease observed in protein content (20%) from summer

Table 17.1 Indicators of protein syntheses, degradation, and strength of black bears during summer, early and late winter

	Summer[g]	Early winter	Late winter
Protein content[a]	530.00 (20.0)	420.00 (18.0)	430.00 (15.00)
Protein synthesis[b]	0.75 (0.07)	0.25 (0.03)	0.26 (0.23)
Protein breakdown[c]	0.53 (0.09)	0.21 (0.01)	0.25 (0.03)
$\delta^{15}N$[d]	0.54 (0.17)	0.64 (0.20)	0.64 (0.15)
DNA[e]	4.51 (0.35)	4.40 (0.44)	4.02 (0.22)
RNA[e]	2.47 (0.32)	1.74 (0.17)	1.52 (0.09)
RNA/DNA ratio	0.52 (0.05)	0.38 (0.26)	0.39 (0.02)
Synthesis/RNA[b,e]	3.28 (0.52)	1.38 (0.26)	1.56 (0.23)
Muscle strength[f]	10.2 (1.50)	6.40 (1.10)	6.40 (0.80)

[a] tissue mass
[b] indicates (nmol of tyrosine incorporated/μg nucleic acid)/mg tissue mass
[c] indicates (nmol of tyrosine released/μg nucleic acid)/mg tissue mass
[d] relative to atmospheric nitrogen
[e] indicates μg nucleic acid/mg tissue mass
[f] indicated gm force/cm^2
[g] indicates significant difference ($p < 0.05$) of summer from early and late winter

to early denning. However the $\delta^{15}N$ signature, RNA content, ribosomal efficiency, and RNA/DNA ratio then remained unchanged throughout the 5 months of denning food deprivation (Table 17.1). Our conclusions are not consistent with the genomics study of muscle biopsied from captive black bears during midsummer and midwinter. Fedorov et al. (2009) reported an overexpression of genes involved in protein biosynthesis without a concomitant overexpression of genes for protein breakdown in midwinter suggesting protein accretion during hibernation fasting rather than during summer feeding.

Our results of summer protein accretion are in agreement with Barboza et al. (1997) who reported captive bears to gain protein during the summer and for bears to be in nitrogen balance during both fall hyperphagia and winter fasting with whole body protein synthesis and breakdown being equal. In contrast to the genomics study (Fedorov et al. 2009), Barboza et al. (1997) reported that rates of whole body protein catabolism increased during winter (compared to summer) but synthesis did not; and as in our study bears were in protein balance throughout the winter. It is important to note that while these three studies may have subtly different results, they are not in contradiction. We believe that an increase in whole body catabolism during the winter (Barboza et al. 1997) suggests that protein may be selectively mobilized from some muscle groups and not others and that protein reserves besides skeletal muscle are being used. It is possible that the muscle reported by Fedorov et al. (2009) indeed underwent elevated transcriptional and translational expression of protein synthesis without degradation during the winter, but it occurred at the expense of other more labile endogenous nitrogen sources.

17.4.4.4 Differential Skeletal Muscle Use and Labile Protein Reserves

Confusion concerning the suggestion of higher protein turnover during winter months can be resolved when considering the following points: 1) individual muscles atrophy at different rates and have unique turnover profiles, 2) nonskeletal muscle protein can act as labile sources of nitrogen, and 3) urea nitrogen salvage (UNS) conserves and redirects nitrogen into specific muscle groups over others.

Labile skeletal muscle protein reserves—Selective protein retention by one muscle at the expense of others has been reported on animals during limited mobility and fasting (Cherel et al. 1994; Le Maho et al. 1981; Yacoe 1983). It is apparent that the hibernating black bear has developed a similar strategy. We believe a portion of the nitrogen released from muscles such as the gastrocnemius and biceps femoris may be resynthesized into protein of other muscles like the vastus lateralis and extensor hallucis longus. But, overall, only a small amount of protein needs to be catabolized from any skeletal muscle groups (Barboza et al. 1997) if other, more labile protein reserves are used to meet the protein requirements for energy and water by the fasting bear.

Labile non-skeletal muscle protein reserves—Tissues that have a high turnover rate may act as labile protein reserves with a release of amino acids (Le Maho et al. 1981) and include the skin, viscera, blood albumins (Cherel et al. 1994) as well as extracellular matrix such as collagen (Cherel et al. 1994; Hissa et al. 1998), which is a rich source of amino acids, especially glycine (Hissa et al. 1998). The collagen metabolites hydroxyproline and glycine were markedly elevated in our free-ranging black bears during the late winter (Lohuis et al. 2005) as well as captive, fasted brown bears (Hissa et al. 1998) and were represented by over expressed genes for type I collagen breakdown in fasted captive black bears (Fedorov et al. 2009). In addition, proteolysis of kidney tissue releases large amounts of serine, that we found to be high in bears during the late winter (Lohuis et al. 2005). The GIT may also be a source of labile protein due to its high turnover rate (Secore and Diamond 1998) with associated elevation of blood α-amino acids (Felig et al. 1969) (see also Lignot, Chap. 14). Using ultrasound imaging, we found the thickness of the small intestine endothelium to appear reduced in bears between early and late winter with concomitant elevation of alpha amino acids. Bintz et al. (1979) determined that skeletal muscle accounts for only 52% of the protein catabolized by starving ground squirrels (*Urocitellus richardsonii*) with the remainder provided from viscera, liver, skin, and collagen. If bears use such sources in the same proportions, the amount of protein I calculated earlier in this chapter from skeletal muscle loss alone, would be approximately double from the whole body reserves thereby providing ample amino acids for protein resynthesis in specific skeletal muscles as well as a source of water and TCA cycle intermediates. Given this scenario, elevated gene transcription for protein synthesis as described by Fedorov et al. (2009) could occur in a selected muscle if amino acids are made available by the marginal degradation of other skeletal muscles in concert with proteolysis of organs, collagen, and smooth muscle. As a result, there would not be an overall increase in whole body protein accretion during the winter

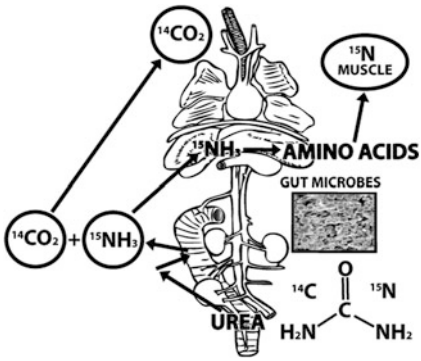

Fig. 17.4 Schematic of body organs depicting the fate of administered nitrogen and carbon labeled urea from the bladder into skeletal muscle and exhaled air

by food deprived bears, only a redistribution of nitrogen between tissues in the amino acid protein pool. One way to accomplish this redistribution is through recycling of amino acid nitrogen by UNS.

UNS—Remarkably, bears do not urinate during the 3–5 months of hibernation (Nelson et al. 1975) due to the process of UNS, which is a symbiotic relationship between a host and its GIT ureolytic microbes that have the enzyme urease. UNS requires three key mechanisms: 1) urea transport out of the bladder into the blood and then into the lower GIT, 2) GIT ureolytic microbial hydrolysis of urea into CO_2 and ammonia, and 3) ammonia transport via blood to the liver for synthesis of new amino acids (Fig. 17.4). The pathway for efficient UNS became evident upon the identification of a family of urea transport (UTB) protein within the GIT tissue that we recently identified in the bladder, and GIT of small mammal hibernators (Greller 2010).

It has been hypothesized that UNS may upregulate during low nitrogen intake and fasting. Indeed, we found ^{15}N labeled nitrogen from exogenously administered ^{15}N-urea in Wyoming ground squirrels (*Spermophylus elegans*) to be enriched in specific tissues, with values elevated 10- to 40-fold during fasting and hibernation (Fig. 17.5). The greatest enrichment of ^{15}N, occurred in tissue with the highest turnover rate such as the GIT, liver, and then skeletal muscle. While bears salvage almost all (99%) of their urea during the winter hibernation fast they cannot do this during a summer euthermic fast (Barboza et al. 1997; Nelson 1980), perhaps due to seasonally altered mechanisms of urea retention and increased UNS. We believe hibernating bears, as the ground squirrel, upregulate the urea transporters in the bladder and GIT during fasting that elevate urea concentrations in the gut as a substrate to enhance the ureolytic microbial community hydrolysis and consequent hepatic reammination of ammonia into nonessential amino acids and potential harvest of gut microbes for essential amino acid synthesis. Indeed, the genomic study by Fedorov et al. (2009) showed a midwinter underexpression of hepatic genes for urea cycle enzymes by bears, thereby preventing a redundant conversion of ammonia back to urea, but rather an enhanced hepatic amino acids synthesis. As a result, nitrogen recycled from protein in smooth muscle, organ, or more labile

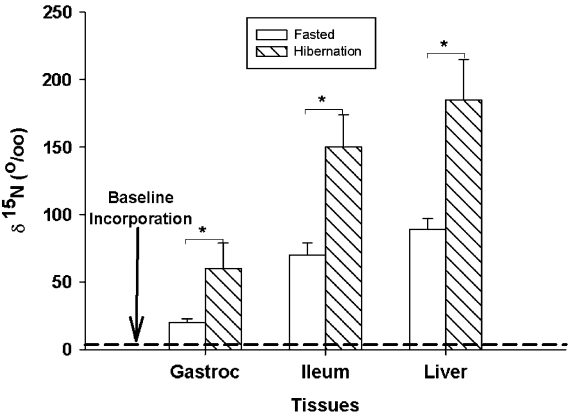

Fig. 17.5 ^{15}N enrichment (expressed as $\delta^{15}N$) of skeletal muscle (gastrocnemius), intestine (ileum), and organs (liver) from ^{15}N labeled urea administered to fasted and hibernating Wyoming ground squirrels. Asterisk represents significance difference ($P < 0.05$)

skeletal muscle groups may provide the substrate for protein synthesis in other muscles such as the vastus lateralis or extensor hallucis longus.

17.4.4.5 Strength Retention

If skeletal muscle protein and fiber structure are conserved by bears during prolonged food deprivation and immobility, then a concomitant retention of strength would be expected. We measured muscle performance of bears in vivo when applying electrical stimulation of the common peroneal nerve that elicited dorsiflexion of the tibialis anterior muscle, resulting in a maximal force reading from the foot when snugged to a platform transducer while the bear was anesthetized at the den site (Fig. 17.6). Muscle strength declined only 23% over the winter (Harlow et al. 2001) that is far less than the 80% drop expected of inactive human and laboratory animals over the same interval (Fig. 17.7). These results are remarkable considering the bear experienced multiple stressors of complete food deprivation and immobility that have a compounding effect on muscle atrophy.

We also conducted an in vitro study on biopsies from the vastus lateralis taken from bears during midsummer, early and late winter (Lohuis et al. 2012). In agreement with protein content and turnover of this muscle, strength reduced from the summer to early winter. Interestingly, strength was completely retained during the winter hibernation (Table 17.1). In partial agreement with our study, Hershey et al. (2008) found that captive brown bears had no loss in muscle strength when measuring in vitro biopsies of the biceps femoris muscle from midsummer active and midwinter hibernating bears. As discussed earlier, we feel there are seasonally unique biochemical mechanisms associated with protein and strength expression for summer accretion and winter conservation. These distinctions may not have been picked up in the Hershey et al. (2008) study because they reported an 8.2% loss of protein over this period that would be expected to result in a detectable decrease in the force of contraction. We believe the protein loss they recorded

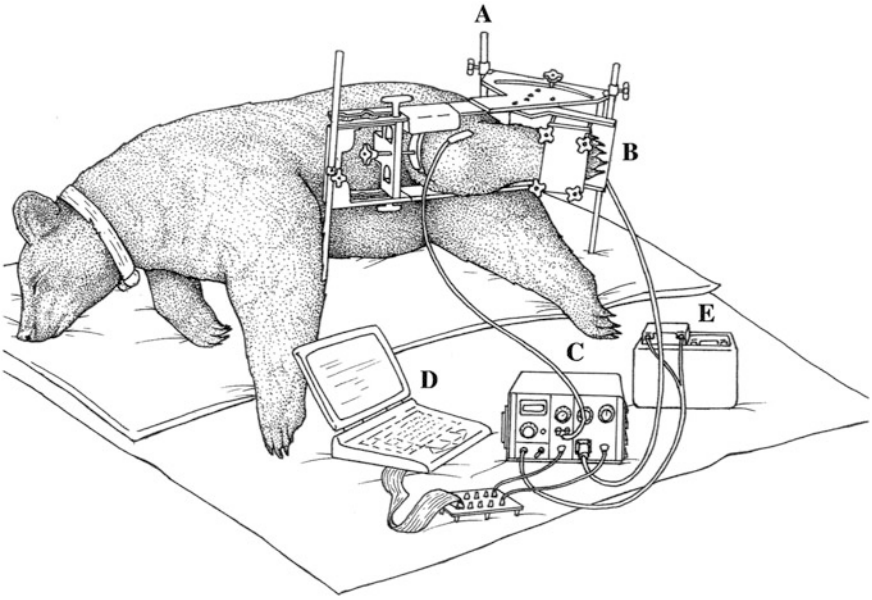

Fig. 17.6 In vivo apparatus for measuring muscle strength of black bears showing: A-leg brace, B-foot pressure plate, C-stimulator, D-data logger, E-battery power source (Harlow et al. 2001)

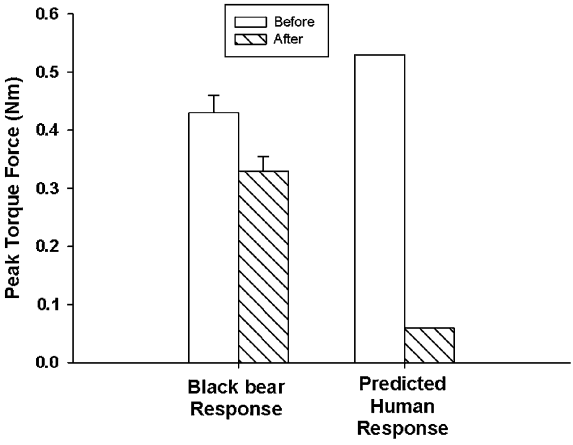

Fig. 17.7 Peak torque force (Nm) of black bear muscle during early and late winter as well as predicted force by inactive humans over the same interval (Harlow et al. 2001)

could have occurred between summer and fall as in our study, and once in the midwinter mode, both protein and strength are retained through the long fast.

We must look at atrophy not only as a mosaic of individual muscles responding uniquely to fasting and immobility, but also as a continuum of circannually changing biochemical events dependent upon the extent of food availability and scarcity. Overall, while skeletal muscles reported on bears may behave with subtle

differences, the unified response reported by researchers is one of the strength retention as a result of protein and fiber conservation during the winter.

17.4.4.6 Muscle Activity within the Den

In order for muscles to retain strength, they require a certain amount of contraction episodes to maintain compliance. Small mammal hibernators engage in violent shivering bouts every 10–15 days (Rourke et al. 2006) during arousal from torpor that bears do not do (Fig. 17.2). In order to determine whether bears shiver, we implanted electromyogram (EMG) loggers (Laske et al. 2005) in bears to monitor muscle activity along with temperature loggers in the bear's abdominal cavity and on the surface of their skin as well as at the entrance of the bear's den. The den demonstrated rhythmic diel changes in temperature, but the body temperature remained at a constant mildly hypothermic state throughout the winter. During this period we observed from 3 to 5 daily, irregular elevations in the skin temperature (5–15°C) (Fig. 17.2) with concomitant EMG signals associated with muscle contractions (Laske et al. 2005). Similar temperature profiles were recorded when the same loggers were placed on the skin surface of humans during short bicycle rides, suggesting the episodes of skin temperature increase represented a fairly rigorous muscle activity by bears during the winter with associated peripheral vasodilation and heat loss to avoid a core temperature increase (Harlow et al. 2004). Therefore, bears do exert overwintering muscle activity to help retain compliance and these bouts are thought to be linked to RSA synchronous with periods of rapid heart rate and breathing (Toien et al. 2011).

17.5 Transition from Protein Sparing to Late Phase III and Exhaustion

When bears leave the den they are often very lean and face food scarcity that is limited to emerging grasses and forbs and availability of carrion. If food resources are insufficient, bears may be unable to make a timely recovery and ultimately enter a state with exhausted body reserves equivalent to a late phase 3 fast, potentially resulting in death. But, even if food is available as they leave the den, bears do not show an immediate switch to accretion of fat and skeletal muscle protein. It has been suggested that this is a period of spring 'walking hibernation' similar to that during the summer to fall transition (Fig. 17.1). Toien et al. (2011) made this point clear when they demonstrated that black bears emerging from the den maintained a body temperature still mildly hypothermic (36.6°C), metabolic rates that were 52.9% of BMR with RSA, and a heart rate that was only 43% of summer levels. This physiological state persisted for several weeks while the bears were actively foraging and consuming food. This transition state seems to

represent biochemical adjustments by the bear needed for full resumption of GIT capacity to digest and assimilate food (Fedorov et al. 2009; see also Jenni-Eiermann and Jenni, Chap. 11).

17.6 Conclusions

Overwintering black and brown bears are adaptively obese and exhibit remarkable protein conservation mechanisms during 5 months without food or water while confined within a winter den. RSA maintains cardiac muscle tone and bouts of isometric and isotonic contraction retain compliance and fiber-type composition of skeletal muscle. UNS recycles urea nitrogen from labile protein sources such as smooth muscle and collagen to be used in the synthesis of skeletal muscle protein for relatively uncompromised strength retention and locomotor capacity over the winter. Areas of future focus should address why certain skeletal muscle groups are lost at the expense of others and how cardiac muscle is completely protected from atrophy during winter fasting. Bears need to be monitored throughout the year to understand their biochemical processes of 'waking hibernation' as they undergo transition into and out of winter torpor. How is it that the bear is the only nonruminant capable of recycling almost 100% of its urea nitrogen? Compound specific ^{13}C and ^{15}N stable isotope analysis tracer studies are needed to determine the source of essential amino acids for protein synthesis at a time of no energy or nitrogen intake: is it collagen and other labile protein reserves or the 'harvest' of microbes as seen in some hind gut fermenters? Continued investigation of gene expression by food-deprived bears is essential but they must be linked to studies determining their protein product to elucidate biochemical pathways that allow bears to deal with seasonal hypothermia, hypometabolism, and protein conservation. Understanding these mechanisms in bears has application in preventing muscle disuse atrophy of humans during periods of food deprivation, immobility, and weightlessness associated with hospital confinement and space travel.

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Chapter 18
Seasonal Starvation in Northern White-Tailed Deer

Duane E. Ullrey

18.1 Introduction

18.1.1 Geographical Distribution

White-tailed deer (*Odocoileus virginianus*) are ruminants that acquire their food primarily by browsing. They are native to southern Canada, the United States (except for Nevada, Utah, California, Hawaii, and Alaska), Central America, and South America as far south as Peru, and possibly Bolivia (Baker 1984). Such widespread geographical distribution is evidence that this species is adaptable to a variety of environmental circumstances, including ambient temperature, moisture supply, day length, and food availability. Whether living in a north temperate zone or a tropical environment, white-tails favor habitats with shrubs and low trees for browse and cover (Halls 1984).

18.1.2 Northern Ecosystem

In northern regions of the U.S. and southern Canada, white-tails tend to use the forest edge and nearby agricultural land in non-winter months. Grasses may be consumed in early spring on open-land habitat, but grasses are replaced in the diet by forbs, leaves of shrubs, and agricultural crops as these become available in late spring and summer. In late summer and fall, as forbs dry and crops are harvested, deer switch to green leafy browse, crop residues, and if available, acorns and wild fruits (McCullough 1984). When autumn frosts suspend access to green leaves of forbs, shrubs, and low-hanging

D. E. Ullrey (✉)
Departments of Animal Science and Fisheries and Wildlife,
Michigan State University, East Lansing, MI 48824, USA
e-mail: dsullrey@aol.com

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Fig. 18.1 Winter deer-yard in northern Michigan. Photo credit DEU

trees, deer respond by concentrating in heavy coniferous cover, where snow accumulation is limited by overhanging branches, wind is blocked, and convective and radiant heat losses are minimized.

Here, deer are forced to subsist on nutritionally poor small twigs and evergreen fronds (Ullrey et al. 1964, 1967, 1968, 1971, 1972). Northern white cedar fronds (*Thuja occidentalis*) are the best of these, followed by balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), and large-toothed aspen shoots (*Populus grandidentata*)—items found in conifer swamps, conifer uplands, and nearby cutover areas, although deer generally prefer to yard in cedar swamps when these are available (Fig. 18.1).

The apparent digestible energy (DE) in winter browse from white cedar or aspen, fed to captive, pregnant white-tailed does, was found to be 59 or 52% of browse gross energy (GE), respectively. Estimated metabolizable energy (ME) in these browses was 46 or 28% of GE, respectively (Ullrey et al. 1972). However, voluntary daily browse intakes were only 1.16 kg of cedar or 0.30 kg of aspen.

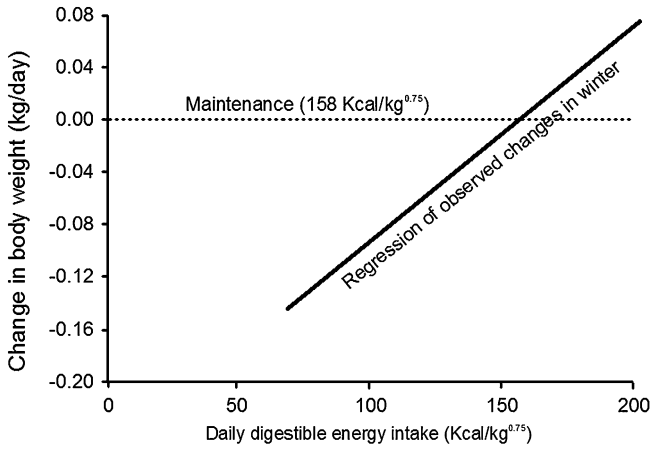

Fig. 18.2 Digestible energy requirements for winter maintenance of adult white-tailed deers determined by regression of average daily bodyweight gain or loss on average daily DE intake. Redrawn from data presented in Ullrey et al. (1970)

Fig. 18.3 Deer browse line in Michigan white cedar swamp. Photo credit DEU

Adult does were previously shown (Ullrey et al. 1970) to require 57 kcal DE/kg body weight/day (158 kcal DE/kg $BW^{0.75}$/day) for maintenance during a Michigan winter (Fig. 18.2). By adjusting for energy losses in urine and methane, requirement estimates for winter maintenance become 47 kcal of ME/kg of body weight/day (131 kcal ME/kg $BW^{0.75}$/day). Thus, 0.91 kg of cedar fronds or 1.36 kg of

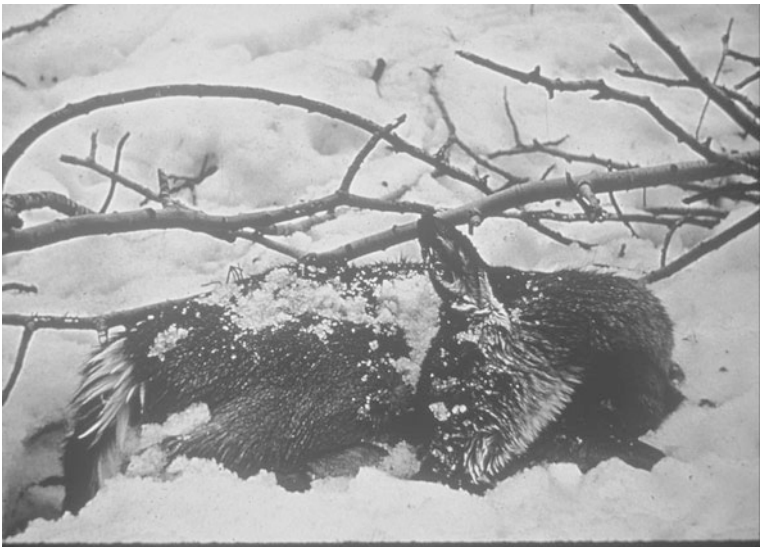

Fig. 18.4 White-tailed deer dead of starvation in region where woody browse was the only winter food available. Photo credit DEU

aspen shoots must be consumed per day to prevent more than a 25% loss of body weight during a 100-day wintering period. Assuming normal body fat stores at the onset of winter and no undue delay in spring green-up, most deer restricted to white cedar for winter browse would be expected to survive. However, the outlook for deer largely dependent on aspen would be decidedly grim.

Access to these meager food resources may also be limited by high populations and over-browsing (Fig. 18.3)—placing food out of reach, particularly for fawns and yearlings. If predators are present, such as wolves, bears, coyotes, or uncontrolled dogs, additional energy expenditures would be induced (Vreeland et al. 2004). These problems are exacerbated by deep snow and increased energy demand for movement. As a consequence, survival and fecundity are highly dependent upon accumulation of adequate body energy stores during summer and autumn. Otherwise a significant number of deer will die of starvation (Fig. 18.4; Verme and Ullrey 1984).

18.2 Energy Budget

18.2.1 Conservation Measures

The various strategies that animals use to deal with insufficient food energy have been reviewed by McCue (2010). As discussed above, the DE and ME requirements for winter maintenance of white-tailed does in Michigan (Ullrey et al. 1969, 1970)

and the DE and ME supplied by typical winter browse (Ullrey et al. 1964, 1967, 1968, 1971, 1972) are markedly disparate. To conserve energy, deer lie down on a sunny, wind-sheltered hillside or seek shelter on cloudy days to reduce convective heat loss. At night, deer seek protected locations such as cedar swamps where they curl up—to minimize exposed skin surface—under overhanging branches that restrict radiant heat loss from warm skin to cold sky (Marchinton and Hirth 1984). In this way, body energy is conserved and basal metabolic functions are sustained, chiefly by drawing upon fat reserves. This assumes that sufficient fat has been stored during the previous summer and autumn to support life until suitable food becomes available in the spring.

When winter food energy is in short supply, energy stores in body tissues are catabolized to support vital functions. Glycogen stores are only a small fraction of the energy reserve and tend to be exhausted early, leading to utilization of the nonstructural lipids in separable fat, muscle, and viscera (Kleiber 1975). Urinary urea nitrogen excretion increases, and muscles begin to atrophy due to catabolism of body protein as nutritional deprivation is prolonged and other energy sources are depleted (DelGiudice and Seal 1988). Analyses of urine deposited in snow have been used in attempts to assess the nutritional status of stressed, free-ranging deer (DelGiudice et al. 1988).

Neither white cedar nor aspen support energy balance in winter (4-week weight loss of 383 or 541 g/day, respectively), but the rumen fluid of captive deer fed white cedar fronds had significantly higher concentrations of total nitrogen, soluble nitrogen, and protozoa than those fed aspen shoots (Ullrey et al. 1972). Captive white-tails restricted to woody browse in winter also have low rumen volatile fatty acid production—indicative of low DE intake—and exhibit biochemical and clinical signs of starvation (Verme and Ullrey 1984).

A study was conducted of the body composition of free-ranging deer collected in the southern lower peninsula of Michigan between October 19 and January 19. The deer were totally dependent on natural foods, and their numbers were in balance with their food supply. A fawn, yearling, and adult of each sex was examined. A mean of about 69% of body GE was found in nonstructural fat—presumably metabolizable for energy—in adipose tissue separable by gross dissection from muscle and viscera. The approximate proportions of body GE in nonstructural fat, not separable by dissection, was 19% in muscle, 7.4% in viscera, 2.1% in skin, and 2.4% in long-bone marrow (McCullough and Ullrey 1983). The marrow of cortical bone tends to be fat-rich until other fat reserves are used (Ransom 1965), but when it is deep red and gelatinous at necropsy, mortality from starvation is a reasonable diagnosis (Fig. 18.5).

18.2.2 When Energy Conservation Fails

Increases in mortality and declines in deer population due to winter food scarcity have been reported in Ontario, Michigan, New York, Vermont, and Wisconsin

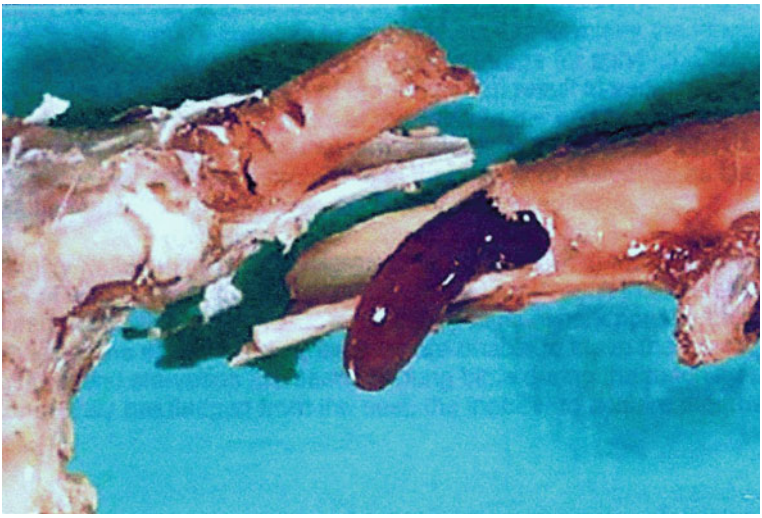

Fig. 18.5 Femur bone marrow from well-nourished deer on *left* and starved deer on *right*. Photo used with permission by Michigan DNR-Wildlife Disease Lab

(Fagerstone et al. 1984). In the early 1950s, deer populations were thought to be at their peak in the region including Michigan, Minnesota, and Wisconsin, and each state experienced annual die-offs of 20,000–50,000 white-tails. Despite a large state-financed winter feeding program in Wisconsin from 1934 through 1954, widespread starvation persisted, leading to attempts at population management, including introduction of antlerless deer harvest (Creed et al. 1984).

Deaths from starvation in northern Michigan deer are most likely in late winter or early spring if body fat reserves are inadequate and green-up is delayed. Fawns are more susceptible to winter stress and food deprivation than adults because they are less competitive and have the smallest fat reserves (Watkins et al. 1992; Marchinton and Hirth 1984) and greatest relative heat loss (Kleiber 1975). Individuals can experience weight loss of 25–30% and still survive, but death is imminent (McCullough and Ullrey 1983; Watkins et al. 1990–1992). Winter malnutrition may also inhibit attainment of typical fertility in young deer and fecundity in older does (Table 18.1) unless overcome by good summer and autumn range (Verme 1967).

18.2.3 Adaptation to Impending Energy Shortage

Controlled experiments demonstrate that physiological adaptations to seasonal changes in food supply are related to changes in day-length. Hand-reared doe fawns were raised, from near birth in May and June, under controlled light (16L:8D) to October 15. They were then paired by weight, and one of each pair

Table 18.1 Relation of autumn nutritional plane to white-tail doe productivity

	Fawns per doe			
Nutritional plane	Yearling does	Two-year-old does	Prime-age does	All does
Low	0.05	0.50	1.31	0.54
Moderate	0.84	1.40	1.85	1.43
High	1.18	1.53	1.78	1.50

Table created from data presented in Verme (1967)

was assigned to early short days (8L:16D) until February 6. The second of each pair was kept on long days (16L:8D) until December 5 and then placed on late short days (8L:16D) until February 6. Pelage change was delayed in deer on the extended summer period and was completed within 3–5 weeks after initiating the experimental photoperiods. When exposed to short photoperiods, either early or late, food intake, weight gain, and sexual maturity were stimulated (Budde 1983).

The specific mechanisms underlying these processes are still under investigation, but it is clear that control of the seasonality of appetite is complex (Anukulkitch et al. 2007; Rhind et al. 2002). In apparent anticipation of the predictable, seasonal reductions in food availability with which these deer evolved, captive populations of Michigan white-tails, with natural environmental exposure and ad libitum access to a nutritionally complete diet, exhibited hyperphagia during late summer and early fall (Watkins et al. 1983). Pre-winter hyperphagia appears to be regulated by circulating hormonal signals, neural signals from the gut, liver, and fat depots, and neural communication between various regions of the brain (see also Jenni-Eiermann and Jenni, Chap. 11). A light-sensitive organ in the hypothalamus, the pineal gland, is responsive to changes in day-length and has long been considered to play a significant role (Morgan et al. 1994).

Fawns hand-reared in indoor pens and acclimated to 16L:8D from June 1 to September 9, and then subjected to either this light schedule or to 8L:16D until December 16, diverged in feed intake, weight gain, and accretion of fat in muscle and viscera (Abbott et al. 1984). On December 16, ether extract was 12.2% of muscle dry matter (DM) and 29.0% of viscera DM for long-day (16L:8D) deer, and 33.8% of muscle DM and 54.0% of viscera DM for short-day (8L:16D) deer. These measures were all significantly greater, and serum prolactin concentrations significantly lower, in short-day deer. Other potential triggers for hyperphagia are being studied, such as ghrelin receptors that stimulate appetite, and leptin receptors that suppress it (Rhind et al. 2002).

18.2.4 Photoperiod and Body Composition

Studies were conducted in Michigan Department of Natural Resources facilities near Houghton Lake, MI (44.4° north latitude, 84.7° west longitude) involving captive deer, subjected to natural outdoor temperatures and photoperiod, and fed a

nutritionally complete diet ad libitum. Food intake, rates of lipogenesis, and lipid accumulation in skeletal muscle and viscera increased in relation to declining daylength from mid-June to late fall (Ullrey et al. 2005). Most of the deer were born in May and June, and body composition was examined after euthanasia at approximate ages of 7, 9, 13, 15, or 17 months. Average body weights trended downward from December to February in both genders, trended upward somewhat to June, and then rose appreciably up to late October or early November. Weights of skeletal muscle and viscera exhibited the same patterns, apparently a consequence of increases in lean body mass characteristic of structural growth and the influence of season on accumulation of body fat. Mean ether extract concentrations in muscle plus the ether extract concentrations in viscera, expressed as percentages of live body weight, were lowest when collected on June 24 or 26, near the summer solstice (2.7% in females; 1.9% in males), and highest when collected on December 7 or 19, near the winter solstice (10.8% in females; 8.8% in males) in both genders (Table 18.2).

Measurements of lipogenesis in subcutaneous and perirenal adipose tissue also exhibited photoperiod effects (Table 18.2) with highest activity (within gender) during the period from longest to shortest days. Lipogenic activity tended to be higher in the adipose tissue of females than in males, although the data were gathered in separate studies and were not compared statistically.

18.2.5 Additional Environmental Factors

Infectious diseases have seldom created a mortality problem in northern Michigan deer, although during the mid 1950s and 1970s epizootic hemorrhagic disease was seen in several northern Michigan counties. Internal parasites, such as nose bots and meningeal worms, are generally well tolerated, and biting lice are occasionally encountered without serious consequence in otherwise healthy deer.

Photoperiod changes are less obvious at lower latitudes, but seasonal behavior and physiological differences have been observed in relation to environmental temperature and water availability. Texas, representing the lower latitudes of the U.S., has a very diverse ecosystem, including both dry-land and humid environments. The daily forage intake of yearling deer in south Texas increases in late spring, declines in summer in association with hot, humid weather, and increases again in fall (Fulbright and Ortega-S 2006). Death from starvation may be associated with prolonged declines in food intake during periods of extreme drought—a consequence of low plant density, diminished nutritional quality, and poor digestibility—synergistically limiting food energy availability. Disease, high deer populations, and competition for a limited food supply can complicate the situation further (Fulbright and Ortega-S 2006). Starvation in other wild ruminants on several continents, associated with winter weather or drought, has been reviewed by Young (1994).

Table 18.2 Effects of age, gender, and season on body weights, percent fat content in skeletal muscle and viscera (minus ingesta), and lipogenesis in subcutaneous (SC) and perirenal (PR) adipose tissues (mean ± SEM) of captive white-tailed deer fed complete diet ad libitum

Age (months)	Sex	*N*	Date	Weight (kg)	Muscle (kg)	Fat (%)	Viscera (kg)	Fat (%)	Lipogenesis SC	Lipogenesis PR
7	F	5	7-Dec	34 ± 2.5	14.2 ± 1.2	7.1 ± 0.6	5.0 ± 0.4	3.7 ± 0.4	108 ± 18	62 ± 11
7	M	5	19-Dec	40 ± 2.5	18.0 ± 1.2	5.5 ± 0.5	6.0 ± 0.4	3.3 ± 0.4	30 ± 9	61 ± 9
9	F	5	19-Feb	30 ± 2.5	12.4 ± 1.2	3.2 ± 0.6	3.5 ± 0.4	1.9 ± 0.4	9 ± 7	1 ± 1
9	M	5	6-Feb	37 ± 2.5	16.5 ± 1.2	3.7 ± 0.5	5.6 ± 0.4	2.9 ± 0.4	20 ± 8	17 ± 2.8
13	F	5	24-Jun	39 ± 2.5	15.9 ± 1.2	1.7 ± 0.5	5.0 ± 0.4	1.0 ± 0.4	256 ± 118	245 ± 116
13	M	4	26-Jun	45 ± 2.8	18.6 ± 1.3	1.1 ± 0.5	5.7 ± 0.4	0.8 ± 0.4	21 ± 32	18 ± 33
15	F	5	25-Aug	46 ± 2.5	18.3 ± 1.2	2.9 ± 0.5	5.9 ± 0.4	1.6 ± 0.3	661 ± 104	1264 ± 334
15	M	5	27-Aug	57 ± 2.5	23.9 ± 1.2	1.9 ± 0.6	7.7 ± 0.4	1.1 ± 0.4	162 ± 58	144 ± 65
17	F	5	3-Nov	54 ± 2.5	22.1 ± 1.2	4.4 ± 0.6	8.1 ± 0.4	2.3 ± 0.4	1515 ± 726	616 ± 329
17	M	4	31-Oct	59 ± 2.8	25.1 ± 1.3	3.3 ± 0.7	9.0 ± 0.4	2.2 ± 0.5	167 ± 37	186 ± 40

Lipogenesis rates reflect $^{3}H_2O$ incorporation into adipose tissue (nmol/100 mg) over a 2-h period. Table recreated from data presented in Ullrey et al. (2005)

18.3 Conclusions

Adaptation of white-tailed deer to the seasonal changes in food supply, characteristic of northern Michigan and southern Canada, is basic to deer survival in this ecosystem. To successfully survive winter starvation, food of adequate quantity and quality must be available in late summer and fall. During lean periods, deer use both behavioral and physiological means to survive. They minimize extraneous activity and employ adaptive thermoregulatory strategies to conserve energy. Increased food intake and increased rates of tissue lipogenesis prior to these lean times result in accumulation of energy in body fat stores which, if large enough, will sustain life during the winter period of negative energy balance. However, if spring green-up has been delayed, or if stores of body fat are inadequate, death by starvation is likely. This chapter provides evidence that this cyclical accumulation and depletion of body fat reserves occurs even when food supplies are unlimited, and is regulated primarily by photoperiod.

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Chapter 19
Fasting Physiology of the Pinnipeds: The Challenges of Fasting While Maintaining High Energy Expenditure and Nutrient Delivery for Lactation

Cory D Champagne, Daniel E Crocker, Melinda A Fowler and Dorian S Houser

19.1 Introduction to Pinniped Foraging and Fasting Periods

Pinnipeds forgo feeding opportunities when they voluntarily haul-out to breed or grow new pelage. The timing of haul-outs does not necessarily coincide with reduced prey availability in foraging areas; rather, haul-out patterns are dictated by endogenous rhythms or environmental factors. Thus, the fasting periods in pinnipeds are regular and predictable for individuals and tied to important life history events. Therefore, we usually refer to pinnipeds as "fasting" rather than "starving" (Castellini and Rea 1992; McCue 2010). Although environmental changes (e.g. El Niño events) can influence prey availability and periodically impose nutritional stress on pinnipeds (Crocker et al. 2006; Le Boeuf and Crocker 2005; Trillmich and Limberger 1985; Trites and Donnelly 2003). The rare combination of marine feeding with terrestrial breeding and pelage synthesis regularly requires that fasting occur simultaneously with energetically costly activities. This review describes the current understanding of the metabolic adjustments that occur in fasting pinnipeds under the constraint of competing nutrient demands.

C. D. Champagne (✉) · D. S. Houser
National Marine Mammal Foundation, 2240 Shelter Island Dr 200,
San Diego, CA 92106, USA
e-mail: champagn@biology.ucsc.edu

D. E. Crocker · D. S. Houser
Department of Biology, Sonoma State University, Rohnert Park, CA 94928, USA

M. A. Fowler
Institute of Marine Science, UC Santa Cruz, Santa Cruz, CA 95060, USA

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19.1.1 Pinniped Life Histories Represent a Continuum of Fasting Abilities and Energetic Constraints

Fasting patterns vary among pinniped groups and are influenced by phylogeny, habitat (especially the physical stability of the haul-out site), and body size, among other factors (Boyd 1998; Schulz and Bowen 2005; Trillmich and Weissing 2006). Some species fast continuously through lactation (many phocid seals—for example, elephant seals *Mirounga* spp and monk seals *Monachus* spp) while others frequently leave their pup to forage intermittently (otariids—e.g. California sea lion *Zalophus californianus*). These two strategies are often referred to as "capital" and "income" breeding, respectively (Fig. 19.1). Several authors have discussed the evolutionary factors influencing these reproductive patterns in pinnipeds (Bartholomew 1970; Costa 1993; Schulz and Bowen 2005; Stephens et al. 2009). We refer to general patterns in this review, but there is frequently a continuum of these features within groups and even within species.

Phocids—"true" seals—often have a large body size that allows them to store substantial blubber reserves. Most phocid seals feed on offshore prey, requiring prolonged foraging trips to sea. Thus, females usually exhibit a capital breeding strategy, fasting for the duration of a brief lactation period. Some species, however, forage opportunistically during lactation, especially the smaller phocids (Bowen et al. 2001). The length of lactation in phocids ranges from only a few days in hooded seals, *Cystophora cristata* (Bowen et al. 1985) to over a month in some species. Because mothers are nearly always in attendance, pups feed continuously for the duration of lactation. Pups are weaned abruptly when the mother departs to sea for distant foraging grounds. Once weaned, pups undergo a postweaning fast of varying length, depending on the species.

Otariids—sea lions and fur seals—feed intermittently over a prolonged lactation period. Their generally smaller body size limits the amount of energy they may store as fat. The length of lactation varies widely between species, from 4 months in Antarctic, *Arctocephalus gazella*, and northern fur seals, *Callorhinus ursinus*, to over a year in others (Gentry and Kooyman 1986). Starvation is imposed on pups when mothers are foraging at sea. Many otariids feed on abundant nearshore resources making frequent foraging trips of 1–7 days. Some, however, like the subantarctic fur seal, *Arctocephalus tropicalis*, feed at distant foraging grounds up to 1,800 km from the rookery (Georges and Guinet 2000). In this species, females must repeatedly leave pups for several weeks and maternal foraging trips of over 2 months are common (Verrier et al. 2011a). The fasting duration required of subantarctic fur seal pups approaches that of many phocid seal pups during their postweaning fast and illustrates the continuum of fasting and lactation strategies exhibited by pinnipeds.

Many pinniped species breed in large colonies while others do not congregate during breeding, instead hauling out on small ice flows. Polygynous breeding systems favored extreme sexual dimorphism in several species, especially those breeding on land. This sexual dimorphism is often exhibited in the larger body size

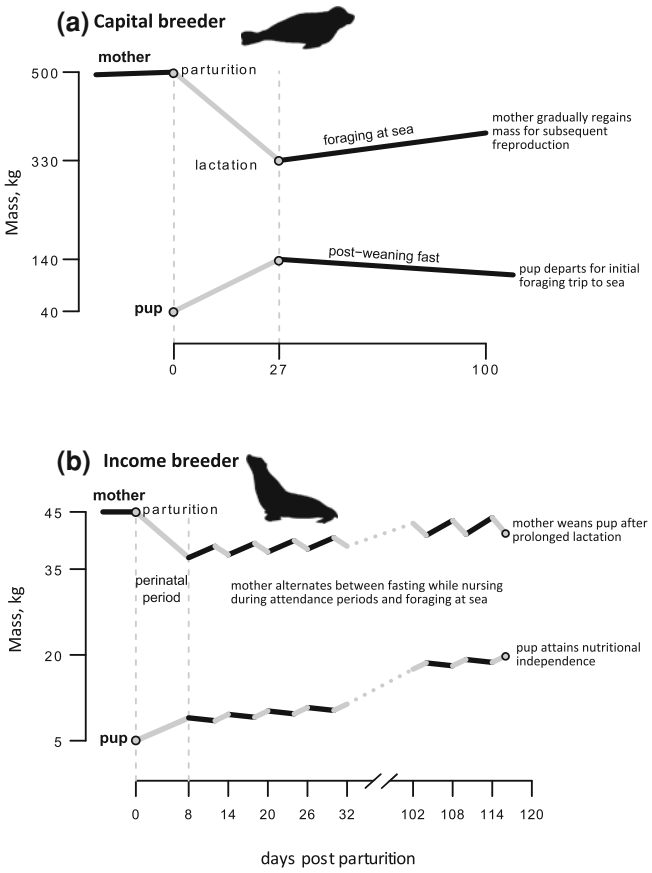

Fig. 19.1 Lactation strategies of **a** capital and **b** income breeding pinnipeds. The approximate periodicity and mass change in mothers and pups (*top* and *bottom line* in each graph) is shown. *Gray lines* indicate periods of female attendance when mothers lactate simultaneous with fasting; *black lines* indicate periods of at-sea foraging in females and starvation is imposed on pups. Phocid seals tend to exhibit a capital breeding strategy—females fast for the duration of lactation and pups endure a prolonged fast when mothers depart for sea. Otariids exhibit income breeding—after a short perinatal period following parturition, females alternate brief attendance periods at the rookery with longer foraging trips to sea; thus, otariid pups alternate feeding and fasting throughout maternal dependence

of males, which is advantageous in both physical competition and the tolerance for fasting—the latter of which also allows for longer attendance at breeding sites (Bartholomew 1970). Breeding males defend territories at polygynous breeding colonies as long as their energy reserves permit and opportunities for copulation remain. Polygynous breeding and territorial defense by males spans both phocid and otariid groups. In species where females do not assemble in large breeding colonies, however, there is no onshore resource to defend and complete fasting is likely not necessary.

19.1.2 Challenging Metabolic Processes During Fasting

Pinnipeds must fast simultaneous with energetically costly activities associated with their life history stage—for example, lactation, breeding, and post-natal development. This review describes our current understanding of the metabolic features observed in seals, sea lions, and fur seals maintaining "normal" or even elevated energy output while fasting as part of their normal life histories. There are inherent difficulties in studying free-ranging animals that often inhabit remote areas and tolerate inclement weather conditions. Studying these animals, however, provides insights into natural fasting adaptation, rather than fasting after obesity pathology (Henry et al. 1988).

19.1.2.1 High Rates of Nutrient Output for Lactation

Lactation is the most costly component of reproduction (Gittleman and Thompson 1988). Rather than conserving body tissues during fasting, seals must actively mobilize tissue for milk synthesis to provision offspring. Maternal energy reserves that were obtained on previous foraging trips support the energetic costs of both lactation and maternal maintenance metabolism. By increasing the rate of milk delivery and decreasing lactation duration, females reduce the metabolic overhead of lactation (the energy spent on processes other than milk production, for example, maintenance metabolism) and optimize energy investment in offspring (Fedak and Anderson 1982). Therefore, lactation in phocids is characterized by rapid nutrient delivery to offspring despite fasting. The short lactation period is further truncated in species that breed on unstable substrate, as many ice seals do. For example, the hooded seal, which breeds on unstable pack ice, has the shortest lactation duration of any mammal—pups are weaned in as little as 3–5 days (Bowen et al. 1985).

Females fasting simultaneous with lactation lose substantial body mass, often a third of their postpartum mass (Crocker et al. 2001; Lydersen and Kovacs 1996; Mellish et al. 2000; Wheatley et al. 2006). Energy expenditure is high during lactation and many species sustain metabolic rates over five times that predicted by body mass, based on Kleiber's equation, throughout fasting and lactation (Crocker et al. 2001; Lydersen and Kovacs 1996). In some instances energetic expenditure may be much higher, more than 10 times predicted in hooded seals fasting only a few days (Mellish et al. 1999). Over the course of fasting simultaneous with lactation, females may lose 50% of their initial energy reserves (Crocker et al. 2001; Mellish et al. 2000; Wheatley et al. 2006). The majority of the mobilized nutrients are used for milk synthesis with a lesser amount used to support maternal energy demands (Costa et al. 1986). This high nutrient output by females is complemented by highly efficient energy assimilation by pups—they may gain over 70% of maternal mass loss (Lydersen and Kovacs 1996); gray seals, *Halichoerus grypus*, assimilate 75% of milk energy into body tissue (Lydersen et al. 1995) and northern elephant seal pups store as much as 85% (Crocker et al. 2001).

The rapid nutrient delivery from mother to pup is accomplished by the production of large amounts of high-energy milk. Milk composition in pinnipeds (high fat and low carbohydrate) has been driven by the constraints of fasting (Oftedal 1993). The fat content of seal milk is exceedingly high: 30–50% in otariids (Arnould and Hindell 1999; Costa and Gentry 1986; Gales et al. 1996; Georges et al. 2001; Trillmich and Lechner 1986) and over 50% in several phocid species (Iverson et al. 1993, 1995; Lydersen et al. 1996; Riedman and Ortiz 1979). This rate of lipid energy output in phocids requires extensive lipid mobilization from adipose reserves. Some studies have suggested that the fatty acid composition is similar in maternal blubber and milk (Iverson et al. 1995; Smith et al. 1997) potentially increasing the rate of lipid transfer and incurring little energetic cost in modifying fatty acids for export in milk (Costa and Trillmich 1988). There is, however, some controversy over the similarity in blubber and milk fatty acid constituents in marine mammals (Grahl-Nielsen 1999; Grahl-Nielsen et al. 2000; Smith et al. 1999) and more data on this issue is warranted.

The protein content in pinniped milk is among the highest of any mammal and can compose 10–18% of the milk volume (Cane et al. 2005; Davis et al. 1995; Oftedal 1984; Sharp et al. 2005). Thus, female pinnipeds fasting while lactating do not spare protein as most fasting animals do. Rather, seals must actively mobilize amino acids from lean tissue for delivery to mammary tissue, incorporating as much as 15% of their total body protein into milk protein over the course of lactation (Costa et al. 1986; Oftedal 1993). The protein content and constituent amino acids appear stable across lactation (Cane et al. 2005; Davis et al. 1995) and only trace amounts of carbohydrate are found in pinniped milk. Theoretically, decreasing the carbohydrate content of milk would reduce the need for gluconeogenesis during fasting.

19.1.2.2 High Rates of Energy Metabolism for Intrasexual Competition

Pinnipeds display some of the most extreme examples of polygyny in mammals. Terrestrial breeding and extreme polygyny favored high degrees of sexual dimorphism in pinnipeds—males may be over five times as large as females (e.g. several fur seal species, and northern elephant seals). Large body size is advantageous in intrasexual competition (e.g. display and combat), but it also confers the ability to fast for longer durations due to a decrease in mass-specific metabolic rate and the potential for increased energy stores. A longer fasting duration permits longer tenure at the breeding colony and added reproductive opportunities. Males of several species fast for the duration of the breeding season while competing for and defending territories or harems. Fasts may last a few weeks, as in gray seals (Beck et al. 2003) and hooded seals (Kovacs et al. 1996), or exceed 2–3 months, as in many otariids (Riedman 1990) and in elephant seals (Deutsch et al. 1990; Galimberti et al. 2007).

In polygynous species, males compete for reproductive opportunities, frequently engaging in intense physical combat. Several studies have measured mass

loss in males across the breeding season to investigate the energetic costs associated with this breeding strategy (see Table 19.1). Despite the high level of activity, males typically lose less than 1.0% of body mass per day during the breeding season. Deutsch et al. (1990) weighed male northern elephant seals during the breeding season and calculated mass loss across the duration of fasting. The average fasting duration was ~90 days and males lost 0.4% of body mass each day. Although the daily rate of mass loss in elephant seals is lower than in other pinniped species, over the entire breeding season animals lost over a third of their initial body mass. One large male weighed 2,266 kg on arrival; after controlling a harem of 350 females he departed for sea 106 days later weighing 1,050 kg—less than half his initial body mass.

Rarely have reproductive energetics in male pinnipeds been measured. Gray seals expended 29% of their total body energy (59 MJ/day) during the three week breeding season, far less than the 50% expended by females over a far shorter lactation period (Beck et al. 2003). Similar data on Antarctic fur seals, however, indicate that males may expend as much as 50% (42 MJ/day) of their initial total body energy while holding a territory for 1 month (Boyd and Duck 1991). Studies of breeding energetics in adult male elephant seals suggest that their energetic expenditure is directly related to the position that a seal maintains in the dominance hierarchy. For example, dominant males have extremely high rates of energy expenditure that appear to be related to both the number of reproductive events and the time spent in defending their harems. Successful males approach the levels of sustained energy expenditure suggested as "metabolic ceilings" in nonfasting species (Deutsch et al. 1990; Crocker et al. 2012).

19.1.2.3 Tissue Reorganization During Development and Molting

As described in this volume, many adult animals fast but extended fasting is far less common in young animals. Young seals undergo substantial reorganization of tissues in preparation for the physiological challenges of foraging at sea, including thermoregulation, aquatic locomotion, and the need for adequate breath-hold capacity. Once departing the rookery, young must retain sufficient lipid for thermoregulation and energy stores to meet their metabolic requirements until they are successful at acquiring prey. One hallmark of diving mammals is their capacity to store large quantities of oxygen in their tissues. This oxygen storage capacity is characterized by a large blood volume, high hematocrit, and elevated concentrations of respiratory pigments, hemoglobin and myoglobin, relative to nondiving mammals (Kooyman and Ponganis 1998). Neonates are not born with the oxygen storage capacity of adults; young seals must substantially increase their oxygen storage capacity before foraging (Burns et al. 2007). Gray and northern elephant seals increase mass-specific levels of hematocrit, hemoglobin, myoglobin, and blood volume during the postweaning fast (Bennett et al. 2010; Noren et al. 2005; Thorson and Le Boeuf 1994). This increases oxygen storage capacity and occurs simultaneous with an increase in diving behavior and greater diving capability

Table 19.1 Selected fasting durations, mass loss, and body composition during breeding, molting, and development in phocids and otariids

Species	Age class	Fasting duration	% Mass loss	% Initial lipid	Reference
Gray seal	Adult female	2.5–3 w	31	31	(Mellish et al. 2000; Reilly et al. 1996)
	Breeding male	3–8 w	14	28	(Tinker et al. 1995)
	Weanling	2–3 w	28	47	(Noren et al. 2008; Reilly 1991)
Harp seal	Weanling	4 w	31	39	(Nordøy et al. 1993)
Hooded seal	Adult female	3–4 d	10	28–44	(Iverson et al. 1995; Mellish et al. 1999)
Northern elephant seal	Adult female	5 w	36	38	(Crocker et al. 2001)
	Adult male	3 m	26–36	33	(Deutsch et al. 1990; Wenzel 2008)
	Weanling	2–2.5 m	24	44	(Houser and Costa 2001; Noren et al. 2003)
Southern elephant seal	Adult female	4 w	36	29	(Arnbom et al. 1993)
	Adult male	2–3 w	26	na	(Galimberti et al. 2007)
	Weanling	5–6 w	42	na	(Carlini et al. 2001)
	Juvenile	3–4 w	30	27	(Field et al. 2005)
Weddell seal	Adult female	6–7 w	39–42	40	(Wheatley et al. 2006)
Antarctic fur seal	Adult female	3 d	17–22	14	(Arnould et al. 2002; Costa and Trillmich 1988)
	Adult male	31 d	25	24	(Boyd and Duck 1991)
	pup	5 d	19	na	(Arnould et al. 2001)
Galapagos fur seal	Adult female	5 d	8	26	(Costa and Trillmich 1988)
Subantarctic fur seal	Adult female	4 d	14	na	(Guinet et al. 2004)
	pup	33 d	21	49	(Verrier et al. 2009)
Steller sea lion	Juvenile	7–14 d	8–21	21	(Rea et al. 2007)

Fasting duration is reported in days (d), weeks (w), or months (m)
"na" indicates data not available

(Bennett et al. 2010). Similar increases in oxygen stores have been reported in otariids, although they occur at slower rates and across a longer period of maternal investment (Richmond et al. 2006; Spence-Bailey et al. 2007; Verrier et al. 2011b).

Molting often occurs gradually, over several months, and may not be associated with extended haul-outs or periods of fasting, especially among the otariids. A few pinniped species, namely elephant and monk seals, undergo a rapid "catastrophic" molt where animals replace their entire skin surface in approximately 1 month. These species fast completely during their annual molts. Three studies have investigated body composition changes and energetics in elephant seals molting while fasting (Boyd et al. 1993; Slip et al. 1992; Worthy et al. 1992). Adult female northern elephant seals lost 24% of their initial body mass over a mean of 32 days during the spring molt. Somewhat surprisingly, body composition did not change across molting in either northern or southern female elephant seals, remaining around 25% lipid throughout. By contrast, the proportion of body lipid decreases markedly during lactation, illustrating the substantial material cost of milk production. Protein loss accounted for 16% of the total mass loss while molting in female but only 10% in male elephant seals.

Several field investigations have reported low metabolic rates during molting (Boyd et al. 1993; Slip et al. 1992; Worthy et al. 1992). Concurrent changes in activity, feeding, insulation and thermoregulation, and body mass complicate estimates of the true energetic and material costs of molting. Nevertheless, the evidence suggests that free-ranging seals are able to synthesize new skin and pelage simultaneous with reduced or absent food intake at a nominal energetic cost and that most of the apparent loss of lean tissue is due to a reorganization of protein for the purpose of skin synthesis (Worthy et al. 1992).

19.2 Metabolic Strategies for Fasting

Numerous metabolic alterations, including changes in adipose, protein, and carbohydrate metabolism occur with the progression of fasting in pinnipeds. These processes have been investigated in multiple seal species by various methods—including monitoring changes in mass, body composition, and the measurement of specific metabolic processes using isotopic tracers. Comparing whole-animal processes among individuals is complicated by their varying body size. In addition, because marine mammals tend to have large fat stores that change drastically over the course of fasting, mass-specific values of metabolism may be especially misleading (see also Bar and Volkoff, Chap. 6). While claims of adipose as being metabolically inert are exaggerated (Anghel and Wahli 2007), this tissue has far different metabolic requirements than does lean tissue. Packard and Boardman (1999) argued convincingly that mass-specific rates of metabolism are an inappropriate metric of whole-animal metabolism—dividing by mass does not remove the effects of body size. They suggested ANCOVA to compare physiological measurements among

groups of varying size and we agree with their assessment. When referencing published values, however, this approach is frequently not available. Thus, in the following sections we often report mass-specific values along with body size.

19.2.1 Lipolysis Supports High Energy Expenditure

The primary adaptation that enables extended high-energy fasting in pinnipeds is the ability to generate the majority of ATP through lipid oxidation. High levels of lipid oxidation are inferred widely from mass and body composition changes during fasting (Table 19.1), but actual measures of lipolysis or lipid oxidation are rare in pinnipeds (Castellini et al. 1987; Houser et al. 2007). Nevertheless, several measurement approaches suggest that lipids provide 80–95% of the energetic demand during fasting in a wide range of pinnipeds (Arnould et al. 2001; Boyd and Duck 1991; Crocker et al. 2001; Field et al. 2005; Guinet et al. 2004; Houser and Costa 2001; Mellish et al. 1999; Nordøy et al. 1993; Noren et al. 2003; Reilly 1991; Reilly et al. 1996; Verrier et al. 2009).

19.2.1.1 Lipolysis and Metabolism

A reliance on lipid oxidation is supported in part by high rates of lipolysis. Reported circulating values of nonesterified fatty acids (NEFA) are greater in fasting pinnipeds than those reported for any other animal (as high as 3.2 mM) and NEFA increases over the fast in some species, particularly during lactation (Houser et al. 2007; McDonald and Crocker 2006; Verrier et al. 2009). Plasma glycerol levels show similar increases and are potentially available as a substrate for gluconeogenesis. Collectively, these results suggest a very high rate of lipolysis in fasting pinnipeds. However, turnover studies suggest that plasma NEFA levels may be independent of the rate of lipolysis, providing evidence that circulating plasma concentrations do not accurately represent substrate utilization (see also Price and Valencak, Chap. 15). For example, lactating elephant seals undergo dramatic increases in plasma NEFA despite increases in milk fat content and stable rates of lipolysis across lactation (Houser et al. 2007). These findings suggest alterations in NEFA oxidation and reesterification across the lactation fast, possibly in response to changing demands for lipid substrates.

The fat-based metabolism of pinnipeds is facilitated by high levels of adiposity and metabolic adaptations that promote lipolysis and the tissue uptake of NEFA. As in terrestrial mammals, the adiposity at the onset of a fast has been shown to influence protein sparing and a reliance on lipid oxidation in a variety of marine mammal species (Atkinson et al. 1996; Bennett et al. 2007; Cherel et al. 1992; Goodman et al. 1980; Noren et al. 2003, 2008; Reilly 1991). Juveniles and neonate seals with low lipid reserves exhibit uncharacteristically high levels of protein catabolism (Arnould et al. 2001; Houser and Costa 2003) and lipid reserves at

weaning have been shown to be an important determinant of the duration of the postweaning developmental fast (Noren et al. 2003, 2008; Sokolović et al. 2010)

Ketones, primarily β-hydroxybutyrate, accumulate somewhat during fasting in weaned elephant seals (Champagne et al. 2005), gray and harp seals *Pagophilus groenlandicus* (Nordøy et al. 1993; Nordøy and Blix 1991), and Weddell seals, *Leptonychotes weddellii*, (Rea 1995) and subsequently decline rapidly as the end of the fast is approached (Castellini and Costa 1990). This suggests that ketones may contribute to energy metabolism during long-term fasting, although levels are significantly lower than nonfasting adapted species and never reach levels affecting acid–base balance or causing ketoacidosis. Particularly striking in this regard are data from lactating female and breeding adult male northern elephant seals—despite elevated NEFA levels over the fast, elephant seals exhibit consistently low β-hydroxybutyrate values across the breeding fast <0.80 mM; (Castellini and Costa 1990; Champagne et al. 2006). Recent studies in fasting elephant seal pups found increases in β-hydroxybutyrate : acetoacetate ratios across the postweaning fast suggesting altered ketone metabolism with development. Data on circulating ketone levels in otariids are sparse. Fasting, lactating subantarctic fur seal females exhibit low, stable, ketone levels across their short lactational fasts (Guinet et al. 2004). In contrast, their pups exhibit a 2-fold increase in β-hydroxybutyrate across 30 days of fasting (Verrier et al. 2009). These increased ketone levels, however, were only demonstrated in juvenile animals of relatively small body size.

Relatively few studies have examined changes in blood lipid profiles in the context of natural, long-term fasts. In general, marine mammals have elevated cholesterol levels which may reflect the high cholesterol content of their diet. Under conditions of fasting, hepatic and intestinal synthesis of cholesterol is markedly reduced or absent and the hypercholesterolemia associated with food deprivation in species that are not adapted to long-term fasting is representative of cholesterol mobilization from adipose tissue (Savendahl and Underwood 1999; Sokolović et al. 2010). A recent study in fasting adult male elephant seals revealed highly elevated cholesterol levels that declined with adipose tissue stores across the fast (Tift et al. 2011). Male elephant seals were able to defend high density lipoprotein (HDL) levels despite dramatic reductions in total cholesterol and low density lipoproteins (LDL) with fasting. This selective depletion of serum LDL across the fast suggests the metabolic adaptation to fasting may include alterations in cholesterol metabolism (see also Price and Valencak, Chap. 15).

19.2.1.2 Lipid Mobilization for Milk Delivery

Pinnipeds are not only capable of fasting for prolonged periods but produce one of the most lipid-rich milks found in nature, even while fasting (Oftedal 2000; Riedman and Ortiz 1979). The mammary gland is capable of synthesizing fatty acids from glucose or ketones (Mepham 1987) and many seal species have high plasma glucose levels and rates of glucose production (see Sect. 19.2.3), and increasing ketone concentration during fasting. Glucose and ketones, therefore, are

available for fatty acid synthesis by the mammary gland. In the mammary gland, fatty acid synthesis from glucose and ketones results in short and medium chain fatty acids containing fewer than 12 carbons (Neville and Picciano 1997). These short and medium length fatty acids, however, have not been detected in pinniped milk, suggesting that there is little de novo lipid synthesis in the mammary gland (Debier et al. 1999; Iverson et al. 1995; Riedman and Ortiz 1979; Wheatley et al. 2008). These data suggest that NEFA delivery and uptake by the mammary gland is responsible for milk fat content.

The production of high fat milk while fasting is, therefore, dependent on two processes—the release of NEFA from adipose stores into circulation and the uptake of circulating NEFA by mammary tissue. Within the adipocyte, nearly all of the lipolytic activity is driven by a pair of enzymes that regulate the mobilization and release of NEFA from triacylglycerol (TAG) adipose stores—hormone sensitive lipase (HSL) and adipose triacylglyceride lipase (ATGL) (Schweiger et al. 2006). Lipoprotein lipase (LPL) is responsible for the uptake of circulating TAG into tissues, including mammary (Hamosh et al. 1970). Increases in mammary LPL activity simultaneous with milk lipid suggest that LPL is involved in regulating milk lipid content in hooded and gray seals (Iverson et al. 1995; Mellish et al. 1999, 2000). This relationship, however, was not present in elephant seals (McDonald and Crocker 2006). This suggests that factors other than mammary gland uptake, such as lipid mobilization by HSL and ATGL, are involved in driving milk lipid content in some seal species. These lipolytic enzymes have never been quantified in pinnipeds; research investigating the activity of these enzymes may determine their importance in lipid mobilization during lactation and any relationship to milk lipid content.

19.2.2 Conflicting Demands for Protein

The fate of endogenous protein stores during fasting is partially dependent on the life history stage of the fasting pinniped (Crocker et al. 1998; Rea et al. 2009). All fasting pinnipeds mobilize protein stores to meet certain basal metabolic demands (e.g. gluconeogenesis). In addition, simultaneously fasting and lactating females direct protein to milk synthesis, sexually competing males direct protein to wound healing and sperm production, fasting pups direct protein to the de novo synthesis of erythrocytes and respiratory pigments, and fasting and molting pinnipeds direct protein to pelage synthesis. Indeed, in the one pinniped in which circulating amino acids have been profiled across the life history stages, evidence suggests variability in amino acid metabolism is related to the peculiar demands of each stage (Houser and Crocker 2004). As in terrestrial mammals, the greater the relative and absolute fat reserves at the beginning of the fast, the more efficiently pinnipeds spare protein and the longer that periods of food deprivation can be tolerated (Fig. 19.2; Arnould et al. 2001; Carlini et al. 2001; Noren et al. 2003, 2008; Rea and Costa 1992). In some phocid pups, the initial body condition at weaning is believed to be critically important to dictating the time and material committed to the

physiological ontogeny of diving (Burns et al. 2007; Noren et al. 2008; Thorson and Le Boeuf 1994). In some intermittently nursing otariids, the ability to spare protein may be constrained to only a few days because of limitations on fat reserves (Rea et al. 2000). Although, otariid pups adapted for prolonged fasting, like subantarctic fur seals, show efficient protein sparing during monthlong fasts (Verrier et al. 2009). By contrast, phocid pups such as that of the northern elephant seal, may fast up to 3 months with consistently less than ~2% of the metabolic rate being met through protein catabolism (Houser and Costa 2001; Pernia et al. 1980). Similar trends are observed in lactating elephant seals where the demands of lactation and body composition dictate the rate of protein catabolism (Crocker et al. 1998). In phocid seals, it is only under conditions of severe body store depletion that the ability to spare protein is lost. This has been observed in natural settings—at the end of lactation when adult female elephant seals have lost up to 40% of their mass, and in abandoned pups that are undernourished and which eventually die of starvation (Crocker et al. 1998; Houser and Costa 2003).

Potentially unique to the pinnipeds may be a "pre-adaptation" to the fasting condition, and protein sparing in particular—i.e., seals seem to continuously exhibit the metabolic characteristics of Phase II fasting. Throughout their evolution in the marine environment, marine mammals have subsisted on prey that is high in lipid, protein, and water content, but essentially devoid of carbohydrate. Under these conditions, substrate partitioning favors lipid fuel use. Elephant seal pups have been shown to meet less than 4% of their metabolic demand through protein catabolism while suckling (Houser and Costa 2001). Low rates of protein catabolism may be due, in part, to the ability of neonates to spare protein but the high fat content of the diet, ~55% fat by midlactation (Riedman and Ortiz 1979), provides ample substrate to induce a state of fuel oxidation predominantly dependent on lipid. Similar observations have been made in gray seal pups, which improve protein sparing during supplemental feeding with herring (Bennett et al. 2007). Whether similar patterns are observed in adult pinnipeds remains to be seen, but evolving on a diet devoid of exogenous carbohydrate seems likely to have constrained fuel substrate partitioning under both fasting and feeding conditions.

19.2.2.1 Protein Requirements During Development

Many pinniped neonates must develop the physiological capacity for diving in the face of either periodic bouts of fasting (e.g. intermittent suckling and fasting periods, as in most otariids) or a continuous fast following abrupt weaning (e.g. most phocids). During these fasts, the physiological downregulation of protein catabolism to meet metabolic demands or to serve as a source of gluconeogenic precursors is opposed by a need to mobilize protein for the de novo synthesis of tissues supporting breath hold diving. Elephant seal pups spend only 2% of the day in the water at weaning but increase this to 52% of the day by the 10th week of fasting (Thorson and Le Boeuf 1994). Across the postweaning fast, oxygen stores increase by 47%; the blood volume increases by 33 ml/kg, myoglobin increases by

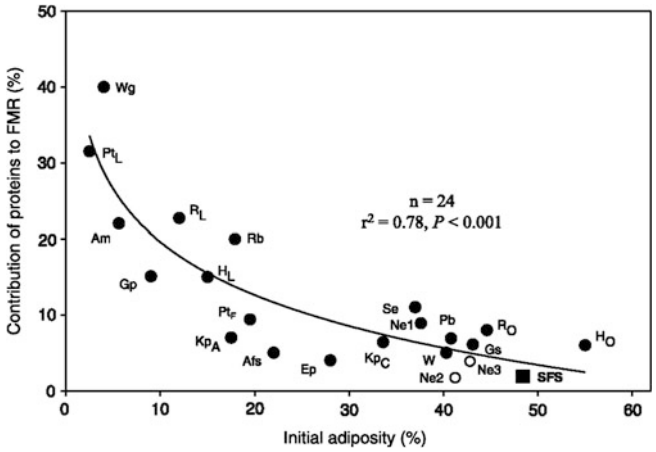

Fig. 19.2 The contribution of protein to field metabolic rate (FMR) decreases with initial adiposity in various bird and mammal species. Figure reproduced, with permission, from Verrier et al. (2009); see original for references. Species abbreviations: *Wg*, William grous; Pt_L, lean ptarmigan; *Am*, American marten; R_L, lean rat; *Rb*, rabbit; *Gp*, gentoo penguin; H_L, lean human; Pt_F, fat ptarmigan; Kp_A, adult king penguin; Afs, Antarctic fur seal pup; Ep, emperor penguin; Kp_C, king penguin chick; *Ne*, Northern elephant seal pup [Ne1 (●); mass balance method; Ne2 and Ne3 (○), urinary urea turnover method]; *Se*, Southern elephant seal pup (mass balance method); *Pb*, polar bear; *W*, woodchuck; *Gs*, grey seal pup; R_O, obese rat; H_O, obese human; SFS, subantarctic fur seal pup

1.7/100 g of muscle, and hemoglobin increases by 3.6 g/dL of blood (Thorson and Le Boeuf 1994). Similar trends are noted in other pinniped species, both phocids and otariids, although the magnitude of changes in blood volume and respiratory pigments varies (Bryden and Lim 1969; Horning and Trillmich 1997; Sepulveda et al. 1999). During the postweaning fast, the increases in the blood volume and respiratory pigments are necessarily derived from endogenous protein stores that were acquired during the suckling period.

19.2.2.2 Protein Requirements During Lactation

As previously noted, many female phocid seals combine fasting with lactation. Unlike most terrestrial mammals, the lipid, water, and protein for lactogenesis in these seals is derived from endogenous stores without replacement during lactation. The nutrient demands of lactogenesis, like that of the ontogeny of breath hold capacity in fasting pups, are in direct conflict with the downregulation of substrate use common to fasting. The protein content of phocid milk is approximately 10–11% protein by mass and remains fairly consistent across lactation (Lydersen et al. 1995, 1996; Lydersen and Kovacs 1996; Riedman and Ortiz 1979). The rate of milk consumption is high in suckling phocids and ranges from 3.5 to 7.6 kg of milk/day depending on the species (Lydersen et al. 1995, 1996; Lydersen and

Kovacs 1996; Riedman and Ortiz 1979). This results in 0.4–0.8 kg/day of protein lost from the mother as a function of lactogenesis and milk transfer. Thus, despite effective sparing of protein from use in oxidation, lactating phocid females can lose >25% of total body protein stores during the fast (Crocker et al. 2001). Across species, the amino acid composition of the milk is approximately 40% essential amino acids and is dominated by the amino acids leucine, proline and glutamate (Davis et al. 1995). Thus, not only is the loss of protein in the milk substantial, but the composition, in the form of essential amino acids, potentially provides a severe limitation on the amount of the protein that may be lost.

Protein sparing ability while concomitantly fasting and lactating has been suggested as being dependent on the duration of lactation and the energy content of the milk. Indeed, static measures of blood urea nitrogen (BUN), which are typically thought to be reflective of protein catabolism, have been monitored in simultaneously lactating and fasting phocid species, and have been thought to support this hypothesis (Mellish and Iverson 2001). Gray seals, with moderate lactation durations (16 days), maintain low BUN throughout lactation and are thought to effectively guard protein from catabolism. By contrast, hooded seals have lactation periods of 3–5 days and have higher circulating BUN levels. This has been suggested to indicate a higher rate of protein catabolism, which is tolerated by the hooded seal because of the shorter lactation period. Although this may be the case, static measures of BUN can be a misleading indicator of protein catabolism. Studies of urea flux in fasting/lactating northern elephant seals demonstrate that even though BUN remains stable across lactation, the rate of protein catabolism increases 50% above midlactation rates just prior to weaning and the resumption of foraging by the mother (Crocker et al. 1998). In this latter case, it appears that the initial body reserves of the mother are critical to determining the duration of lactation and that the demands of lactation are sufficient to cause entrance into Phase III fasting, a condition that has not been observed in this system during other life history stages. Whether lactation demands are similarly constraining in other phocid systems remains to be determined.

19.2.3 Carbohydrate Metabolism

Some tissues—for example, the central nervous system, erythrocytes, renal medulla—cannot catabolize lipids and must be supplied with glucose or ketone bodies during prolonged fasting. Carbohydrate stores, primarily in the form of glycogen, are only sufficient to provide glucose for a few days of fasting. Thus, glucose demands by glucose-dependent tissues must be supplied through gluconeogenesis. Amino acids directed to gluconeogenesis can lead to lean tissue loss and compromise vital organ function (Owen et al. 1998). Animals enduring a prolonged fast may preserve organ function by limiting carbohydrate use to glucose-dependent tissues and gluconeogenesis from amino acids. Paradoxically, circulating glucose concentrations appear somewhat elevated in pinnipeds,

especially relative to their body size (Umminger 1975). Reported glucose concentrations in phocids range from 5 to 10 mM among several species including harbor seals, *Phoca vitulina* (Davis 1983; Davis et al. 1991), harp seals (Nordøy et al. 1993), northern elephant seals (Costa and Ortiz 1982; Fowler et al. 2008; Viscarra et al. 2011b), and Weddell seals (Burns and Castellini 1996; Castellini and Castellini 1989; Mellish et al. 2011). Glucose concentrations appear similar in otariids. Plasma glucose concentrations only slightly decreased from ~9.5 mM at the onset of fasting to 8 mM within a few days in Antarctic fur seals (Arnould et al. 2001) and remained above 6 mM in subantarctic fur seal pups for over 30 days fasting (Verrier et al. 2009). Steller sea lions, *Eumetopias jubatus*, both pups fasting up to 3 days and juveniles fasting up to 5 days, maintained plasma glucose values over 6 mM (Rea et al. 2009, 2000). By comparison, circulating blood glucose concentrations are higher in seals that have fasted for over a month than they are in humans only a few hours after a meal (Frayn et al. 1993).

19.2.3.1 Glucose Oxidation

Despite abundant plasma glucose being available, its complete oxidation to CO_2 appears to be low in seals, as is expected in fasting adapted animals. Several studies have measured respiratory quotient (RQ) in both phocids (Davis et al. 1985; Kohin et al. 1999) and otariids (Arnould et al. 2001; Verrier et al. 2009). Measured RQ values are continuously low in seals, between 0.71 and 0.80, consistent with metabolism primarily based on lipid oxidation. Antarctic fur seal pups decreased RQ to 0.72 with the onset of fasting, but then increased to 0.77 after only 4–5 days fasting—a typical fasting duration in this group (Arnould et al. 2001). Subantarctic fur seal pups fasting for more prolonged periods, over 30 days, had stable RQ of 0.80 throughout fasting (Verrier et al. 2009). These same pups had exceptionally low rates of protein catabolism. Similar rates of glucose oxidation have been found in phocids. Northern elephant seals show consistently low RQ values, around 0.74, throughout their prolonged postweaning fast and in 10-month-old seals after their first foraging trip (Kohin et al. 1999). Keith and Ortiz (1989) administered [$^{14}C_6$]-glucose and used breath testing to measure the appearance of $^{14}CO_2$ in expired air. Even after several hours, less than 1% of the infused ^{14}C had appeared in expired air, suggesting very low rates of glucose oxidation. These data indicate that the rates of glucose oxidation are indeed low in seals. Thus, although glucose remains available in circulation throughout fasting, peripheral tissues preferentially oxidize fat. Carbon loss from glucose oxidation is minimal and the nervous system is probably responsible for most of the glucose oxidized.

19.2.3.2 Glucose Production and Carbon Recycling

Endogenous glucose production (EGP) has been measured using isotopic tracers in a few seal species amenable to repeated sampling—namely elephant seals, gray

seals, and harbor seals. Reported rates of glucose production are typically from 8 to 15 μmol per min per kg. EGP has been investigated most extensively in elephant seals during their postweaning fast. Weaned elephant seal pups decreased EGP with fasting by as much as 25%, even when accounting for the reduction in body mass over the 2-month fasting duration (Champagne et al. 2005). Adult northern elephant seals, however, did not show the same suppression of EGP with time fasting (Champagne et al. 2006).

Several studies have investigated protein catabolism in elephant seals, as discussed above (Adams and Costa 1993; Crocker et al. 1998; Houser and Costa 2001). These rates of protein loss have been used to estimate the maximal contribution of protein to EGP in elephant seals of various age classes. We estimate that protein contributes only a small proportion of the total EGP, only ~2% in weaned pups, potentially as much as 14% in lactating females but probably substantially less. The high rates of fat catabolism and lipolysis in elephant seals suggested that glycerol, available from the hydrolysis of TAG, would substantially contribute to EGP. Our studies, however, using various isotopic methods, indicate that glycerol is also a minor contributor, supplying only ~5% of EGP, in both weaned pups (Champagne 2011) and adult females (Houser et al. 2007).

Together, these investigations suggest high rates of glucose carbon recycling in fasting seals. Rates of EGP do not appear to decrease with fasting to the same extent observed in other mammals (Fig. 19.3a); glycerol and protein-based gluconeogenesis only account for a small fraction of the total rate of EGP, and the proportion of glucose oxidized is small. These findings suggest that most glucose carbon released from the liver is recycled via 3-carbon intermediates (e.g. lactate). Glucose recycling during fasting has been of interest for quite some time (e.g. Cahill et al. 1966) as this process may transfer the energetic burden of glucose dependent tissues from carbohydrate to lipids since lipid is the ATP and reducing source for glucose production (Fig. 19.3b; Frayn 2010, p 245). Directly measuring these rates of recycling using isotopic tracers, however, has proven problematic due to isotopic exchange, especially via the tricarboxylic acid (TCA) cycle (Radziuk and Lee 1999). Nevertheless, the rates of adipose and lean-tissue loss, rates of protein catabolism, maintenance of plasma glucose levels, and EGP measurements all support low rates of glucose oxidation and substantial amounts of glucose carbon recycling in seals. The function of sustained rates of EGP that appear to exceed the needs of glucose-dependant tissues with extended fasting is, as yet, unknown.

19.3 Regulation of Fasting

Studies on hormonal and fuel regulation during fasting have suggested that some species of phocid seals may exhibit high rates of lipid oxidation characteristic of Phase II fasting throughout their lives. In all pinnipeds, fat is an important energy source—during early development as high fat milk and throughout adulthood in a

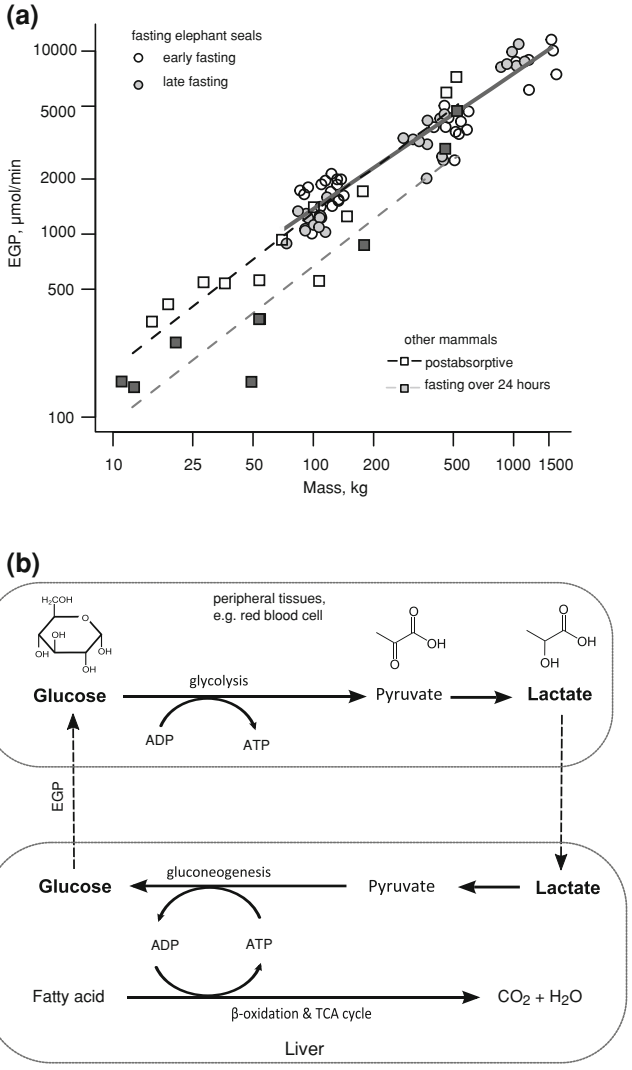

Fig. 19.3 **a** Phocid seals do not appear to suppress endogenous glucose production (EGP) to the same extent as terrestrial mammals. Northern elephant seals are represented by *white* (early) and *gray* (late fasting) *circles*; the relationship between EGP and mass is shown with a *solid gray line*. Rates of EGP from other mammals were drawn from the literature—postabsorptive mammals (*white squares* and *black dashed line*) and mammals fasting longer than 24 h (*gray squares* and *gray dashed line*). EGP in northern elephant seals fasting for 1–3 months is similar to EGP in these terrestrial animals in the postabsorptive state. References: (Anwer et al. 1976; Brockman et al. 1975; Chaiyabutr et al. 1982; Champagne 2011; Champagne et al. 2005, 2006; Cowan et al. 1969; Davis 1983; Evans 1971; Freminet et al. 1977; Issekutz 1977; Katz et al. 1974; Kreisberg et al. 1970; Kronfeld and Raggi 1964; Muller et al. 1983; Nordøy and Blix 1991; Trayhurn et al. 1981; Weber et al. 1997; White and Luick 1976). **b** Schematic of glucose carbon recycling. The difference between EGP and glucose oxidation in fasting seals suggests high rates of glucose carbon recycling (e.g. Cori cycle) via 3-carbon intermediates, such as lactate. We hypothesize that glucose carbon recycling in this manner supports typical postabsorptive rates of EGP throughout prolonged fasting in elephant seals and probably other phocids as well

high fat diet of marine prey and while using fat stores when fasting. Studies of protein conservation during suckling (Houser and Costa 2001) and the role of insulin in glucose regulation (Kirby and Ortiz 1994) suggest that seals may be "pre-adapted" for fasting. In contrast, several studies in otariids have revealed a distinct transition from Phase I to Phase II, suggesting a more typical mammalian metabolic response to food deprivation (Arnould et al. 2001; Verrier et al. 2009).

The reliance on fat metabolism and the resultant high concentration of circulating NEFA in seals potentially influences glucose metabolism (Lam et al. 2003). Low insulin concentrations, impaired glucose clearance, low insulin to glucagon (I:G) ratios, and reduced insulin sensitivity have been demonstrated during several life history stages (Fowler et al. 2008; Viscarra et al. 2011b). Typically, insulin concentration and I:G ratio decrease in fasting seals. Recent investigations have begun to describe alterations in the insulin signaling pathway of northern elephant seals during their postweaning fast. Using adipose tissue from blubber biopsies, Viscarra and co-authors characterized changes in several components of the insulin signaling pathway including the relative abundance of insulin receptor and insulin receptor substrate, phosphoinositol 3-phosphate, and Akt (Viscarra et al. 2011b). These insulin signaling components all decreased across the postweaning fast. Despite these decreases, total GLUT4 content and phosphorylated 5′-AMP-activated protein kinase (AMPK) increased across the same period (Viscarra et al. 2011b). However, the location of the GLUT4 transporter—whether intracellular or associated with the plasma membrane—could not be determined; so the influence on glucose uptake is not yet known.

Experimental work has found that northern elephant seals vary their hormonal response to a glucose tolerance test (GTT) across fasting. Adult females released insulin in response to a GTT early in lactation, but at the end of lactation no insulin response was detected; suggesting that females functionally reduce insulin response with declining adipose tissue stores as lactation progresses (Fowler et al. 2008). Glucagon, however, declined in response to the GTT both early and late in fasting simultaneous with lactation. The separate responses of insulin and glucagon—no response of insulin detected late in lactation but a glucagon response both early and late—indicates that there is some decoupling of the two hormones. Viscarra et al. (2011a) found a similar trend in pups during the postweaning fast where the timing of insulin secretion was delayed at the end of the fast. These GTTs suggest impaired insulin response and glucose clearance in both fasting pups and adults. Late in the fast, weaned elephant seal pups exhibited elevations in AMPK and peroxisome proliferator-activated receptor γ (PPARγ) in response to a GTT despite reductions in insulin signaling proteins. Additionally, hormone challenge studies also suggest important impacts of development, reproduction, and condition on responses to hormones. For example, fasting elephant seal pups do not exhibit a gluconeogenic response to a pharmacological dose of glucagon. In contrast, lactating and molting adult females exhibit a delayed but significant increase in blood glucose levels in response to the same challenge but this response declined with fasting duration (Crocker et al. 1998, 2001, 2006). Similarly, insulin response to a GTT declined with the level of adiposity. Adult females at the end of

their fast exhibited insulin responses to glutamine administration but not to a GTT, suggesting that pancreatic function is maintained during the fast but may not be responsive to alterations in plasma glucose (Crocker et al. 1998, 2001, 2006).

In general, studies on fasting pinnipeds have reported significant increases in growth hormone and cortisol associated with fasting (Ortiz et al. 2001a, b; Guniet et al. 2004; Champagne et al. 2005). Elevations in growth hormone may help reduce the protein wasting impacts of cortisol elevations while permitting elevated rates of lipolysis. Thyroid responses to fasting have been widely variable between species and life history stages. Thyroid levels are higher and may increase across the fast in developing pups, possibility due to thyroid impacts on erythropoiesis (Ortiz et al. 2003; 2001b; Woldstad and Jenssen 1999). In general, thyroid levels have been higher and stable across the fast in adults, possibly to support the high metabolic rates associated with breeding (Hall 1998; Oki and Atkinson 2004).

Over the past decade, evidence has grown that many of the hormonal changes in response to fasting may be impacted by the starvation-induced decline in leptin—a peripheral signal involved in the regulation of food intake, body adiposity, metabolic rate, and immune function in mammals. Few studies, however, have examined these features in fasting adapted wildlife. Leptin levels were stable during fasting in elephant seals, suggesting leptin may have a reduced role in the regulation of fasting metabolism (Ortiz et al. 2001a). Studies in subantarctic fur seals, however, detected rapid declines of leptin with the onset of fasting, suggesting this hormone may play a role in the transition from Phase I to Phase II fasting in this group (Arnould et al. 2002).

19.4 Future Directions

19.4.1 Ketosis

Pinnipeds provide a unique fasting model that couples the demands of energetically costly activities with the substrate conserving mechanisms required of the fasting state. An area that has received relatively little attention, but which is of particular interest for medical reasons, is the fact that pinnipeds do not become ketotic even after prolonged periods of high rates of lipid oxidation. Ketoacidosis is problematic in several medical conditions, the most notable of which is diabetes mellitus. During fasting, increases in circulating ketone levels can increase their oxidation by nervous tissue, in particular the brain. This adaptive response further conserves valuable protein stores that might otherwise be directed toward gluconeogenesis. However, ketosis may become problematic in that it can result in a detrimental state of metabolic acidosis. Future work in pinnipeds will look to investigate how pinnipeds rely heavily on lipid oxidation, potentially for months, without incurring ketoacidosis. Indeed, studies will need to focus on how only modest increases in ketones occur even in the most extreme fasting conditions.

The occurrence of ketoacidosis likely lies in the overloading of the TCA cycle with 2-carbon substrates that result from the β-oxidation of fatty acids. Whether or not the TCA cycle functions at a rate that is capable of dealing with the high rate of β-oxidation in pinnipeds, or whether some compensatory mechanism allows for the elimination of ketones from their blood, remains to be seen.

19.4.2 Hypoxia and Oxidative Stress

One understudied aspect of fasting adaptation is the potential for oxidative stress associated with food deprivation. Prolonged food deprivation in terrestrial mammals increases reactive oxygen species production, oxidative damage, and inflammation, which may be induced by an increase in the renin–angiotensin system. Recent studies in elephant seals have suggested adaptations to reduce the potential for oxidative stress in response to fasting (Vazquez-Medina et al. 2010). Fasting induced dramatic increases in plasma renin activity, angiotensin receptor populations, and prooxidant molecules. Despite these changes, there were no increases in oxidative stress markers. This appears to be the result of 40–60% increases in the levels of antioxidant enzymes in response to fasting. These studies suggest that glutathione biosynthesis increases with fasting and that glutathione contributes to counteracting hydroperoxide production, preventing oxidative damage in fasting seals (Vázquez-Medina et al. 2011). These antioxidant defenses may be linked to defenses against oxidative damage from hypoxia and ischemia in this species.

Hypoxia may also influence various metabolic pathways, including those of lipid and carbohydrate metabolism (Majmundar et al. 2010; Rankin et al. 2009; Zhang et al. 2011). Pinnipeds experience repeated bouts of hypoxia and tissue ischemia during diving (Kooyman and Ponganis 1998; Meir et al. 2009) and in periodic apneas on land (Ponganis et al. 2006; Stockard et al. 2007). The hypoxia inducible transcription factor (HIF) is responsive to oxygen availability and has wide-ranging effects on whole-animal metabolism (Aragones et al. 2009). In conditions of low oxygen availability, the oxygen sensors prolyl hydroxylase domain-containing enzymes (PHDs) and factor inhibiting HIF (FIH) cease to target HIF for enzymatic degradation. HIF then dimerizes and has extensive effects on gene expression, shifting ATP production from aerobic to anaerobic pathways (Miyata et al. 2011). For example, the HIF1α subunit influences gene expression with several effects on carbohydrate metabolism—increasing glycolytic enzymes and lactate dehydrogenase (LDH), and suppressing pyruvate conversion to acetyl CoA and its entrance into the TCA cycle (Wheaton and Chandel 2011). These metabolic effects are adaptive in hypoxic conditions endured by many organisms, including pinnipeds. Many of the features of carbohydrate metabolism discussed above (higher than expected plasma glucose levels, rates of EGP, and glucose carbon recycling) are consistent with upregulation of HIF. In selected species, like seals, some aspects of fasting metabolism may be related to adaptation to hypoxia.

Comparative investigations between animals of varying degrees of diving and fasting ability may clarify this relationship.

19.5 Conclusion

Many species of pinniped fast simultaneous with energetically costly activities as part of their normal life history. This review described many of the metabolic adjustments that allow the resolution of these conflicting energy and material demands. Many metabolic characteristics of seals conform to expectations of fasting adapted animals, including high circulating NEFA, high rates of lipolysis and lipid oxidation, and efficient protein sparing. Much of our understanding of fasting in pinnipeds comes from studies in northern elephant seals. Some of their metabolic features when fasting do not conform to predictions based on terrestrial mammals, such as high plasma glucose levels, their rates of glucose production and glycerol gluconeogenesis, and the lack of ketone accumulation. Comparative studies will allow a better understanding of whether the unique features in this system broadly reflect adaptations in pinnipeds or other free-ranging animals adapted to extended fasting while not torpid.

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Chapter 20
The Use and Application of Stable Isotope Analysis to the Study of Starvation, Fasting, and Nutritional Stress in Animals

Kent A. Hatch

20.1 Introduction

Physiologists have long been fascinated with fasting and starvation in animals (Cahill 1970; Young and Scrimshaw 1971). This is perhaps because fasting and starvation have long been intimate parts of the human condition (see Grant, Chap. 21). While fasting and starvation are related terms, starvation can be understood as a situation where an animal that is willing to eat cannot consume sufficient food to maintain its body condition and therefore enters a catabolic state (McCue 2010). Fasting can be understood as the condition in which an animal has sufficient food readily available but chooses not to eat, becoming catabolic. Starvation can be life threatening, and organisms have developed mechanisms and strategies to enhance their ability to survive periods of starvation (Kirk 1997; McCue 2007a, b; McCue 2010; Rion and Kawecki 2007). By contrast, fasting often appears to be adaptive, allowing the organism to devote its energies to activities other than procuring food, such as mating, nesting, rearing young, predator avoidance, and so on. (Cherel et al. 1988; King and Murphy 1985; Mrosovsky and Sherry 1980). Fasting may also be a response to illness or disease and may enhance the organism's ability to fight infection (Bazar et al. 2005; Chang and Bistrian 1998).

This chapter focuses on how stable isotope analysis (SIA) can be used as a tool to better understand starvation, fasting, and related forms of nutritional stress. There are a number of good reviews of the physiological strategies animals employ in fasting and to survive starvation (Castellini and Rea 1992; Cherel et al. 1988; McCue 2010; Wang et al. 2006), but few of these discuss how nutritional stress affects stable isotope dynamics.

K. A. Hatch (✉)
C.W. Department of Biology, Post Campus, Long Island University,
720 Northern Boulevard, Brookville, NY 11548, USA
e-mail: kent.hatch@liu.edu

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Stable isotope analysis (SIA) has much to offer in helping physiologists understand the physiological and biochemical processes underlying fasting and starvation. It can aid in detecting catabolic states, which in wild animals might not otherwise be obvious. Of the commonly measured isotopes, nitrogen isotope ratios appear to be the most sensitive to changes in metabolic state (Habran et al. 2010; Hare et al. 1991), though $\delta^{13}C$ values may respond as well (Boag et al. 2006; Hatch et al. 1995; McCue and Pollock 2008). Some studies suggest that anabolic states that are associated with higher rates of protein synthesis and positive nitrogen balance, such as gestation (Fuller et al. 2004), and lactation (Fuller et al. 2005), cause $\delta^{15}N$ values to decrease in tissues. Other studies show that disease (Katzenberg and Lovell 1999) can cause $\delta^{15}N$ values of tissues to increase. However, the majority of studies have focused on the effect of nutritional stress, fasting, and starvation on ^{15}N and ^{13}C enrichment of various tissues.

20.1.1 The Importance of Understanding Fasting and Starvation

Fasting is an important adaptive mechanism. It helps animals allocate time and energy better during periods where foraging needs to take a back seat to demands such as feeding, migration, reproduction, sleep, or hibernation. It also allows animals to cope with the cyclic food availability found in boom and bust environments. During times of plenty they can accumulate endogenous resources and then use those endogenous stores during times of scarcity, thereby allowing them to persist in, rather than migrate from a particular habitat (King and Murphy 1985). The ability to determine when animals actually enter a state of fasting will allow biologists to better understand how animals allocate resources to allow for and survive periods of behaviorally or externally imposed periods of scarcity.

The inability to obtain enough food to survive, i.e., starvation, has long been assumed to be the primary factor limiting population sizes (Darwin 1859; Malthus 1798). However, this assumption has rarely been tested (King and Murphy 1985; Newton 2004). Starvation might be the ultimate arbitrator of population size, but in many cases may pose a theoretical, but rarely reached limit. Often populations may actually be limited by other, more immediate factors (King and Murphy 1985) such as predation, weather, or disease (Newton 2004; Peterson et al. 1998; Sullivan 1989). Nevertheless, starvation can be the factor limiting population sizes (Hubbs and Boonstra 1997; Peterson et al. 1998; Sullivan 1989) and can threaten the survival of small, endangered populations (Browne et al. 2011).

The direct threat of starvation to an animal's survival is obvious, but starvation can also adversely affect how an animal allocates its resources to activities such as foraging, predator avoidance, growth, development, mating and reproduction, and may therefore indirectly affect an animal's fitness. Body condition can affect whether an animal mates or does not mate, determine when it lays eggs or gives birth, and influence clutch or offspring size. These characteristics, in turn, affect

the number of surviving offspring and lifetime fitness (Drent and Daan 1980; Gil et al. 2004; Nowicki et al. 2002; Ohlsson and Smith 2001). Furthermore, fasting and starvation reduce fat stores that may be necessary for other physiological functions, such as thermoregulation, water balance, buoyancy, and streamlining the body (Iverson 2009).

Finally, it is important to study the effects of fasting, starvation, and nutritional stress on the isotopic discrimination factors of commonly sampled tissues in various species. Since ^{13}C SIA is frequently used to assess the contribution of isotopically distinct foods to the diet and ^{15}N SIA is frequently used as a measure of trophic level, fasting, starvation, and nutritional stress could distort estimates of dietary composition and trophic level (Hobson et al. 1993; Sears et al. 2009).

20.1.2 Detecting Fasting and Starvation

While fasting and starvation are easily detected in the late stages, earlier detection in wild animals can be difficult. Because of the difficulty of recapturing wild animals, most traditional methods preclude repeated measurements. Direct measurement of body composition is the most reliable method of measuring body condition, but is destructive. Relevant data have often been restricted to body weights and the need for nondestructive methods of assessing body reserves is longstanding (Drent and Daan 1980). In addition, merely encountering animals under nutritional stress may be difficult because of their low survival rate (Ben-David et al. 1999; White 1993).

Nitrogen isotope ratios appear to be particularly sensitive to nutritional stress and models predict that whole body $\Delta^{15}N$ should increase with increased duration of fasting or starvation (Martinez del Rio and Wolf 2005). Tissue enrichment in ^{15}N appears to occur during the synthesis, deamination, and transamination of amino acids (Gaebler et al. 1966; Hare et al. 1991). ^{14}N is preferentially excreted and ^{15}N retained in newly synthesized amino acids and proteins (Steele and Daniel 1978). The effect of fasting on $\delta^{15}N$ is dependent on the absolute rate of protein synthesis of the tissue and the duration of the fast. The faster the rate of protein synthesis, the longer the fast, the more positive the $\delta^{15}N$ of the tissues become (Cherel et al. 2005; Hobson et al. 1993; Martinez del Rio and Wolf 2005).

20.2 $\delta^{15}N$ as an Indicator of Nutritional Stress

It has been suggested that SIA might serve as an index of, or diagnostic tool for detecting nutritional stress (see Gannes et al. 1997, 1998; Martinez del Rio et al. 2009). To date, much of the research using SIA to study fasting has focused on this problem. Though both laboratory and field studies have sought to establish that $\delta^{15}N$ and $\delta^{13}C$ of tissues and substrates increase in response to starvation, fasting, or nutritional stress, the results have been mixed.

20.2.1 Field Studies

Attention was first drawn to the possibility that δ^{15}N values of tissues might be useful indicators of fasting or starvation in wild animals by Hobson et al. (1993). They measured the δ^{15}N of liver and muscle of Ross's geese during their nesting period. These birds do not feed while incubating their eggs. After a resulting 4-week fast the liver and muscle tissues of nesting geese were 2.2 and 1.2‰ more enriched respectively in ^{15}N than those of newly arrived geese that had not yet laid eggs.

The degree to which δ^{15}N may be a useful indicator of fasting, starvation, or nutritional stress under field conditions is unclear. While Fasting for 25 days significantly increased δ^{15}N values of plasma, blood cells, and whole blood in adult king penguins, the increase was small (0.70‰, 0.24‰, and 0.35‰, respectively; Cherel et al. 2005), often falling within the range of measurement error. Such small changes may be particularly difficult to detect in wild populations where interindividual variability of δ^{15}N can be high (Stevens et al. 2006; Ugan and Coltrain 2011). Sometimes results can be mixed. When the δ^{15}N of blood plasma at the beginning and end of a fasting period was compared for different polar bear family groups, Polischuk et al. (2001) found that in three family groups of polar bears δ^{15}N decreased as a result of fasting, while in two family groups δ^{15}N increased as a result of fasting.

20.2.2 Laboratory Studies Suggesting δ^{15}N as an Indicator of Nutritional Stress

Hobson et al. (1993) demonstrated in the laboratory, as well as in the field, that δ^{15}N might be indicative of nutritional stress. When 10-day-old Japanese quail (*Coturnix japonica*) were raised for 18 days on a maintenance diet their liver and bone collagen ^{15}N were enriched by more than 1‰ compared to the tissues of controls fed ad libitum. Muscle and whole blood were also significantly ^{15}N enriched, while feather δ^{15}N values were not significantly different between the two groups. The quail on the restricted diet were arguably nutritionally stressed since they gained no weight during a time when they normally should have experienced rapid growth. While this study suggested that nutritional stress, and thus fasting and starvation, could increase δ^{15}N values of tissues, it was not designed to demonstrate whether δ^{15}N values might provide an index of nutritional stress.

Other laboratory studies similarly demonstrated that δ^{15}N values of tissues or substrates will increase due to fasting, starvation, or other forms of nutritional stress, but likewise were not designed with the purpose of developing a nutritional stress index based on δ^{15}N. Wolf spiderlings (*Paradosa lugubris*) when fasted for 12 days were enriched by 1.34‰ in ^{15}N when compared with hatchlings (Oelbermann and Scheu 2002). Uric acid shows promise as an indicator of nutritional stress caused by starvation. Both American anoles (*Anolis carolinensis*)

and side-blotched lizards (*Uta stansburiana*) showed significant increases in $\delta^{15}N$ of excreted uric acid after 14 days without food when compared with controls fed ad libitum (Castillo and Hatch 2007).

Urine may be a good indicator of catabolism caused by nutritional stress. Urine not only has the advantage of being more sensitive to isotopic changes than bodily tissues (Barboza and Parker 2006), but has the advantage of being noninvasive and easily collected from snow. Consequently, there is some interest in using excreted urine as a measure of nutritional stress in arctic ungulates (see also Ulrey, Chap. 18). In a study of wild, pregnant caribou (*Rangifer tarandus*) held in enclosures in late winter (Gustine et al. 2011), $\delta^{15}N$ measurements of voided urine were used as an index (Barboza and Parker 2006) of nitrogen balance. Although this approach provided evidence that some of the animals were catabolizing endogenous protein stores for energy, additional validation is needed before it can be broadly applied (Gustine et al. 2011).

20.2.3 Laboratory Studies Suggesting $\delta^{15}N$ as an Index of Nutritional Stress

If the enrichment of tissues in ^{15}N is to serve as an index of fasting, starvation, or nutritional stress, it must vary predictably along a continuum with other indicators of nutritional stress or with time fasted. A number of laboratory studies suggest that this might be the case.

Gaye–Siessegger et al. (2007) found that the homogenized tissues of Nile Tilapia (*Oreochromis niloticus*) fasted for 5 weeks or fed 1.2 g $kg^{-0.8}$ day^{-1} were significantly enriched in ^{15}N compared to those fed the highest feeding levels (4.8 and 2.5 g $kg^{-0.8}day^{-1}$ is considered maintenance at 27°C). However, the difference, 0.3‰, was very small. Nevertheless, the effects of different feeding levels were correlated with $\delta^{15}N$ values of whole, homogenized tilapia, as demonstrated by linear regression ($F_{1,\ 3} = 22.01$, $p = 0.\ 018$, $R^2 = 0.88$; Fig. 20.1).

Because of its relatively small pool size, measurements of ^{15}N enrichment of excreted uric acid may be a more sensitive index of nutritional stress than ^{15}N enrichment of tissues. The $\delta^{15}N$ of the uric acid of American anoles and side-blotched lizards collected after 15 days without food were positively correlated with the amount of weight lost by the lizards ($F_{1,14} = 6.116$, $p = 0.028$, $R^2 = 0.32$), whereas the $\delta^{15}N$ of tail tissue newly regenerated during this period showed no such trend and did not differ from that of controls (Castillo and Hatch 2007). Furthermore, when gaboon vipers (*Bitis gabonica*), ball pythons (*Python regius*), ratsnakes (*Elaphe obsolete*), boa constrictors (*Boa constrictor*), western diamondback rattlesnakes (*Crotalus atrox*), and savannah monitor lizards (*Varanus exanthematicus*) were all starved for 168 days, neither the $\delta^{15}N$ values claws nor that of whole carcass were affected by duration of starvation. However, the excreta (mostly uric acid) of all six species became significantly more ^{15}N enriched as the duration of starvation increased (McCue and Pollock 2008).

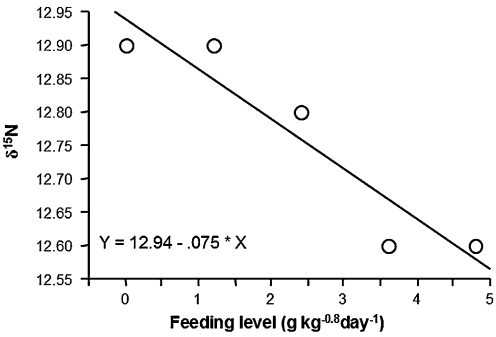

Fig. 20.1 Linear regression of the mean δ^{15}N values of whole tilapia bodies versus. feeding level. Based on data from Gaye-Siessegger et al. (2007)

The fact that in both these studies found that fasting affects the δ^{15}N of uric acid and not whole body tissue suggests that there are two separate pools from which nitrogen may be recycled, nitrogen in the form of proteins and amino acids in circulation, and nitrogen in the tissues of the animal (McCue and Pollock 2008). The former is assumed to be labile, while these studies strongly suggest the latter is not very labile, at least in reptiles. Initially, nitrogen excreted as uric acid apparently came primarily from the labile, circulating pool, but as this was depleted, more nitrogen came from the ^{15}N enriched nonlabile pool, enriching the excreta. In reptiles fasted for 168 days, a linear mixing model suggests that the nonlabile pool initially contributed no nitrogen to the excreta, but by the end of the study was contributing 53% of the excreted nitrogen (McCue and Pollock 2008).

There are also many studies of invertebrates suggesting that δ^{15}N enrichments of tissues may provide an index of fasting or starvation. When a logarithmic regression of the δ^{15}N values of starved daphnia (*Daphnia magna*) against the number of days without food is performed, the relationship is highly significant ($F_{1,3} = 22.04$, $p = 0.018$, $R^2 = 0.88$; Fig. 20.2). The authors attributed the enrichment in ^{15}N to increased reliance on endogenous N sources with decreased availability of dietary N. However, daphnia neonates starved for 5 days (the longest period starved) were only 0.45‰ more enriched in δ^{15}N than newly hatched neonates (Adams and Sterner 2000). In another study that showed a strong relationship between the amounts of time fasted and δ^{15}N of whole body tissues, springtails (*Protaphorura fimata*) were fasted for up to 28 days (Haubert et al. 2005). A linear regression of the mean δ^{15}N values of springtails fasted for different lengths of time demonstrated a significant relationship ($F_{1,5} = 12.66$, $p = 0.162$, $R^2 = 0.72$; Fig. 20.3a) with δ^{15}N increasing with increasing duration of the fast. C/N ratios, which decreased with fast duration, were also correlated with δ^{15}N values ($F_{1,5} = 30.214$, $p = 0.0027$, $R^2 = 0.86$; Figs. 20.3b and 20.4a). While ANOVA of the δ^{15}N of chironomid larvae (*Chironomus acerbiphilus*) fasted 0–12 days did not suggest that δ^{15}N increased significantly with fast duration (Doi et al. 2007), a linear regression of mean δ^{15}N of whole body tissues over time does show that δ^{15}N of the tissues increases significantly with increased fast duration ($F_{1,3} = 13.763$, $p = 0.034$, $R^2 = 0.82$; Fig. 20.4b). However, the increase over the 12-day period is small, about 0.7‰.

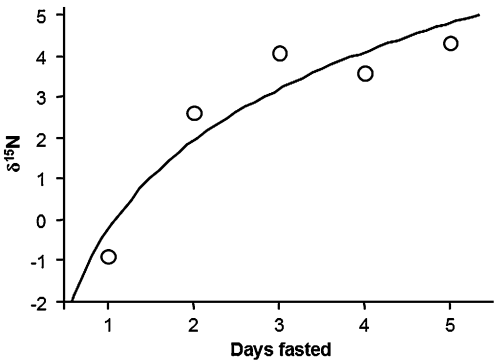

Fig. 20.2 Logarithmic regression of the mean $\delta^{15}N$ values of whole daphnia bodies versus time fasted. Based on data from Adams and Sterner (2000)

New Zealand flatworms (*Arthurdendyus triangulatus*) proved an interesting model organism for studying the effects of starvation on tissue stable isotope ratios. Part of the posterior of the flatworm was removed for SIA while the anterior portion, which remained alive and active, was starved and then analyzed, allowing the individual flatworms to serve as their own controls. The $\delta^{15}N$ of the starved anterior end of the flatworms increased up to a 2.8‰ after fasting for 243 days (Boag et al. 2006). The relationship between the mean $\delta^{15}N$ values of starved flatworms and the number of days starved appeared best explained by a logarithmic regression ($F_{1,2} = 20.273$, $p = 0.046$, $R^2 = 0.91$; Fig. 20.5).

The $\delta^{15}N$ of the tissues of the marine polycheate worm *Nereis virens* increased most rapidly at the onset of starvation and then appeared to reach a plateau. The new equilibrium was reached after approximately 7 days and $\Delta^{15}N$ was approximately 1‰ (Olive et al. 2003).

20.2.4 Evidence Against $\delta^{15}N$ as Possible Indicator of Nutritional Stress

While the above studies suggest that measurements of $\delta^{15}N$ may provide an index of fasting or nutritional stress, there are also a number of studies that do not support this hypothesis. Arctic ground squirrels (*Spermophilus parryii plesius*) suffering nutritional stress, as indicated by weight loss, blood urea nitrogen, and blood glucose concentrations did not differ in $\delta^{15}N$ from Arctc ground squirrels whose food had been supplemented and were under no measurable nutritional stress (Ben-David et al. 1999). While fasting adult king penguins for 25 days did affect $\delta^{15}N$ values (see Sect. 20.2.1), $\delta^{15}N$ values of plasma and blood cells were not affected by the molting fast of adults nor by the long period of undernutrition in chicks (Cherel et al. 2005). When nestling song sparrows (*Melospiza melodia*) fed a restricted diet for 28 days were compared with controls fed ad libitum no difference was found between the $\delta^{15}N$ of the blood, liver, muscle, or feather of the

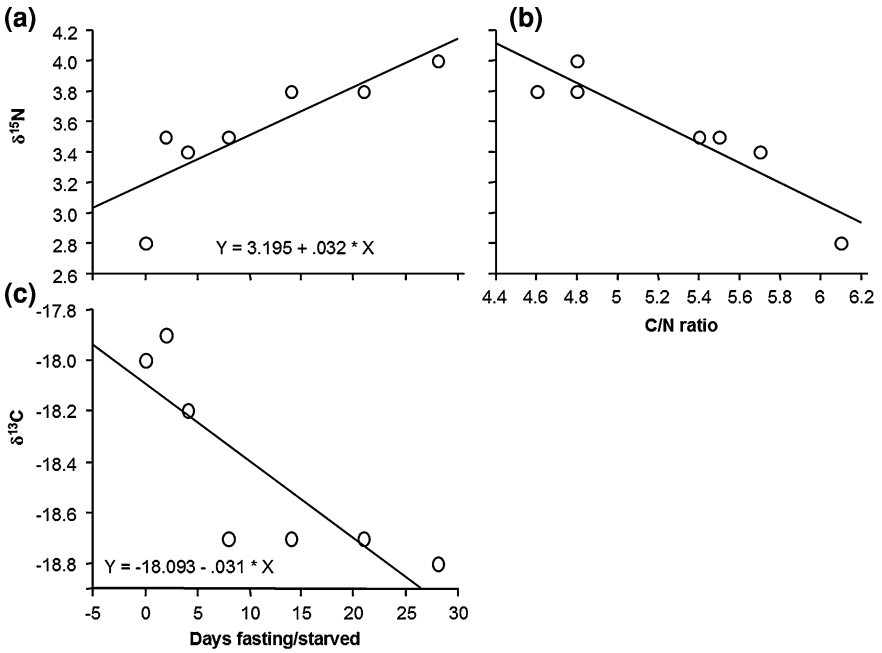

Fig. 20.3 Linear regression of the mean δ^{15}N values of whole springtail bodies versus (**a**) the time they were without food and (**b**) their *C/N* ratios. Linear regression of the mean δ^{13}C values of whole springtail bodies versus (**c**) the time they were without food. Based on data from Haubert et al. (2005)

two groups (Kempster et al. 2007). While starving for 15 days had an effect on uric acid, it had no effect on the δ^{15}N of new tail growth of American anoles and side-blotched lizards (Castillo and Hatch 2007). The whole body of earthworms (*Lumbricus festivus*) starved for 56 days did not change in δ^{15}N over time, despite losing 13.4% of their wet biomass (Schmidt et al. 1999). Fasting the mysid shrimp *Mysis mixta* at 10°C for 5 weeks did not significantly change δ^{15}N values of whole body tissues (Gorokhova and Hansson 1999). Adult cockroaches fasted for 80 days did not show any changes in δ^{15}N (McCue 2008), but any changes might have been masked by the fact that they were observed consuming their feces (M.D. McCue, personal observation).

20.2.5 Continuum Versus Threshold Effects

One possible reason that δ^{15}N values of tissues respond to fasting or nutritional stress in some studies but not in others is that rather than being a continuous index of nutritional stress, there is a threshold effect, below which δ^{15}N values of tissues are not affected but above which ^{15}N of tissues becomes enriched in response

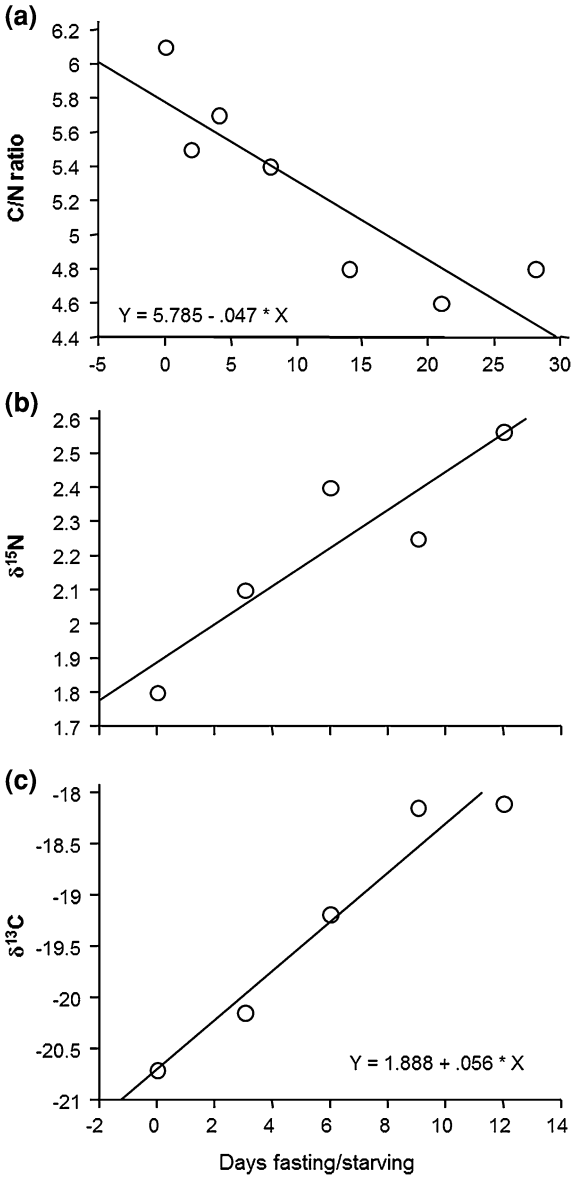

Fig. 20.4 Linear regression of the mean *C*/*N* of whole springtail bodies versuss. days starved (**a**). Based on data from Haubert et al. (2005). Regression of number of days fasted versus $\delta^{15}N$ (**b**) and $\delta^{13}C$ (**c**) of whole body tissues of fourth instar of the chironomid larva *C. acerbiphilus*. Based on data from Doi et al. (2007)

to the stress (Kempster et al. 2007) (see also Bar and Volkoff, Chap. 6). While nutritionally stressed Arctic ground squirrels may have dropped 18.6% in body weight (Ben-David et al. 1999), if this was due primarily to a reduction in lipid stores, the point of significant catabolism and recycling of protein reserves may not have been reached and this may be why the authors found no difference between nutritionally stressed and adequately fed squirrels. No significant differences

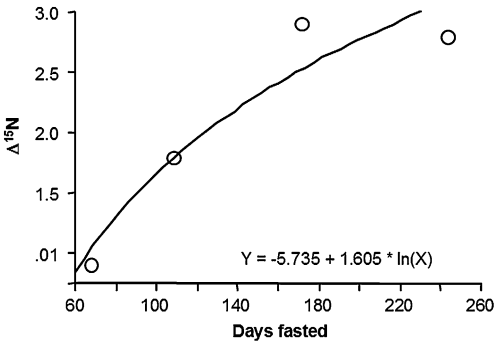

Fig. 20.5 Logarithmic regression of the mean $\delta^{15}N$ of the anterior – $\delta^{15}N$ of the posterior portions of fasted New Zealand flatworms as a function of time. Based on data from Boag et al. (2006)

in tissue $\delta^{15}N$ between controls and song sparrows (Kempster et al. 2007) fed restricted diets may have been found for the same reasons. Models suggest that tissues should become enriched in $\delta^{15}N$ only if fasting or starvation is severe enough that protein loss occurs; catabolism of lipid reserves is not sufficient to induce such a response (Martinez del Rio and Wolf 2005).

The ability to rely on lipid oxidation may also explain the mixed results of the polar bear study above (see Sect. 20.2.1). The first group was, on average older and had greater fat reserves while the second group was younger and leaner (Polischuk et al. 2001). It may be that the first group was relying primarily on lipid reserves and was not catabolizing significant protein reserves while the second group was catabolizing protein reserves. If so, the $\delta^{15}N$ values of polar bear blood plasma in the first group did not increase because they had not yet reached the point of catabolizing significant amounts of protein, while the second group had.

Striped dolphins (*Stenella coeruleoalba*) that died of dolphin morbillivirus showed no relationship between the $\delta^{15}N$ values of muscle and evidence of nutritional stress as measured by blubber lipid content (Gomez-Campos et al. 2011). This outcome may be explained by threshold effects. The large blubber reserves of marine mammals may buffer them sufficiently so that they can long avoid the degree of nutritional stress required to mobilize proteins and cause $\delta^{15}N$ enrichment of tissues.

Threshold effects could explain why it may be difficult to detect nutritional stress in the $\delta^{15}N$ of the tissues of hibernating animals. If a hibernating animal is catabolizing fat reserves, but not catabolizing protein reserves, $\delta^{15}N$ values of tissues might not change significantly. Ben–David et al. (1999) invoked this mechanism to explain the lack of differences in $\delta^{15}N$ values between nutritionally stressed and adequately fed ground squirrels. Additionally, the recycling urea that occurs in some hibernating animals may also mask changes in body condition due to fasting (see also Harlow, Chap. 17). If a hibernating animal is not urinating or defecating, then it is not excreting lighter nitrogen and one would not necessarily expect a change in $\delta^{15}N$ values of its tissues.

A study of the importance of body protein to reproducing, captive musk oxen provides important example of how SIA can provide information on body

condition by looking for threshold effects. These animals face seasonal bouts of starvation. While during the winter food is available and the musk oxen actively feed, it is usually insufficient for them to maintain body mass (Adamscewski et al. 1995) found that the δ^{15}N of red blood cells increased in pregnant musk oxen, but that the δ^{15}N of serum amino acids and the proportion of amino acids derived from body reserves (as estimated using SIA) did not change. Based on SIA, the authors concluded that even though the pregnant oxen were not able to eat sufficiently to maintain body weight, they were well within their adapted limits to periods of seasonal hunger, able to recycle amino acids to maintain their tissues.

20.2.6 Confounding Factors

Factors other than fasting, starvation, and nutritional stress can affect carbon and nitrogen isotope ratios in the tissues of animals. Diet (Best and Schell 1996; Polischuk et al. 2001) is one obvious factor that may confound the use of δ^{15}N as an indicator of stress. Ben-David et al. (1999) found no significant differences between the δ^{15}N of Arctic ground squirrels in poor and excellent body condition. They suggested that diet selection may have obscured any effect nutritional stress or poor body condition may have had on the δ^{15}N values of clotted blood cells. In another study, δ^{15}N of lipid free, whole homogenized tilapia (*Oreochromis niloticus*) fed identical diets but at four different levels decreased with increasing feeding levels. None of the fish were starving and the fish in all four groups gained weight. Therefore, the authors attributed this relationship to protein turnover and an artifact of the difference between the δ^{15}N of endogenous tissues and that of the diet. At lower feeding levels the δ^{15}N of newly synthesized protein more closely approximated the isotopically heavier endogenous stores, while at higher feeding levels it more closely approximated that of the isotopically lighter diet (Gaye-Siessegger et al. 2004).

Trophic level effect (DeNiro and Epstein 1978; Macko et al. 1982; Minagawa and Wada 1984) is another obvious factor that may confound the use of δ^{15}N as an indicator of nutritional stress. It appears that feeding animals large amounts of food or high protein diets can also result in ^{15}N enrichment of tissues, as one would expect from trophic level effects. The effect of fasting, starvation, or nutritional stress on δ^{15}N values of various tissues is often small, as seen in many of the studies sited in this chapter. Trophic level effects average about 3.4‰ for nitrogen (Minagawa and Wada 1984), but can range from −3.22 to 5.89‰ (Vanderklift and Ponsard 2003). Diet quality can have a similar effect. Oelbermann and Scheu (2002) found increased δ^{15}N in the tissues of spiderlings fed different diets to be related to diet quality. Given that trophic level effects are generally larger than changes in δ^{15}N due to nutritional stress, trophic effects may easily mask the effects of fasting, starvation, or nutritional stress.

It is also possible that increased rates of deamination due to high feeding rates can cause increases in the $\delta^{15}N$ of tissues (Sick et al. 1997). Focken (2001) found a significant *positive* relationship between the $\delta^{15}N$ and feeding levels in the tissues of the tilapia *Oreochromis niloticus* fed at just above maintenance levels. He attributed the increase in $\delta^{15}N$ to high deamination rates at high feeding levels. It is unlikely that the enrichment in ^{15}N was due to an artifact of diet, since all the tilapia was raised on the same diet and the only difference was the amount fed. The author suggests that too much protein in the diet causes nutritional stress that may cause increases in tissue $\delta^{15}N$. In any case the difference between the $\delta^{15}N$ of tissues of tilapia fed at the lowest and highest rates was only 0.4‰ (Focken 2001).

Stage of development or growth may be a confounding factor when interpreting $\delta^{15}N$ values. While starving wolf spiderlings fed low quality diets were found to have higher $\delta^{15}N$ than hatchlings, wolf spiderlings fed higher quality diets had still higher $\delta^{15}N$ values. Wolf spiderlings raised on a constant diet were also found to have increasing $\delta^{15}N$ values with increasing age. The authors therefore attributed the high $\delta^{15}N$ values of wolf spiderlings fed high quality diets to the fact that they developed faster and were larger than wolf spiderlings fasted or fed low quality diets, resulting in a greater percentage of their nitrogen pool being replaced by dietary nitrogen than that of the slower growing spiderlings (Oelbermann and Scheu 2002).

Moderate dietary restriction may actually have the opposite effect of severe dietary restriction on $\Delta^{15}N$ of tissues. Rather than increasing the $\delta^{15}N$ of tissues, it may actually deplete the tissues of ^{15}N. Rhinoceros Auklet (*Cerorhinca monocerata*) chicks raised on diets that were 40% of ad libitum for 41 days experienced reduced growth rates when compared with controls fed ad libitum. The $\delta^{15}N$ values of their red blood cells during this period were on average 0.37‰ less than those of the controls. Likewise, the $\delta^{15}N$ values of the feathers of the restricted birds were an average of 0.59‰ less than those of the controls (Sears et al. 2009). Similarly, the $\delta^{15}N$ of whole blood of tufted puffins (*Fratercula cirrhata*) fed restricted diets was lower than that of puffins fed ad libitum (Williams et al. 2007).

Lactation may have confounding effects on $\delta^{15}N$ values. No change was observed in the $\delta^{15}N$ of the whole blood or serum of fasting northern elephant seals that were also lactating (Habran et al. 2010). These animals fast while they nurse their young (Le Boeuf et al. 1973; see Champagne et al., Chap. 19). Lactation is an anabolic process and it may also cause a decrease in the $\delta^{15}N$ of some tissues (Koch 1997; Kurle and Worthy 2002). The authors speculated that the anabolic effect of lactation offset the catabolic effect of fasting in these females, producing no net change in the $\delta^{15}N$ of the blood serum. Thus, it was impossible over the course of the nursing fasting period to detect either an effect of fasting or lactation in the blood or serum of the female seals based on $\delta^{15}N$ values of blood or serum (Habran et al. 2010).

As we have seen, the effects of fasting, starvation, or dietary restriction on $\delta^{15}N$ values of tissues are small and varied. There is ample evidence that other factors can obscure shifts in $\delta^{15}N$ due to nutritional stress, making it questionable as to whether measurements of $\delta^{15}N$ of tissues will ever provide an adequate index

of nutritional stress. If this is so, it brings into question the usefulness of $\delta^{15}N$ measurements as an index of fasting or nutritional stress in the field, as it will likely be difficult to see the effects of nutritional stress amongst the noise.

20.2.7 $\delta^{13}C$ as an Indicator of Nutritional Stress

Some studies have shown that tissues or animal products increase in $\delta^{13}C$ after fasting or nutritional stress. Increases in $\delta^{13}C$ in red muscle (4.1‰), liver (2.6‰), and white muscle (1.3‰) tissues were found in Atlantic salmon after migration and overwintering to the kelt stage, a period of approximately 50 weeks without feeding. However, this change was attributed to depletion of lipid reserves in the tissues rather than fractionation due to protein catabolism (Doucett et al. 1999). Of the six reptile species studied by McCue and Pollock (2008), the carcasses of one species became ^{13}C enriched with increased duration of starvation and time starved was significantly related to increased $\delta^{13}C$ values of the excreta of four of the six species. Oelbermann and Scheu (2002) observed hatchling spiders starved for 12 days were enriched in ^{13}C by 1.46‰ when compared with controls. The $\delta^{13}C$ of lipids of carp (*Cyprinus carpio L.*) increased with decreased feeding levels, but in a curvilinear fashion (Gaye-Siessegger et al. 2004). The authors attributed this response to the differential importance of anabolic versus catabolic pathways at different feeding levels (Gaye-Siessegger et al. 2004). Fasted polycheate worms reached a peak ^{13}C enrichment ($\Delta^{13}C = 0.8 \pm 0.42$‰) after 15 days and then decreased (Olive et al. 2003). Fourth-instar chironomid larvae fasted for up to 12 days actually showed a greater change in $\delta^{13}C$ over time than in $\delta^{15}N$ (see above), increasing by approximately 2‰ (Doi et al. 2007). A regression of the means shows the relationship between $\delta^{13}C$ and the number of days fasted to be highly significant ($F_{1,3} = 58.528$, $p = 0.0046$, $R^2 = 0.951$; Fig. 20.4c). The $\delta^{13}C$ of whole body tissues of springtails that were fasted for up to 28 days showed that fasting may deplete tissues of ^{13}C ($F_{1,5} = 11.78$, $p = 0.0185$, $R^2 = 0.70$; Fig. 20.3c).

20.2.8 $\delta^{13}C$ Not a Useful Indicator of Nutritional Stress

Several studies have found no measurable effect of fasting or starvation on $\delta^{13}C$ values of various tissues. The $\delta^{13}C$ values of the liver or muscle tissues of Ross's geese did not change significantly after fasting for 4 weeks (Hobson et al. 1993). The $\delta^{13}C$ of blood, liver, muscle, or feather did not differ between nestling song sparrows fed a restricted diet for 28 days and controls fed ad libitum (Kempster et al. 2007). Restricting the diets of growing rhinoceros auklet chicks by 40% had no effect on the $\delta^{13}C$ of either red blood cells or feathers (Sears et al. 2009). There was no difference in the $\delta^{13}C$ of new tail growth or of excreted uric acid

of American anoles and side-blotched lizards fasted for 15 days when compared with controls (Castillo and Hatch 2007). The carcasses of five of the six reptile species studied by McCue and Pollock (2008) showed no evidence of ^{13}C enriched with increased duration of starvation. There was no effect of starvation on the $\delta^{13}C$ of claws from any of the six species either. A linear regression of earthworms starved for 56 days did not detect a significant relationship between whole body $\delta^{13}C$ and the duration of food deprivation (Schmidt et al. 1999). Fasting mysid shrimp at 10°C for 5 weeks did not significantly change $\delta^{13}C$ values of whole body tissues (Gorokhova and Hansson 1999). When part of the posterior of New Zealand flatworms was removed to serve as a control for SIA while the anterior portion, which remained alive and active, was starved and then analyzed, the $\delta^{13}C$ of the anterior end of the flatworms did not differ from that of the posterior end after starving the anterior end for 243 days (Boag et al. 2006).

Some studies have found trends in directions opposite of that expected. Tilapia rose on identical diets, but at different feeding rates, all of which were above maintenance, showed increased $\delta^{13}C$ values of lipid extracted homogenized whole fish tissue as well as the lipid extract. The reason for this was unclear. However, it is unlikely that this was due to an artifact of diet, since the tilapia were all raised on the same diet and the study involved no change in diet (Focken 2001). The $\delta^{13}C$ of lipids or lipid free tissue of Nile Tilapia (*Oreochromis niloticus*) fasted or fed at different levels showed no clear trends (Gaye-Siessegger et al. 2007). In tufted puffins, restricting diets decreased rather than increased the $\delta^{13}C$ of whole blood relative to that of puffins fed ad libitum (Williams et al. 2007).

Sometimes results can be mixed. When the $\delta^{13}C$ of blood plasma at the beginning and end of a fasting period was compared for different polar bear family groups, Polischuk et al. (2001) found that in three family groups of polar bears $\delta^{13}C$ decreased as a result of fasting, while in two family groups $\delta^{13}C$ increased as a result of fasting.

20.3 Using SIA to Learn About Fasting and Starvation

Many studies have focused on testing or establishing the use of SIA, especially of ^{15}N as an index or indicator of fasting, starvation, or nutritional stress. Fewer have focused explicitly on using SIA to learn about the physiology of fasting or the ecology associated with fasting. However, SIA can be shown to be a useful tool for understanding nutrient routing and the metabolic changes that occur due to fasting, starvation, and nutritional stress.

20.3.1 Lessons from Studies of Capital Breeders

A special case of fasting or starvation is that of capital breeders. Capital breeders use endogenous stores to produce offspring as opposed to using locally available

food to supply nutrients for reproduction. A capital breeding strategy may evolve when food is not sufficiently available locally, but the advantages of breeding at that time and location outweigh the disadvantages of insufficient food (Drent and Daan 1980; Meijer and Drent 1999). In cases where locally available food is consumed, but insufficient to support reproduction, capital breeding may be considered a form of seasonal starvation (Gloutney et al. 2001; Gustine et al. 2010). In other cases, food may be available, but the parent "chooses" to forgo eating and can be considered to be fasting (Bottitta et al. 2003; Drent and Daan 1980), though the existence of pure capital breeders may be doubtful (Gauthier et al. 2003; Senechal et al. 2011). Capital breeding is also strongly associated with a semelparous strategy whereby endogenous stores are devoted to a single, life-time reproductive effort (Bonnet et al. 1998). In all the above cases, endogenous stores must be catabolized to supply nutrients for reproduction and thus stable isotope studies of capital breeding can provide examples of how SIA may be employed to learn more about nutrient allocation during starvation and fasting in general.

Bird species that are traditionally considered to be capital breeders provide a good example of how SIA can help us understand how nutrients are allocated during periods of starvation. Though some food is locally available and these birds have been observed to feed (Gloutney et al. 2001), they lose significant weight and face the threat of death from starvation during the reproductive period (Ankney and MacInnes 1978; Ryder 1970). It was once assumed that most birds were income breeders, producing their eggs from nutrients derived from concurrently available exogenous resources (food), while a subset of larger bodied birds that bred in marginal habitats were capital breeders (Drent and Daan 1980). Because these habitats had so little locally available food during the breeding period, it was assumed that these birds produced eggs from endogenous stores deposited previous to egg laying and incubation. However, evidence also suggested that many of these birds cannot supply the nutrients for egg production from endogenous reserves alone (Meijer and Drent 1999). Since traditional methods of estimating endogenous contributions to egg production are indirect, they are poorly suited to investigate the extent to which egg production depends on endogenous body stores and concurrently available food (Hobson 2006). SIA of a number of bird species once thought to be pure capital breeders has shown that birds exhibit a continuum between pure capital breeders at one end and pure income breeders at the other end, with almost all "capital breeders" actually having a mixed strategy and lying somewhere in between (Senechal et al. 2011).

The great insight that SIA has provided, when combined with multi source mixing models (Jackson et al. 2009; Moore and Semmens 2008; Phillips and Gregg 2003; Phillips and Koch 2002; Semmens et al. 2009) is that one can quantitatively determine where along the capital-income continuum a species lies. This was first done by Gauthier et al. (2003), who showed that greater snow geese (*Chen caerulescens atlantica*) are not capital breeders and actually are closer to the income breeding end of the spectrum. SIA revealed that only 27–33% of egg production is derived from endogenous stores, while the remainder is derived from locally available food. In addition, SIA revealed that this is variable and egg

production for late breeding birds is slightly more dependent on endogenous stores than that of birds which breed earlier in the season. However, the same proportion of endogenous stores was devoted to developing each egg in a clutch, regardless of clutch size. Therefore, birds laying larger clutches devoted a greater absolute amount of endogenous stores to reproduction than those that laid smaller clutches (Gauthier et al. 2003).

SIA has led other authors to similar conclusions regarding other birds that were once thought to be pure capital breeders. SIA and mixing models revealed that common eiders (*Somateria mollissima*), which fast during the entire incubation period (Bottitta et al. 2003) also use a mixed capital-income strategy (Senechal et al. 2011). SIA also revealed that waders, once thought to be capital breeders, are actually income breeders (Klaassen et al. 2001; Pearson et al. 2003).

Musk oxen are also capital breeders (Moen et al. 2006) and rely on endogenous stores to provide the nutrients for reproduction (Gustine et al. 2010). Endotherms that overwinter harsh arctic conditions need to increase their metabolism to compensate for the colder temperatures. This increased metabolic demand combined with decreased food availability due to grazing on plants no longer growing and/or deep snow cover can combine to make conditions survival and reproduction difficult (see also Zhang et al., Chap. 13). Indeed, when winters are too severe, calves born in the spring are of smaller sizes and the adults may forgo reproduction entirely (Reynolds 2001; White et al. 1997). While feeding is important to their survival and reproduction, there is consistently insufficient food during the winter to support reproduction. Consequently, endogenous stores are the major source of proteins and lipids for gestating young. SIA showed that even under ideal conditions, pregnant musk oxen will route endogenous proteins for reproduction, using dietary resources only for maintenance (Gustine et al. 2010). This suggests that in harsh winters with low food availability musk oxen must either route endogenous resources to maintenance as well (Gustine et al. 2010) or forgo reproduction entirely.

Just as SIA has revealed differences in the proportion of endogenous and exogenous nutrients to reproduction and body maintenance of capital breeders, it has also revealed that endogenous and exogenous resources may be differentially routed to differing components within the egg. Greater snow geese devote a greater proportion of endogenous stores to form the proteins within the eggs than to form egg lipids, as might be expected in herbivorous birds eating a low protein diet (Gauthier et al. 2003). By contrast, in common eiders the lipids in the eggs came primarily from endogenous stores and egg proteins from exogenous diet (Senechal et al. 2011). This pattern, opposite of that of the snow geese above, suggests that carnivorous birds such as eiders allocate reproductive resources differently than do herbivorous birds.

While many studies of capital breeding have been of birds and mammals, capital breeding is even more common among ectotherms (Bonnet et al. 1998). Just as SIA has shown that many endotherms once thought to be capital breeders employ a mixed strategy, SIA revealed that a lizard, the jacky dragon (*Amphibolurus muricatus*), employs a mixed strategy, and the authors suggest such

strategies may be more common among ectotherms than previously thought (Van Dyke and Beaupre 2011; Warner et al. 2008).

Similarly, SIA can provide sound evidence, which would otherwise be difficult to obtain, of extended periods of fasting. The Japanese eel (*Anguilla japonica*) are thought to fast during their migration from the rivers, estuaries, and coastal waters of Japan to their presumed breeding grounds near the West Mariana Ridge (Tsukamoto 2009). Chow et al. (2010) were able to confirm this by comparing the $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue of eels caught in Japan with those of eels caught in the southern part of the West Mariana Ridge. The $\delta^{13}C$ and $\delta^{15}N$ values of the muscle tissue of the eels caught near the breeding grounds was similar to that of eels caught in Japan and showed no evidence of a shift to a marine diet. Given that the migration takes 6 months, there was certainly adequate time for such an isotopic shift should eels feed en route. This lack of evidence supports the conclusion that Japanese eels do not feed as they migrate to their breeding grounds (Chow et al. 2010).

The point here is that just as SIA has shown how both endotherms and ectotherms are surprisingly flexible in how they allocate endogenous and exogenous nutrients in the special case of nutritional stress caused by reproductive effort, SIA may yield similar surprises in studies of nutrient routing in starvation and fasting in general. In contrast with previous approaches, SIA has the advantage of being a direct and quantifiable measure of nutrient allocation at the level of the individual (Hobson 2006; Hobson et al. 2005). Previous approaches were indirect and often overestimated endogenous nutrient allocation to eggs (Hobson 2006; Meijer and Drent 1999). They provided information on at the population level, but not at the level of the individual. SIA also has the potential to show how exogenous and endogenous stores may be routed to tissue growth, tissue maintenance, energy and nitrogenous waste production during periods of fasting, or starvation.

20.3.2 Nutrient Routing and Starvation

Nutrient routing has not received adequate attention in studies of starvation and SIA is particularly well suited to provide insights into nutrient routing (McCue 2011). While fasting animals voluntarily forgo feeding and must use endogenous stores to supply all forms of metabolism and production (e.g., reproduction, tissue turnover, feather or hair growth), starving animals are frequently able to obtain some, though insufficient nutrition from exogenous sources (see also McCue, Chap. 1). Studies of birds with mixed breeding strategies (in between capital and income strategies) demonstrate how SIA can indicate routing of exogenous and endogenous resources towards different components of reproduction, such as yolk versus albumen, or fat versus protein, during times of nutritional stress. A similar approach could be taken to better understand how animals employ endogenous versus exogenous resources to tissue growth, tissue turnover, and energy production during periods of starvation (Hatch et al. 2006).

For example, even during periods of severe nutritional stress, tissue turnover continues, though at a decreased rate (Waterlow 1986). There are also tissues, such as feather or hair, which are of such high priority that they will continue to grow during periods of nutritional stress and general catabolism. SIA has shown that emperor penguins, which molt and begin growth of new feathers while at sea, initially construct their feathers from marine dietary proteins. However, feather growth continues during the fast on land, at which point the feather is constructed from endogenous stored proteins (Cherel et al. 2005). From a clinical or health management viewpoint, such studies could indicate which sources of nutrition are most critical to animals (or humans) during periods of starvation and how better to prevent starvation or help recovery from periods of starvation.

SIA has proven an important tool in understanding important interactions between starvation, bleaching, and how nutrients are provided and used in the coral *Stylophora pistillata*. A combination of controlled feeding experiments and fasting experiments in the presence and absence of light showed that while the planulae of this species have an oral pore, they do not actively feed. Instead, SIA revealed that they receive all of their nutrition from symbiotic zooxanthelae. While adult coral colonies can survive periods of bleaching by increasing predation, the larvae of *S. pistillata* cannot and have to consume endogenous stores in the absence of photosymbionts (Alamaru et al. 2009). Thus bleaching, loss of their zooxanthellae, decreases the likelihood of successful recruitment of these larvae.

20.3.3 Breath Analysis and Fasting

Analysis of exhaled CO_2 ($\delta^{13}C_{breath}$) has been underutilized in studying the physiology and ecology of fasting, but promises to provide valuable insights into understanding the resources animals metabolize while fasting. For example, birds undergo long periods of food deprivation during migration (Battley et al. 2000; Biebach 1990). While much can be learned about substrate metabolism during flight by analyzing blood metabolites (Jenni-Eiermann and Jenni 1991, 1992, 1997; see also Jenni-Eiermann and Jenni, Chap. 11), the degree to which carbohydrates contribute energy to flight and when birds switch from burning exogenous carbohydrates to endogenous stores is difficult to measure using blood metabolites. This is because, unlike mammals, blood glucose levels do not vary much in birds, regardless of fast or flight duration (Schwilch et al. 1996; Swain 1992). Thus, while measuring free fatty acids and β-hydroxybutyrate in blood can provide evidence of increased lipid metabolism (Schwilch et al. 1996), blood metabolites do not provide evidence of decreased carbohydrate metabolism in flying or fasting birds.

By measuring $\delta^{13}C_{breath}$, Hatch et al. (2002b) were able to show the degree to which both resting and flying pigeons were metabolizing carbohydrates as compared to lipids after fasts of varying durations. In both cases, carbohydrate

utilization dropped off dramatically after 12–24 h of fasting, indicating both groups was entering stage II of fasting. Before reaching stage II of fasting the $\delta^{13}C_{breath}$ indicated that flying birds used relatively less carbohydrates and more lipids to fuel metabolism than resting birds, but once stage II was reached, SIA of exhaled breath indicated that neither group was using carbohydrates to fuel metabolism. At the same time, blood metabolite levels indicated both greater protein metabolism and lipid metabolism among flying pigeons than among resting pigeons. However, the $\delta^{13}C_{breath}$ did not differ between or change for either resting pigeons or flying pigeons after fasts of 24–72 h. These results support an earlier hypothesis that protein and lipid metabolism in birds are coupled closely through the citric acid cycle (Jenni and Jenni-Eiermann 1998). The level of both lipid and protein metabolism increased, but the relative amount of lipid to protein metabolized stayed the same. These examples underscore the promise for SIA of $\delta^{13}C_{breath}$ to further our understanding of the metabolic processes that occur during periods of fasting and starvation in both sedentary and exercising animals.

The $\delta^{13}C_{breath}$ can also be used as a nondestructive, noninvasive method to understand the diet previous to and following periods of fasting or starvation. For example, the $\delta^{13}C$ of exhaled CO_2 from cockroaches raised on different diets continued to reflect the differences in those diets, even as the $\delta^{13}C$ of the exhaled CO_2 decreased during food deprivation as the cockroaches switched from metabolizing food to burning endogenous lipids for energy (Miller et al. 1984). In another study, pigeons whose lipid stores were laid down while being fed a C_4 diet were then switched to a C_3 diet. On fasting their $\delta^{13}C_{breath}$ switched from the C_3 signal of the current diet to a C_4 signal, revealing the isotopically different diet that the birds had previously eaten and that this previous diet was recorded in the lipid stores (Hatch et al. 2002a). Thus the isotopic signal of the food the animal was eating when lipid stores were laid down can be revealed in the exhaled CO_2 when those lipid stores are metabolized for energy.

20.3.4 Understanding Metabolic Pathways of Starvation or Fasting

Breath tests have become an important part of medical research and clinical diagnosis. Breath tests have been used to examine such things as hepatic function (Baker et al. 1983), the hydrolysis of various starches (Hiele et al. 1990), metabolism of lipids and fatty acids (Demmelmair et al. 1997; McClean et al. 1993), and the presence of the ulcer causing bacterium *helicobacter pylori* (Conti-Nibali et al. 1990; Delvin et al. 1999; Logan 1998). The use of isotopically labeled substrates in breath tests is becoming increasingly important to studies of animal physiology and ecology (Ayliffe et al. 2004; Passey et al. 2005; Sponheimer et al. 2006; Starck et al. 2004). They promise to shed much light on how organisms route nutrients and the importance of nutrient routing to their ecology and life history (McCue 2010, 2011 ; McCue et al. 2011). Breath tests can

be particularly useful for understanding metabolic pathways of fasting individuals. Tracers labeled with ^{13}C can be administered to individuals. The metabolism of these substrates then becomes apparent with an increase in the $\delta^{13}C$ of the exhaled CO_2 of the individual (Duchesne et al. 1982; Lacroix et al. 1982; Schoeller et al. 1984).

SIA of exhaled CO_2 proved an important tool in understanding the metabolism of lipid chylomicrons and chylomicron remnants (CR) in the blood of normal and obese subjects. A protein-free, ^{13}C-labeled lipid emulsion was injected intravenously into the subject. Increased δ^{13} CO_2 in the breath provided evidence of the metabolism and clearance of CR. Obese mice on normal diets showed impaired CR metabolism when compared with controls, but when put on a restricted diet for 6 weeks such that they experienced weight loss they metabolized CR similarly to the controls. Breath tests showed that CR metabolism occurred along mitochondrial pathways but was markedly lower in obese mice under normal dietary conditions, while restricting their diets increased the rate of metabolism of CR for these obese rodents (Martins and Redgrave 2004).

SIA and breath testing isotopically labeled substrates has also helped identify how fasting affects the metabolism of exogenous nutrients. McCue et al. (2011) showed that after fasting, zebra finches (*Taeniopygia guttata*) fed ^{13}C-labeled glucose, leucine, or palmitic acid metabolized these substrates more slowly than did finches that had not fasted. On refeeding, fasted birds metabolized exogenous glucose more slowly than fed birds. They metabolized leucine about half as fast and palmitic acid one-fourth as fast as fed birds did. These differences were correlated with average losses of 10% body mass, 35% of liver and intestine mass, and an average decrease in body temperature of 2.3°C. These responses suggest that either the previously fasted finches were routing the exogenous nutrients to replenish endogenous stores at a greater rate than the fed birds, or that reductions in the digestive tract caused by fasting decreases their ability to absorb or metabolize food after fasting. During migration the digestive tract of birds may atrophy substantially (Hume and Biebach 1996; McWilliams and Karasov 2001, 2005) (see also Bauchiner and McWilliams, Chap. 12). The ability to route exogenous nutrients to more rapidly replace endogenous stores would be advantageous to migrating birds, allowing them to recover more quickly at stopover sites. However, given gut atrophy, it seems more likely that migrating birds may have a decreased ability to absorb nutrients after fasting. Decreased absorption would adversely affect the rebuilding of stores during feeding at stopover sites. Indeed, it appears that migrating birds may compensate by increasing retention times of digesta (Bauchinger et al. 2009). However, studies following the nonoxidative fates of exogenous nutrients after fasting are required to determine whether the nutrients are being routed to replenish stores, or merely assimilated more slowly (McCue et al. 2011).

Similarly, isotopically labeled tracers have even been used to determine whether corals conserve nitrogen during periods of starvation. Given the relative paucity of nutrients in tropical waters, Piniak and Lipschultz (2004) hypothesized that tropical corals would retain more endogenous nitrogen than temperate corals.

They also tested the hypothesis that nutritional stress and differential catabolism would have different effects on the biochemical pools of host and zooxanthellae. To do this, they acclimated tropical corals (*Oculina diffusa*) and congeneric temperate corals (*Oculina arbuscula*) to three different feeding regimes (i.e., fed, starved, or starved and supplemented with inorganic nutrients). They found no difference between fasted and fed corals in the distribution of ^{15}N among the coral and zooxanthellae biochemical pools. This result was consistent with the hypothesis that corals and their symbionts conserve nitrogen during periods of starvation. The hosts can preferentially catabolize photosynthetic products during periods of starvation, leaving such biochemical pools as lipids or proteins the same in all treatments. They attributed the absence of differences between temperate and tropical corals to the fact that the corals were congeners (Piniak and Lipschultz 2004).

20.3.5 Traditional Diet Analysis

Of course, SIA can be used to understand diet and the ecology of starvation, especially when combined with more traditional techniques, such as stomach flushing. As such, it can contribute importantly to the understanding and conservation of endangered populations and species. For example, yellow-eyed penguins (*Megadyptes antipodes*) are the world's most endangered species of penguin, and the low recruitment of their small populations appears to be due to starvation of their chicks (Browne et al. 2011). Stomach flushing revealed small, low energy meals almost exclusively of blue cod (*Parapercis colias*). However, SIA revealed that opal fish (*Hemerocoetes monopterygius*) were as important to the diet of the chicks as the blue cod. The fact that many stomachs were empty and that stomach flushing provides only an instantaneous view of the diet led to a small sample size and ultimately overlooked the importance of opal fish to the diet of the chicks. SIA of blood and feather tissue provided a time integrated view of the diet and allowed for a larger sample size, revealing the importance of opal fish to the diet of the nutritionally stressed chicks (Browne et al. 2011). While these advantages of SIA are well known, this study shows how dietary SIA can be applied to questions of starvation as it relates to management and conservation biology.

20.4 Conclusions

Stable isotope analysis is proving to be a valuable tool for understanding the physiology and ecology of starving, fasting, and otherwise nutritionally stressed animals. Much effort has been focused on using SIA of ^{15}N and to a lesser degree ^{13}C as an index of nutritional stress. While some studies suggest that this is a possibility, numerous studies detect no effect of starvation, fasting, or nutritional stress on the $\delta^{15}N$ or $\delta^{13}C$ of tissues. Others have found that tissues were depleted, rather than enriched as a result of nutritional stress. Where enrichment of ^{15}N or ^{13}C has been detected, the effect has often been $<1‰$. Given the large number

of potential confounding factors and the fact that a number of these confounding factors can cause changes in $\delta^{15}N$ or $\delta^{13}C$ >1‰, the usefulness of an index of nutritional stress based on either ^{15}N or ^{13}C enrichment seems unlikely. Rather, it seems more important to understand how starvation, fasting, or nutritional stress might affect other inferences based on SIA of tissues. One exception to this may be SIA of excreta, but too few studies exist to be certain.

It has been stated that "animals that are fasting or starving do appear to 'live on their own meat' (Waterlow 1968) and consequently exhibit ^{15}N enrichment, while animals that are malnourished do not," and that a threshold may exist (Kempster et al. 2007). SIA of tissues may provide a means of physiologically drawing a line between a state of malnourishment and a state where undernourishment reaches the point of protein catabolism. This would explain why some studies find an effect of fasting, starvation, or nutritional stress on $\delta^{15}N$ values of tissues and others detect no effect. Although, no study has been published that explicitly tests this hypothesis, this is a potentially valuable avenue of research.

SIA shows considerably more promise as a tool for understanding how starvation and nutritional stress affect the use of endogenous and exogenous nutrients. Studies of "capital breeders" demonstrate the potential of SIA to reveal how nutrients are routed during periods of starvation or nutritional stress, either to tissue turnover, new tissue growth, or reproductive effort. Breath analysis, in particular, is a powerful and underemployed tool for understanding substrate use and oxidation during periods of fasting, starvation, and nutritional stress, especially when used in combination with labeled substrates (McCue et al. 2011). While most studies have used naturally occurring enrichments to do this, the use of labeled substrates in such studies is powerful and will increase in the future (McCue 2011). I repeat the call of others (Gannes et al. 1997; Martinez del Rio et al. 2009) for more studies that will test current assumptions, validate currently developing methods, and explore further use of SIA in the study of starvation, fasting and nutritional stress.

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Chapter 21
Fearing the Danger Point: The Study and Treatment of Human Starvation in the United Kingdom and India, c. 1880–1974

Kevin Grant

21.1 Introduction

The Irish militant trade unionist, James Connolly, was on hunger strike in a Dublin gaol for 7 days in September 1913. Having been arrested for leading a labor strike by Dublin transport workers, he declared that he would starve himself to death unless the British government released him. His daughter recalled, "We had had no experience of the effect of hunger-strike….At that time some strange stories were told of the effect it could have upon the brain and parts of the body after only a few days." Fearful for her husband's life, Mrs. Lillie Connolly made a personal appeal to the viceroy, Lord Aberdeen, who released her husband and gave the couple the use of the viceregal carriage to convey themselves from the gaol to a friend's home (MacEoin 1980). The Connollys' uncertainty about the effects of hunger striking is surprising, given the regular media coverage of some 200 hunger strikes by British and Irish militant suffragists over the previous 3 years. In fact, Irish militant suffragists were the inspiration for Connolly's hunger strike (Anderson 1994).

The anxiety about hunger striking was typical of not only strikers and their supporters, but also prison medical officers and private physicians in Great Britain, Ireland, and India. Experts in the burgeoning field of nutritional science recognized the limitations of their understanding of starvation, a fact that Francis Benedict, Director of the Nutrition Laboratory in Boston, attributed "to the difficulty of securing willing human subjects" (Benedict 1907). Prison medical officers were especially preoccupied with a stage of starvation on which researchers had cast no light. This was the so-called "danger point" at which every starving person could presumably suffer a sudden, deadly collapse.

K. Grant (✉)
Hamilton College, 198 College Hill Road, Clinton, NY 13323, USA
e-mail: kgrant@hamilton.edu

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21.2 The Need for a New Science

This chapter recognizes the distinction between involuntary starvation and voluntary fasting for purposes such as health, religion, or politics (*sensu* McCue 2010). By the end of the nineteenth century, the study of starvation by chemists and physiologists had been underway for at least 75 years (see also Lignot and Le Maho, Chap. 2), but no studies of any consequence had examined subjects of involuntary starvation, despite the opportunities presented by desperate poverty in the United Kingdom and famines in Ireland and India. There had been influential dietary studies of the British poor, and of prisoners and soldiers in the United Kingdom and India, but these mainly addressed the effects of under-nutrition on labor productivity and socio economic stability (Arnold 1993; Carpenter 1994, 2006; Hall-Matthews 2008). The recognition of under-nutrition as a measure of poverty was established by Seebohm Rowntree in his study of the working class in York, *Poverty*: *A Study of Town Life*, published in 1901. Following the revelation that many British men were physically unfit for service in the South African War (1899–1902), the British government established the Inter-Departmental Committee on Physical Deterioration, which laid the groundwork for new programs, such as free school meals, to improve the nutrition of British children (Vernon 2007). None of these projects, however, addressed the physiological underpinnings or the clinical symptoms of starvation.

21.3 Starvation: A Protein Deficiency

Nutritional scientists in the early twentieth century regarded protein as the most important nutrient, and they accordingly treated starvation as, primarily, a protein deficiency. They believed that starvation became acute after the body exhausted its stores of carbohydrates and fats in the production of heat and energy, then turned to the combustion of muscle, its essential store of protein, for survival. Although they studied amino acids as components of protein, they did not fully appreciate their nutritional value until the 1920s. There was speculation about vitamins, but the isolation and laboratory synthesis of vitamins did not occur until the 1920s and 1930s. In the golden age of nutritional science between the wars, scientists recognized that deficiencies in amino acids and vitamins were more critical than protein deficiency in starvation (Hutchison 1948). However, as Ancel Keys observed, "…Extremely little was known in detail about either chronic starvation or relief feeding" (Keys 1946). This changed during and soon after the Second World War, when scientists examined starvation with new urgency to save the starving populations of occupied Europe (Simmons 2008). The scientists found that starvation was primarily a calorie deficiency rendered deadly in diverse ways by other factors ranging from diseases to environmental exposure. The new science of starvation would progressively influence medical practice in subsequent

decades, particularly in the treatment of famine victims and war refugees. In the case of hunger strikes in British prisons, however, it appears to have been slower to influence medical officers, who continued to cast a wary eye toward the "danger point."

Research on the effects of starvation on metabolism began in 1825 with a study of the urea output of an insane patient who fasted for 18 days. Chemists and physiologists then conducted intermittent studies of fasting humans and numerous studies of starving animals until the end of the nineteenth century, when a series of important studies examined the physiologies and metabolisms of individual "hunger artists" (Benedict 1907). The understanding of starvation as a protein deficiency was based on the work of Justus von Liebig, who asserted in the 1840s that protein was "the only true nutrient," because it provided not only for the growth and maintenance of the body, but also, as fuel, for the body's work (Carpenter 2003a). At the turn of the twentieth century, nutritional scientists believed that the body more efficiently digested animal proteins than proteins from grains (gluten) and green vegetables (albumen) (Carpenter 2006). Researchers over the next 20 years came to understand that there was no distinction to be drawn between animal and vegetable proteins, but they and physicians generally maintained a strong preference for animal protein in feeding starving people (Carpenter 2003b, 2006; Lusk 1928).They recognized that carbohydrates and fats were mainly responsible for the body's production of heat and energy, but they believed that only protein could restore tissue lost in starvation. They understood, furthermore, that in the long run even a protein-rich diet had to be replaced by a properly proportioned "mixed diet" that included the other three major groups of nutrients: carbohydrates, fats, and minerals. Carl von Noorden acknowledged that, as of 1907, there were no studies to explain why a "mixed diet" was critical to health, but, he noted, "simple clinical experience forms a sufficient guide" (Von Noorden 1907).

21.4 A New Paradigm

The tenets of contemporary nutritional science are found in Robert Hutchison's *Food and the Principles of Dietetics*, which, after its publication in 1900, went through 10 editions and was reprinted 20 times by 1950. Hutchison, a physician at the London Hospital, initially compared the body to a steam engine, stating that, "The building material of food corresponds to the metal of which the engine is constructed, the energy-producers to the fuel which is used to heat the boiler. Where the body differs from the engine is that it is able to use part of the material of its construction (proteid) for fuel also." He divided nutrients into two categories: organic and inorganic. In the former category, he placed nitrogenous nutrients (e.g. proteins and albuminoids) and non-nitrogenous nutrients (e.g. carbohydrates and fats). In the latter category, he placed minerals and water. Tissue formation depended on the combination of proteins, "mineral matters," and water. "Work

and heat" were produced by proteins, albuminoids, carbohydrates, fats, and "maybe" minerals and water. As proteins could produce both tissue and energy, Hutchison hailed their "physiological omnipotence" (Hutchison 1911).

Hutchison's description of the body as a potentially self-consuming engine and his description of its nutritional fuel reflected the prevailing scientific understandings of the physiology and metabolism of starvation. Researchers understood that glycogen metabolism precedes protein metabolism in the absence of ingested food and that the store of glycogen is generally sufficient for 1 day, after which the body continues to burn fat and protein as necessary (Lusk 1906). They had observed that the breakdown of fat produces acetone bodies, which are excreted in measurable quantities by the kidneys and detectable in the smell of a fasting person's breath (Von Noorden 1907). They understood that the breakdown of protein increases the excretion of nitrogen, and particularly urea, through the kidneys (Carpenter 2006). Studies further suggested that the human body could regulate its metabolism in response to either prolonged under-nutrition or starvation (Lusk 1906)."The longer the fast [lasts] the smaller [is] the protein decomposition," Von Noorden explained. "The protein [is] spared, the less valuable fat [is] sacrificed" (Von Noorden 1907).

Researchers suspected that the body increased protein metabolism after its fat stores were exhausted, but there were no long-term studies of humans to confirm this (Von Noorden 1907). They believed that advanced starvation places excessive strain on the heart, leading to the most common, immediate cause of death by starvation: organ failure (Lusk 1906). They observed that women lost 20–30% less nitrogen than men during the early stages of starvation, probably due to their greater percentage of fat, but they did not then speculate that women were likely to endure starvation longer than men. In fact, prison medical officers worried intensely after 1909 that female British and Irish militant suffragist hunger strikers would suffer heart attacks. Why female strikers should have been more vulnerable to heart failure than male strikers is not clear. Von Noorden emphasized, "No long and systematic investigations have been made on women, though satisfactory data for single days of starvation have often been published" (Von Noorden 1907).

21.5 Progression of Symptoms

Researchers recognized that the clinical symptoms of starvation developed in stages. In the first week, there were stomach pains and dizziness. Also, the tongue became furred, which they regarded as a general sign of declining health. After the second week, the subject's capacity for physical excursion decreased and the skin became waxen and blotchy. The subject's temperature dropped; a sign that the body was exhausting its fat. Starving people commonly complained of cold, and it was clear that a cold environment worsened their condition. In the absence of citrus juice, subjects eventually developed scurvy. All researchers recognized that starving people suffered psychological difficulties, most commonly depression.

Throughout the process of starvation, the subject would lose weight, but more quickly in the beginning than later, due to the initial loss of water and fat (Von Noorden 1907) (see also Hall, Chap. 22). Researchers observed that the starving human body did everything in its power to avoid compromising the brain and nervous system. Some hunger strikers remained lucid for several weeks. Even after more than 70 days on hunger strike in 1920, Terence MacSwiney, an Irish republican, rejected offers of food as he slipped in and out of consciousness.

These findings were of limited use to most physicians who actually treated starving people. From their standpoint, critical questions remained unanswered. What was the percentage of weight loss that normally precipitated death? How long could a person normally survive starvation? They did not know the amount or precise kind of food necessary to sustain patients who were markedly emaciated. Moreover, in the absence of long-term empirical studies, they had little confidence in their knowledge of women's physiological and metabolic responses to starvation (Von Noorden 1907). They knew of cases in which people had lived for 6 to 8 weeks without food, but they did not treat these cases as normative (Benedict 1907; Roose 1890). Von Noorden remarked tellingly, "The extent to which loss of tissue can proceed, and the degree of emaciation that can be tolerated for long periods, is really surprising" (Von Noorden 1907). Although no one was sure how to anticipate the "danger point," experimentation on animals suggested that the temperature of a starving human might drop dramatically a few days before death (Lusk 1906; Roose 1890) (see also Hohtola, Chap. 10). Physicians generally agreed that the levels of acetone and urea in the subject's urine were probably the best indicators of imminent demise. The "danger point" supposedly followed a decrease in acetone, indicating the exhaustion of fat stores, and an increase in urea, indicating the greater combustion of protein. It was then marked decisively by a drop in urea that indicated the exhaustion of protein stores and the reduction of the patient to skin and bones.

21.6 A Social Awakening

Before the late nineteenth century, there were advocates of nutritional science and statistics who attempted to reform the treatment of starving people in the United Kingdom and India (Vernon 2007). Until the twentieth century, however, the British public, policy makers, and imperial officials did not generally address starvation in clinical terms, but as a look. This look was skeletal and "hollow-eyed"; it transformed its victims into "phantoms" (Kennedy 1908). In published exposés, one finds starvation represented through visual impressions of bodily decay and suffering. In a series of articles entitled "Starving London," published in *The Globe* newspaper in February 1886, Krausse found confirmation of a women's sad story "…in the presence of [her] gaunt and hungry-looking children" (Krausse 1886). Krausse was working within a well-established *genre* of literature by middle-class "social investigators" who explored Britain's poverty-stricken,

working-class neighborhoods after the midnineteenth century (Vernon 2007). Starvation was a common theme in these works, often represented metaphorically, rather than clinically, as a symptom of unemployment. Likewise, sympathetic British witnesses of famine victims in India identified starvation in predominantly visual, impressionistic terms, though this time the source of suffering was drought. During a terrible famine in India in 1899–1900, Vaughn Nash, writing for the *Manchester Guardian*, recounted "a procession of the most pitiful phantoms, some thirty of them, starved beyond belief, their lips drawn back over their teeth, their eyes burning with fever in their deep-sunk sockets" (Nash 1900).

British officials who managed famines in Ireland and India in the Victorian era proved far more interested in principles of political economy than dietetics (Hall-Matthews 2008; O'Grada 1999). This began to change in India after 1880, when the British regime initiated a uniform famine code. Political economy remained paramount, but the regime also began to use research on Indian prison diets to establish a standard dietary for famine victims (Arnold 1993). In response to widespread starvation, the Government of India, in cooperation with missionaries, set up "famine camps" to which Indians were required to travel for aid. In the camps, people labored for wages that corresponded to the current market value of the foods contained in an official scale of rations. Wages and rations were graduated in accordance with the strength and physical requirements of each person, and allowances were made for nonworking members of a working person's family. The Reverend J. E. Scott, chairman of one of the missionary relief committees, explained, "The code defines rations that are to be given to certain classes—as the full ration, for the able-bodied; the minimum ration, for weakly laborers and adult dependents; the penal ration, for those refusing to work; the proportional rations, for children of various ages and requirements" (Scott 1904).

The dietary scales of the Indian famine code were determined by the availability of foods, the rules of religion and caste, and the government's determination to spend as little money as possible to provide famine victims with the minimal nutrition required for health. The dietary scales also reflected the influence of nutritional science. See, for example, the scales issued by the Government of India in 1883 (Table 21.1). The "full ration" provided protein through flour or rice, pulse, and vegetables. It provided carbohydrates mainly through the flour or rice, and it provided fat through *ghee* (clarified butter) or oil. The salt combined with protein to form tissue, and it also enabled, in Hutchison's words, "the proper constitution of fluids"(Hutchison 1911). The scales reflected proportional differences between the nutritional requirements of men, women and children, and the different requirements of those who labored and those who were idle. While there were debates over the size of the rations, no one disputed that the dietary scales provided starving people with the four major nutrients (i.e. proteins, carbohydrates, fats, and minerals). The "penal diet" offered virtually no fat, and arguably inadequate protein and carbohydrates, but this was, as the name suggested, a punitive diet for allegedly able-bodied people who refused to work.

There was no clinical diagnosis involved in the administration of these dietary scales. British famine relief officers implemented policy in accordance with the

Table 21.1 Dietary scales of the Government of India, 1883, presented in Scott (1904)

	For a man (oz)	For a woman (oz)	For children
Full ration			
Flour or cleaned rice	1 lb. 8	1 lb. 4	3/4, 1/2, or 1/4, of ration, according to age and requirements
Pulse	4	4	
Salt	1/2	1/2	
Ghee or oil	1	1/2	
Condiments and vegetables	1	0	
Minimum ration			
Flour or cleaned rice	1 lb.	14	3/4, 1/2, or 1/4, of ration, according to age and requirements
Pulse	2	2	
Salt	1/8	1/8	
Ghee or oil	1/8	1/8	
Condiments and vegetables	1/2	1/2	
Penal ration			
Flour, grain, or rice	14	12	Not stated
Pulse	1	1	
Salt	1/4	1/4	

look of famine victims, a method that regularly failed in cases of advanced starvation. The Reverend J.E. Robinson commented, "The best efforts prove futile in numberless instances" (Scott 1904). For all the recognizable symptoms of starvation, the British had difficulty in seeing the "danger point," particularly in the many cases aggravated by diseases such as cholera and dysentery. Starvation remained both ghastly and mysterious, its victims waiting pathetically for a lurking death to cut them down. In the absence of any quantitative, diagnostic measure of starvation, the "danger point" appeared perpetually imminent. Scott recalled, "The people suddenly fall in the midst of conversation, and rapidly sink" (Scott 1904).

21.7 Fasting and Starvation in Prisons and Asylums

In Britain, doctors in insane asylums had the most experience in treating starvation. It was not uncommon for inmates to starve themselves. Doctors in asylums rarely worried about the "danger point," but instead employed forcible feeding, generally by stomach tube or sometimes in the form of a "nutrient enemata" administered by rectum (Hutchison 1911). Forcible feeding was less common in prisons, but by no means remarkable. Between 1904 and 1909 there were at least 82 men and 30 women forcibly fed in British prisons; one male prisoner was forcibly fed for over 2 years (Home Office 1909). In most of these cases, the prisoners were deemed insane; in all of them the prisoners had been convicted of ordinary crimes. The increasing use of hunger striking by prisoners after 1909 presented medical officers with a new dilemma in three respects. First, most

strikers had been convicted of politically motivated offences, and their treatment in prison was monitored closely by vocal supporters on the outside. Parliamentary advocates of the militant suffragists, for example, provoked controversy by likening forcible feeding to torture. Second, many strikers were from the "respectable classes," and doctors proved far more reluctant to coerce them into eating than they did working-class prisoners. Finally, for these reasons and others, government officials often forbade the forcible feeding of political strikers, thus prolonging starvation (Grant 2011).

Prison medical officers and government officials in Britain, Ireland, and India were fearful of failing to anticipate the "danger point" in caring for hunger strikers. This was largely due to their worry that the death of a striker in their care would open them to a charge of manslaughter (Grant 2006). The first suffragette hunger striker, Marion Wallace Dunlop, was released after just 91 h for fear that she would be seriously harmed or killed by starvation. Doctors agreed that a fast of 24 h would do no harm, but they continually debated how soon forcible feeding should begin after 48–72 h of fasting. It appears that they subscribed to the belief among researchers that katabolism generally began on the second day, after stores of glycogen were exhausted in the first 24 h. From this forward point, doctors watched intently for signs of "sudden collapse."

The standard criteria of diagnosis for a hunger striker is presented in reports on the suffragette Harriet Kerr by a prison medical officer in June 1913. The officer evaluated her mental state at the outset of her strike on 18 June, describing her as "somewhat eccentric". On a daily basis he checked her pulse and heart beat and evaluated the quality of her sleep. On 21 June he noted the "odour of malnutrition" on her breath, the smell of acetone. Two days later he observed that she had lost 10 pounds and was experiencing pain in her limbs. On the following day, she had "gastric pains," her tongue became "furred," and again he noted the "odor of malnutrition." He wrote, "I consider it would be inexpedient to detain her after tomorrow morning as she is an elderly woman [aged 54] and might have a sudden collapse" (Home Office 1914). Kerr was released on 25 June, after a strike of 7 days.

21.8 Emerging Diagnoses and Treatments

In November 1919, as militant republican hunger strikes proliferated in Ireland during the Anglo-Irish War (1919–21), the Governor of Mountjoy Prison sent to the General Prisons Board of Ireland an excerpt from a medical officer's journal that listed the main indicators of the "danger point." According to the officer, temperature was not a significant factor, unless it indicated fever. The "compressibility" of the pulse could indicate declining health, but the pulse rate was not significant. The officer observed, "Acetonaemia may be expected about the end of the third or on the fourth day. It is generally accompanied by mental hebetude and is a sign that the body tissues are being used up." Furthermore, he warned,

"A heart which responds to normal demands when it is nourished normally may be either fibrous or fatty and only reveals the state post mortem." He finally noted that hunger striking tended "to wake up latent disease" (Governor of Mountjoy Prison 1919). The officer's journal illuminates a gap between the scientific study of starvation and the actual, medical treatment of starvation in prisons. Medical officers did not have the option of evaluating urine samples, so they could not measure the excretion of urea. More remarkable, however, is the silence regarding the prisoner's weight. Given that there were no guidelines on weight loss and mortality, it is, nonetheless, striking that emaciation was not a main indicator of the approaching "danger point." Apparently, a person need not be a phantom to starve to death.

In Britain and Ireland, hunger strikers were generally fed a liquid mixture of milk, eggs, beef, and brandy. There were variations, with an item removed or added. Glucose was a common supplement, and in cases of advanced starvation doctors gave strikers citrus juice to prevent scurvy. Prison medical officers mainly attempted to combat starvation with protein, which, in combination with minerals and water, was presumably the only nutrient that could restore tissue. Milk, a source of both protein and fat, appears to have been included in almost every forcible feeding, along with brandy. Brandy, taken in moderation, was believed to have multiple benefits. It was a well-known *digestif* and a mild anasthetic. Hutchison explained that, in cases of disease, the "volatile ethers" of brandy could have "a most valuable stimulating influence on the exhausted brain and heart" (Hutchison 1911). It was also believed to relieve hunger pangs in the stomach. When Kerr was released after her week-long hunger strike, she was given a shot of brandy before being placed in a cab (Home Office 1914). The feeding of long-term strikers did not change significantly over the next 30 years. When the Irish republican David Fleming ended his 19-day hunger strike in June 1946, prison doctors gave him brandy, milk, and glucose (New York Times 1946).

Very few hunger strikers or their supporters demonstrated a clear understanding of the process or potential duration of starvation. In November 1923, Ernie O'Malley, an Irish republican striker, wrote to a friend, "I really thought I should not last more than twelve days and here I am on my 22nd." Four days later he reflected, "Two weeks ago the doctor gave me two days to live." On the 35th day of his strike, he wrote, "…I really thought I would be dead before the 21st." He then asked his friend to speak with a previous hunger striker, Mary MacSwiney, Terence MacSwiney's sister, and ask her to outline for him the symptoms of advanced starvation. "…Or would it be better for me not to know," he wondered (English and O'Malley 1991).

Hunger strikers learned to anticipate at least some features of starvation. O'Malley asked for lemons (English and O'Malley 1991). He understood that citrus juice would help him to fend off scurvy, though of course he was not aware of Vitamin C, which was not isolated until 1932. Strikers knew that they would become cold, so they requested warm blankets. They knew that they would suffer bed sores, so they requested air beds or water mattresses—and sometimes received them (English and O'Malley 1991; Home Office 1920). Experienced strikers were aware of the dangers of an inappropriate, excessive diet in recovering from a long-term strike (MacEoin 1980). They knew that starvation often entailed depression

or other mental and emotional problems that threatened to undermine morale. Sheila Humphreys, an Irish republican, recalled the failure of a mass strike in which she participated for 31 days in 1923: "We were flattened….The tinted trappings of our fight were hanging like rags about us" (MacEoin 1980).

Two cases of self-starvation in custody in India in the 1930s offer perspective on British diagnostic measures of starvation and changing ideas regarding the dietary rules of forcible feeding between the wars. The first case, reported in *The Lancet* in 1936, involved a 19-month hunger strike by a convict, Munshi Khan, in the central prison of Bareilly, United Provinces. According to Basil Rosair, a medical officer of 15 years' experience who was then superintendent of the prison, Khan remained on strike between May 1934 and January 1936. Rosair ordered occasional forcible feedings until July 1934, when he began a regular regimen of feedings that continued until the strike ended. Having begun his strike at 130 pounds, Khan reached a low point of 85 pounds in December 1934, after which his weight fluctuated between 85 and 105 pounds, depending, Rosair noted, "on the quality and quantity of liquid food given." The diet consisted of various combinations of the following: milk, soup, raw eggs, *mung dal*, barley water, Glucose D-Roboleine, orange juice, cod-liver oil, and olive oil. The progressive influence of nutritional science can be seen in Rosair's advice to other doctors: "In feeding hunger-strikers it is important to avoid vitamin deficiency and to remember the value of fats and carbohydrates compared with proteins." Within 6 weeks of ending his strike, Khan had regained almost all of his weight. "…I have often had to order and conduct forcible feeding", Rosair stated, "and I am convinced that there are no permanent ill-effects either upon mental activity or muscular strength" (Rosair 1936).

The fasts of Mohandas Gandhi are especially instructive, because he cooperated with the government's doctors by providing urine samples. Gandhi undertook his "Epic Fast" on 20 September 1932 over the issue of the representation of *dalits* (untouchables) in provincial legislatures. He declared that he was ready to "fast unto death." His secretary, Pyarelal Nayyar, recalled, "Great anxiety was felt when Gandhiji commenced his fast whether he would be able to stand the physical strain of it for any length of time. For one thing, he was not the same man as he was when he undertook his twenty-one days' fast at Delhi in 1924 [at age 55]" (Pyarelal 2007). On the 6th day of the fast the doctors stated, "The disturbing features are that both acetone and urea contained in his urine have increased, the latter by 1.5%. We are definitely of opinion that this portends entry into the danger zone." For the benefit of Indian legislators who had inquired about Gandhi's condition, Gilder explained,

> …The words 'Danger Zone'…we have used in this sense that Mahatmaji [Gandhi] had not got much fat reserve, he has used that up; and now he is living really on his muscles and apart from an accident like a sudden collapse or a stroke of paralysis that may intervene at any time, we are of the opinion that he has now entered into that stage of his illness where every day increases the danger and where, even if the fast is broken, some danger will still remain (Bombay Legislative Council 1932).

Gandhi broke his fast on the same evening by taking a small amount of citrus juice, after all parties agreed to his demands.

21.9 A New Perspective

The understanding of starvation was advanced in the era of the Second World War by a series of scientific studies. The most influential of these was a 9-month study of the "semi-starvation" and rehabilitation of 32 male volunteers, all conscience objectors, at the University of Minnesota in 1944 (Keys et al. 1950). Led by Ancel Keys, the researchers sought to replicate the famine diets of people in occupied Europe. Most of the findings correspond with the scientific and medical understandings of starvation in the early twentieth century, but there are also two major differences. First, Keys and his colleagues discounted the role of protein in general, and, second, they determined that deficiencies in amino acids and vitamins were not primary factors in starvation. They determined that starvation was fundamentally a calorie deficiency. Contradicting long-standing medical practice, Keys stated, "In relief feeding, calories are of overwhelming importance" (Keys 1946). Researchers and physicians had been moving toward these findings in the 1930s, but the war provided Keys and others with a terrible opportunity to establish these findings through studies of unprecedented length and scope. Their work contributed to the improvement of famine and refugee relief programs in the new era of the United Nations, especially in the creation of standardized, nutritious rations (New York Times 1968).

Keys' study did not address total starvation, and it did not offer guidance to the "danger point," which was of the greatest concern to physicians managing hunger strikers. The limited influence of the new science of starvation on hunger strikes is reflected in the experience of Dolours and Marian Price, sisters who were imprisoned in England in 1973 for their roles in an Irish republican bombing campaign. The two conducted a hunger strike of 205 days, during which they were forcibly fed approximately 400 times. Marian Price recalls that after prison guards tied her into a chair and pried open her mouth, the medical officer inserted a rubber stomach tube down her throat. Then, according to Price, "They throw whatever they like into the food mixer—orange juice, soup, or cartons of cream if they want to beef up the calories. They take jugs of this gruel from the food mixer and pour it into a funnel attached to the tube" (Breen 2004). Although the apparent emphasis on calories, rather than protein, reflected the new science of starvation, the fact remains that the same concoction might have been fed to a striker in the 1920s, with a shot of brandy.

21.10 Escaping the "Danger Point"

The continuing, illusive nature of the "danger point" was perhaps never made so clear as in early June 1972, during a hunger strike of several weeks by republican prisoners in Northern Ireland. One of the strikers, Robert Campbell, had been convicted of armed robbery in 1971 and had served less than 1 year of his 11-year

sentence. He seemed to be nearing a deadly collapse, so prison officials transferred him to the nearest intensive care unit at Mater Hospital in Belfast. The doctors would not allow police or paratroopers into the unit, so gards were posted outside the doors. Campbell jumped out of a first-floor window, into a waiting Ford Cortina, and made good his escape (Northern Ireland Office 1972). For all of the scientific research on starvation by 1972, a hunger striker could still feign the "danger point." As in 1919, prison medical officers, and even hospital physicians, believed that a person need not be a phantom to starve to death. Campbell recognized this, and disappeared.

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Chapter 22
Quantitative Physiology of Human Starvation: Adaptations of Energy Expenditure, Macronutrient Metabolism and Body Composition

Kevin D. Hall

22.1 Introduction

On October 19, 2003, magician David Blaine emerged from a clear plastic box that had been suspended 40 feet over the River Thames in London after 44 days of self-imposed starvation. His weight had been trimmed by 24.5 kg and his blood and urine showed the tell-tale signs of prolonged starvation (Jackson et al. 2006; Korbonits et al. 2005, 2007), despite the skepticism of much of his worldwide audience. In a world where one of the most pressing health issues is the rise of a global obesity epidemic (Swinburn et al. 2011), it is understandable how such prolonged fasting would be met with disbelief by a mostly well-fed western world.

Mr. Blaine's fast was a recent addition to a long history of 'hunger artists' who starved professionally for entertainment. This phenomenon reached its peak of popularity in the late nineteenth and early twentieth centuries and was popularized in the 1922 short story *A Hunger Artist* by Franz Kafka (see also Lignot and LeMaho, Chap. 2). Prolonged starvation has also been used as a form of protest by 'hunger strikers' who have occasionally died as a result (Leiter and Marliss 1982) (see also Grant, Chap. 21). Of course, famine is the most common historical cause of human starvation which continues to the present day despite the fact that the world wastes an enormous amount of food (Hall et al. 2009; Stuart 2009).

Prolonged periods of food scarcity were likely frequent occurrences over the course of human evolution (Prentice 2005) and have resulted in complex physiological adaptations that allow humans to survive for extended periods between feeding opportunities (Chakravarthy and Booth 2004). Modern scientific investigation of human starvation began in the late nineteenth and early twentieth

K. D. Hall (✉)
Laboratory of Biological Modeling, National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD, USA
e-mail: kevinh@niddk.nih.gov

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centuries with the most comprehensive being Francis Gano Benedict's 1912 study of Agostino Levanzin's 31-day fast in Boston (Benedict 1915). In this chapter, I will simulate Benedict's classic starvation experiment using a recently developed computational model of human metabolism (Hall 2006, 2010). In particular, I will use the model simulations along with Benedict's data to illustrate the coordinated metabolic response to starvation with a focus on the dynamic changes of energy expenditure, metabolic fuel utilization, body weight, and body composition. This chapter will also illustrate how the greatly expanded energy stores of obese people can allow them to survive for remarkably long periods of starvation.

22.2 Computational Model of Human Metabolism

Maintaining life and performing physical work requires energy and the body derives its energy from the controlled combustion of three macronutrients: carbohydrate, fat, and protein. These macronutrients are obtained from the diet with about 50% of the energy derived from carbohydrate, 35% from fat, and 15% from protein (Austin et al. 2011). However, these average diet proportions can vary widely from person to person and also from day to day. Complex physiological mechanisms maintain normal functioning of the body despite marked fluctuations of diet quantity and composition. These mechanisms are required to operate both in periods of food surplus as well as scarcity.

While the molecular, cellular, and physiological mechanisms underlying the regulation of human metabolism and body weight are exceedingly complex, the whole-body system obeys thermodynamic laws that constrain its dynamics in ways that make the overall system amenable to mathematical modeling (Chow and Hall 2008). For example, the first law of thermodynamics requires that energy is conserved and therefore changes in the body's energy content must be associated with an imbalance between the rate of food energy intake and the rate of energy expenditure. Similarly, macronutrient imbalances between dietary intake and metabolic utilization underlie changes in stored fat, glycogen, and protein and result in changes in the body composition.

I recently developed a mechanistic computational model of human macronutrient metabolism and body composition change (Hall 2006, 2010). The model was designed to quantitatively track the metabolism of all three dietary macronutrients and their interactions within the human body. The main model assumptions were that energy must be conserved and that changes in the body composition result from imbalances between the intake and utilization rates of fat, carbohydrate, and protein along with intracellular and extracellular fluid changes. The model was developed using published human data from over 50 experimental studies and was the first to model all three dietary macronutrients and accurately simulate the metabolic responses to various diets in a wide variety of subject groups, including lean and obese men and women.

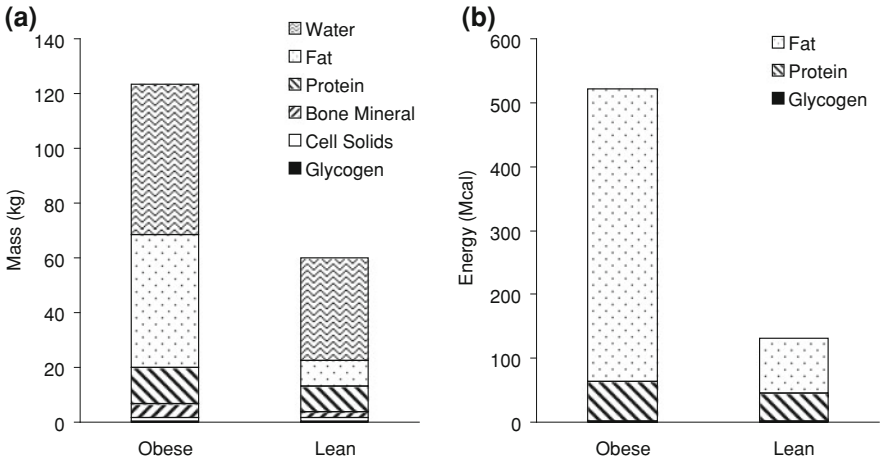

Fig. 22.1 **a** The chemical composition of an obese man (*left*) differs from that of a lean man (*right*) primarily as a result of the increased fat mass. **b** The body energy stores are greatly expanded in the obese man as a result of the increased body fat mass

The computational model predicts how diet changes result in adaptations of whole-body energy expenditure, metabolic fuel selection, and various whole-body metabolic fluxes (e.g., lipolysis, lipogenesis, gluconeogenesis, ketogenesis, protein turnover, etc.) that ultimately give rise to changes in body weight and composition. I previously used the computational model to better understand the metabolic changes that take place during prolonged semi-starvation and re-feeding (Hall 2006), exemplified by the classic Minnesota experiment of Keys (Keys 1950). In this chapter, I present the first simulation of the quantitative changes in macronutrient metabolism and body composition that take place during complete starvation in lean and obese humans.

22.3 Human Body Composition

Figure 22.1a illustrates the chemical composition of an average obese male along with the composition of the lean subject of Benedict's prolonged fasting study. Water is typically the greatest body component of both obese and lean men, but obesity is characterized by a greatly expanded body fat mass that can become the majority body constituent with morbid obesity. The absolute masses of body protein and bone mineral are also increased in obesity, but to a much lesser extent. Glycogen and cellular solids, such as potassium and nucleic acids, contribute a small fraction of the body's overall mass.

Body fat, protein, and glycogen comprise the stored energy of the body and these stores must be mobilized when the diet is insufficient to meet the body's

energy requirements. Figure 22.1b illustrates the composition of the body in terms of its energy content with fat stored in adipose tissue providing the overwhelming majority of the available stored energy, especially in obesity. Despite dietary carbohydrate providing the majority of the body's energy demands on a daily basis, glycogen represents a relatively insignificant store of energy ($\sim$2,000 kcal). Thus, the transition from fed to fasted states must coincide with a substantial shift of metabolic fuel utilization away from carbohydrate oxidation and toward fat oxidation. Otherwise, glycogen could only provide enough fuel to survive for a few days. Body protein represents a substantial amount of energy, but in humans it is not a storage pool in the same sense as adipose tissue triglyceride (compare with Bauchinger and McWilliams, Chap. 12). Rather, body proteins are functionally important and cannot be depleted by a significant fraction without serious complications and death. In contrast, fat stores represent a considerable energy reserve and body fat can be depleted to very low levels without substantial functional impairments (Friedl et al. 1994; Leiter and Marliss 1982).

22.4 Body Weight Loss During Starvation

A continuous supply of energy is required to maintain life and perform physical work. When dietary intake of macronutrients is insufficient to provide for the energy needs of the body, the deficit is taken from the stored energy pools and weight loss ensues as these storage pools decrease in size. Figure 22.2a shows the time course of weight loss during Levanzin's 31-day fast (closed circles) as well as the average weight loss in 18 obese men (open circles) observed by Runcie et al. (Runcie and Hilditch 1974) over the course of a 30-day fast. The computational model simulations of body weight change are shown in the curves and agree reasonably well with the data despite the fact that no model parameters were adjusted and these data were not used in model development (Hall 2006, 2010).

Over the first week of the fast, weight loss was rapid as a result of significant body water decrease. This water loss was the result of decreased glycogen, which binds $\sim$3 grams of water per gram of glycogen, as well as loss of extracellular water to maintain sodium homeostasis in the absence of dietary sodium. Following the rapid water loss, weight was lost linearly over the remaining weeks at similar rates in both the lean and obese subjects. However, the rate of body fat loss was substantially greater in the obese ($\sim$1.4 kg per week) compared to the lean man ($\sim$1 kg per week). The increased rate of fat loss in obesity was the result of the greater energy deficit elicited by the fast since the obese man had a higher energy expenditure rate (Fig. 22.2b) and the greater resulting energy deficit during the fast was met with increased mobilization of energy stores from body fat.

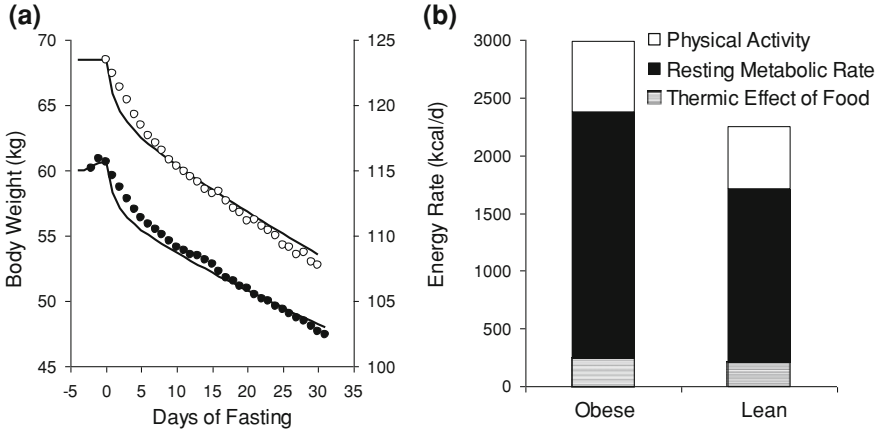

Fig. 22.2 **a** Weight change during starvation in Benedict's lean subject (●, *left axis*) and the average weight of obese subjects during starvation (○, *right axis*). The curves correspond to the computational model simulations. **b** Components of the baseline energy expenditure rate in the average obese man (*left*) and the lean man (*right*)

22.5 Energy Expenditure

22.5.1 Components of Energy Expenditure

Figure 22.2b illustrates three components of human energy expenditure at baseline in relatively sedentary lean and obese men. The obese man required several hundred additional kcal/d to maintain his weight compared to the lean man. Typically, the smallest component of the total energy expenditure rate is the thermic effect of food (sometimes also called 'diet induced thermogenesis' or 'specific dynamic action') defined as the increase of metabolic rate observed for several hours following the ingestion of a meal. The thermic effect of food is believed to represent the energy cost of digestion and absorption as well as the storage and metabolic fate of dietary macronutrients (Westerterp 2004). While the precise mechanisms underlying the thermic effect of food are not understood, there is a clear dietary macronutrient hierarchy in the magnitude of the metabolic rate increase after feeding, with protein causing a greater increment than carbohydrate which is greater than that of fat (reviewed in McCue 2006).

The resting metabolic rate corresponds to the energy expended by the body when not performing physical work and typically is the largest contribution to the total energy expenditure. Contrary to popular belief, obese people generally have higher absolute resting metabolic rate compared to lean people (Fig. 22.2b). The main contributor to the resting metabolic rate is the fat-free mass of the body which is elevated in obesity along with the increased adipose tissue mass which also contributes to their increased resting metabolic rate. The linear relationship

between resting metabolic rate and fat-free mass is identical in obese and lean people (Cunningham 1991; Weyer et al. 1999). This means that the elevated resting metabolic rate in obesity is in line with what is expected for their body composition.

Physical activities typically involve locomotion and the energy costs are determined by the duration and intensity of physical activity in proportion to the overall body weight. Thus, obese and lean people can have similar daily energy costs for physical activity (Fig. 22.2b) despite obese people typically being less active. With weight loss, it costs less energy to perform most physical activities and therefore the energy expended for physical activity typically decreases with starvation unless the quantity or intensity of physical activity increases to compensate.

22.5.2 Measurement of Energy Expenditure by Indirect Calorimetry

Human energy expenditure is typically measured using indirect calorimetry where oxygen consumption, carbon dioxide production, and nitrogen excretion provide quantitative measurements of the macronutrient oxidation rates that supply the body's energy needs (Bursztein et al. 1989). Oxidation of dietary macronutrients yields chemical energy and Hess's law states that the energy released is the same regardless of whether the macronutrients are combusted in a bomb calorimeter or catabolized via the oxidative phosphorylation in the cellular mitochondria. Thus, the energy released from oxidation of macronutrients in the body is identical to that measured using direct calorimetry in the laboratory. However, there is an important caveat. Not all macronutrients in food are completely absorbed by the body. Furthermore, the dietary protein that is absorbed does not undergo complete oxidation in the body, but rather produces urea and ammonia. In accounting for these effects, the 'metabolizable' energy content of carbohydrate, fat, and protein is slightly less than the values obtained by bomb calorimetry (Livesey 1984; Livesey and Elia 1988).

The theoretical basis of indirect calorimetry relates oxidation of carbohydrate, fat, and protein and the heat produced by these processes to the measured oxygen consumption, carbon dioxide production, and nitrogen excretion as follows:

$$1\text{ g Carbohydrate} + 0.831\text{ L } O_2 \rightarrow 0.831\text{ L } CO_2 + 0.6\text{ g } H_2O + 4.2\text{ kcal}$$

$$1\text{ g Fat} + 2.03\text{ L } O_2 \rightarrow 1.43\text{ L } CO_2 + 1.09\text{ g } H_2O + 9.4\text{ kcal}$$

$$1\text{ g Protein} + 0.966\text{ L } O_2 \rightarrow 0.782\text{ L } CO_2 + 0.45\text{ g } H_2O + 0.16\text{ g N} + 4.7\text{ kcal}$$

Therefore, the volumes of oxygen consumed and carbon dioxide produced (VO_2 and VCO_2, respectively) are primarily determined by the amount of

carbohydrate, fat, and protein oxidized. Also, nitrogen excretion, N, is determined by protein oxidation. Therefore, we have the following three equations for the quantities measured via indirect calorimetry in terms of the carbohydrate, fat, and protein oxidized in grams (C, F, and P, respectively):

$$VO_2(L) = 0.831(L/g) \times C(g) + 2.03(L/g) \times F(g) + 0.966(L/g) \times P(g)$$

$$VCO_2(L) = 0.831(L/g) \times C(g) + 1.43(L/g) \times F(g) + 0.782(L/g) \times P(g)$$

$$N(g) = 0.16 \times P(g)$$

These three linear equations for C, F, and P can be solved in terms of the VCO_2, VO_2 and N measurements:

$$C(g) = 4.07(g/L) \times VCO_2(L) - 2.87(g/L) \times VO_2(L) - 2.58 \times N(g)$$

$$F(g) = 1.67(g/L) \times VO_2(L) - 1.67(g/L) \times VCO_2(L) - 1.92 \times N(g)$$

$$P(g) = 6.25 \times N(g)$$

Therefore, the total energy expended, E, is the sum of the energy released during the oxidation of carbohydrate, fat, and protein:

$$E(kcal) = 4.2(kcal/g) \times C(g) + 9.4(kcal/g) \times F(g) + 4.7(kcal/g) \times P(g)$$

$$E(kcal) = 3.745(kcal/L) \times VO_2(L) + 1.285(kcal/L) \times VCO_2(L) + 0.484(kcal/g) \times N(g)$$

While other metabolic processes, such as de novo lipogenesis and gluconeogenesis, also contribute to respiratory gas exchange, their relatively minor contribution is typically ignored for calculation of the total energy expenditure rate.

22.5.3 Dynamics of Energy Expenditure During Starvation

The curves in Fig. 22.3a illustrate the computational model simulations and the open and closed circles represent the measured daily oxygen consumption and carbon dioxide production rates, respectively, during the 31-day fasting experiment of Benedict. Both the model and the data show the characteristic decline of respiratory exchange which translates into a decrease of daily energy expenditure shown as open circles in Fig. 22.3b. By measuring respiratory exchange rates when the subject was at rest, the indirect calorimetry equations provide a measure of resting metabolism. The closed circles in Fig. 22.3b illustrate that the measured resting metabolic rate also slows as the fast progresses in agreement with the computational model simulation.

The mechanism for the slowing of resting metabolism during the fast can only partially be explained by the reduction in body weight and metabolically active

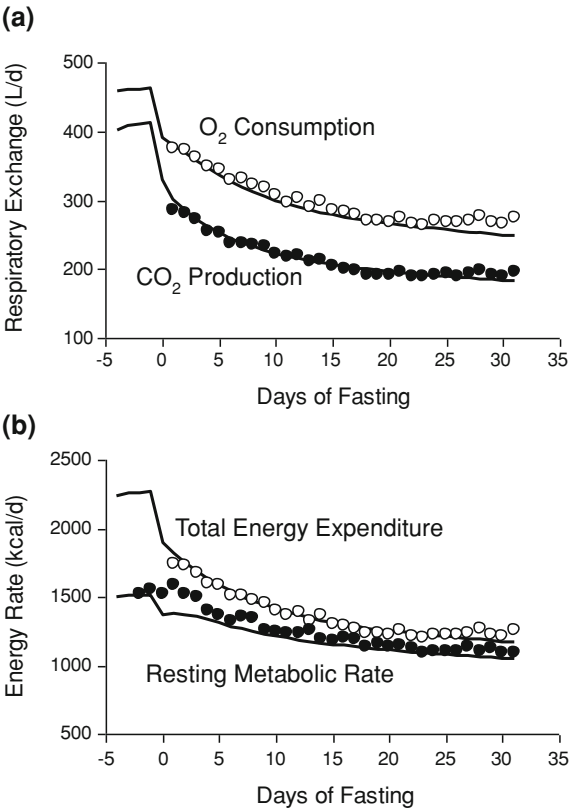

Fig. 22.3 **a** Oxygen (O_2) consumption rate (○) and carbon dioxide (CO_2) production rate (●) measured in Benedict's lean subject during starvation. The curves illustrate the computational model predictions. **b** Measured total energy expenditure rate (○) and resting metabolic rate (●) along with the computational model predictions (*curves*)

lean tissue mass. Decreased flux through energy-requiring metabolic pathways (e.g., de novo lipogenesis and protein synthesis) also contributes to reduced energy expenditure with starvation, but the observed reduction is greater than can be explained based on these changes (see also McCue et al., Chap. 8). Rather, a metabolic adaptation to the dietary energy deficit appears to take place that further slows metabolic rate and this energy savings can amount to several hundred kcal/d (Doucet et al. 2003, 2001; Leibel et al. 1995). The mechanistic basis for such an adaptive decrease of thermogenesis is unknown, but may involve reduced sympathetic tone, thyroid activity, and decreased circulating leptin (Rosenbaum et al. 2000; Weinsier et al. 2000).

It makes evolutionary sense to improve the energy efficiency of the body during periods of food scarcity to optimize the survival time until the next feeding opportunity. Our teleological expectation is that greater energy stored in body fat (Fig. 22.1b) should allow for greater starvation survival time in obesity (see Sect. 22.8) and the requirement for metabolic slowing would not be as critical for survival in the obese. Therefore, we might expect that the magnitude of metabolic slowing would be attenuated by the body fat mass. However, the computational

model predicts that the magnitude of the metabolic slowing is proportional to the decrease of energy intake from baseline (Hall 2006, 2010). Since baseline energy intake is typically greater in obese versus lean people, this means that the model predicts a greater adaptive metabolic slowing during starvation in the obese. While much work remains to properly validate this model prediction, I have previously demonstrated that the model accurately predicts metabolic adaptations to underfeeding in both lean and obese people (Hall 2006, 2010). If the model predictions are correct, this suggests that there has been little evolutionary pressure to attenuate the metabolic slowing based on the body fat mass—much to the dismay of obese dieters.

22.6 Metabolic Fuel Selection and Mobilization

Regulation of metabolic fuel selection plays a prominent role in the physiological adaptation to starvation. For example, while carbohydrate is the majority macronutrient of the western diet, only a few days worth of glycogen is stored in the body which necessitates dramatically decreasing carbohydrate oxidation shortly after the onset of starvation and switching to fat and protein metabolism to provide the metabolic fuels of the body. Thus, a coordinated metabolic response ensues during starvation to increasingly draw energy from fat stores to provide the bulk of the body's energy requirements.

The ratio of carbon dioxide production to oxygen consumption provides an index of metabolic fuel selection since this ratio, called the respiratory quotient (RQ), is equal to 1 for pure carbohydrate oxidation and 0.7 for pure fat oxidation. Thus, the value of the RQ provides an objective estimate of the metabolic fuel mix. Figure 22.4a shows the simulated and measured RQ during the Benedict experiment and indicates the prominence of carbohydrate oxidation prior to the fast and a rapid switch to fat oxidation over the first several days of fasting.

Figure 22.4b illustrates the simulated rates of carbohydrate, fat, and protein oxidation in the Benedict experiment as the fast progresses. These shifting macronutrient oxidation rates over the course of the fast are determined by the altered fuel delivery rates from the carbohydrate, fat, and protein reserves stored in the body tissues. In the first days of starvation, circulating glucose and insulin concentrations decrease, glucagon levels increase, and glycogen is mobilized from the liver and skeletal muscle. As glycogen depletes, glycogenolysis decreases (Fig. 22.5a). The decrease of insulin results in an increase in adipose tissue lipolysis (Fig. 22.5a) thereby resulting in increased mobilization of free fatty acids and glycerol (see also Price and Valencak, Chap. 15). The glycerol is transformed into glucose via gluconeogenesis in the liver and kidneys which also use some amino acids (alanine in particular) as gluconeogenic substrate (Fig. 22.5c). Increased gluconeogenesis ensures that the decrease of circulating glucose is not so severe as to seriously impair brain functioning which primarily relies on glucose as its metabolic fuel in the fed state and in the initial days of starvation.

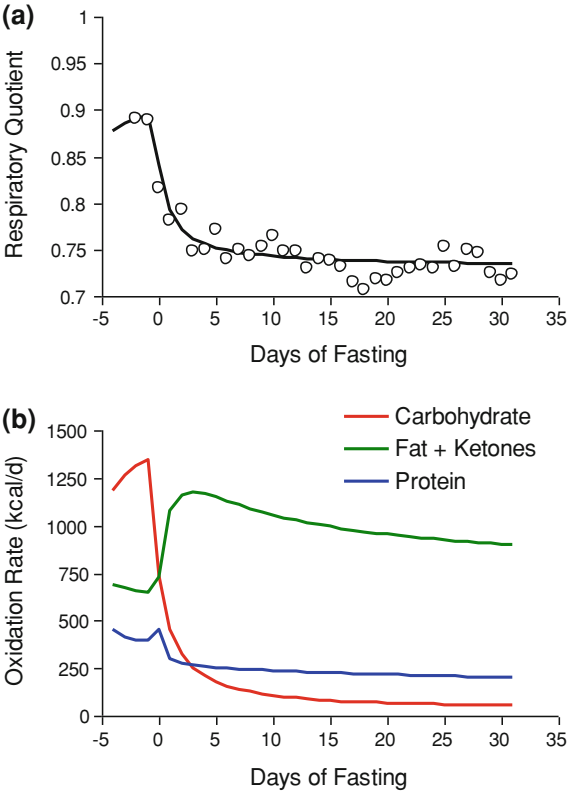

Fig. 22.4 **a** Measured respiratory quotient (○) in Benedict's subject during starvation along with the computational model prediction (*curve*). **b** Computational model predictions of the rates of carbohydrate, fat, ketone, and protein oxidation during starvation

The augmented adipose tissue lipolysis increases free fatty acid supply to the liver which, in the context of low circulating insulin, results in an increase in ketogenesis over the first week of starvation (Fig. 22.5c). Apart from glucose, ketones are the only major fuel source for the brain and the vast majority of the ketones produced by the liver are oxidized. However, as the circulating ketone levels exceed the renal threshold a small fraction are spilled in the urine as shown in the curve in Fig. 22.5d along with the corresponding data from Benedict's subject. These coordinated hormonal and metabolic adaptations to starvation were unknown to Benedict at the time of his fasting experiment. Fifty years later, the classic work of George Cahill and colleagues led the way in elucidating these regulatory mechanisms of human starvation (reviewed in (Cahill 1970, 2006)) that were simulated by the computational model.

22.7 Macronutrient Imbalance and Body Composition Change

During starvation, the macronutrient utilization rates described above define the net macronutrient imbalances depicted in Fig. 22.6a. The fat imbalance contributed the majority of the energy imbalance following the first few days of the fast

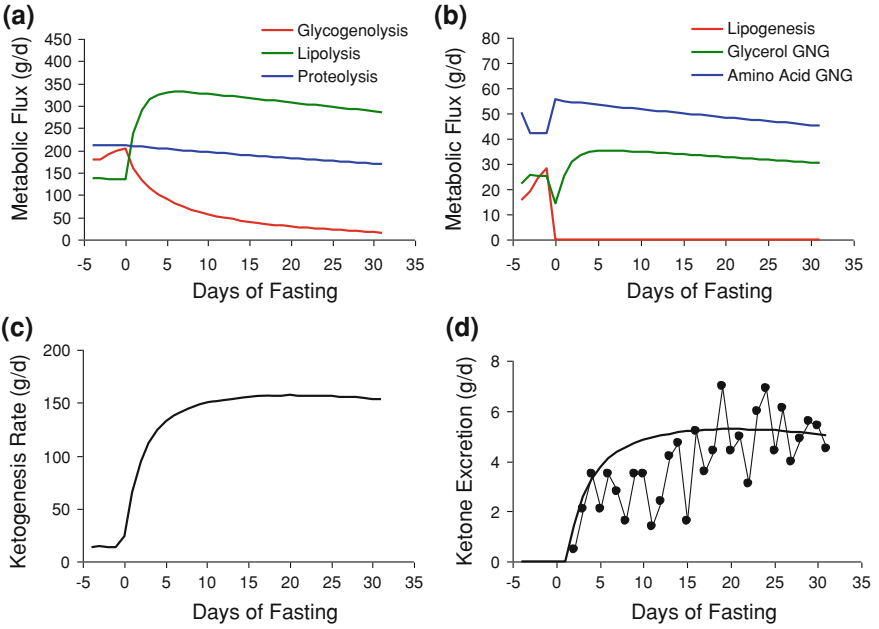

Fig. 22.5 **a** Computational model simulations of whole-body glycogenolysis, lipolysis, and proteolysis during starvation in Benedict's lean subject. **b** Simulated rates of de novo lipogenesis and gluconeogenesis (GNG) from glycerol and amino acids. **c** Simulated ketone production rate during starvation. **d** Measured ketone excretion rate (●) along with the computational model simulation (*curve*)

whereas the carbohydrate imbalance was transient and the protein imbalance was modest but relatively sustained over time. These imbalances were accounted for by decreases of stored glycogen, fat, and protein in the body and Fig. 22.6b illustrates the predicted changes over the course of Levanzin's fast.

About 3 grams of intracellular water are bound to each gram of stored glycogen, and about 1.6 grams of water are bound per gram of protein. These intracellular water changes, along with the extracellular fluid shifts corresponding to maintenance of sodium homeostasis, resulted in the model predicted the fat-free mass changes shown in Fig. 22.6c. Interestingly, while most of the energy imbalance was accounted for by fat, and fat loss was quantitatively larger than the losses of body protein or glycogen, more fat-free mass was lost than fat mass as a result of body water changes. The vast majority of the extracellular water loss occurred within the first several days of the fast and was the major contributor to the early loss of fat-free mass and rapid initial weight change. Thereafter, both fat mass and fat-free mass decreased approximately linearly.

Figure 22.6d illustrates the predicted average fat mass and fat-free mass changes in the fasting obese men investigated by Runcie. Similar to the Benedict data, fat-free mass had a rapid initial reduction due to extracellular fluid loss followed by a linear phase of parallel loss fat-free mass and fat mass. About 1.8 kg more fat mass

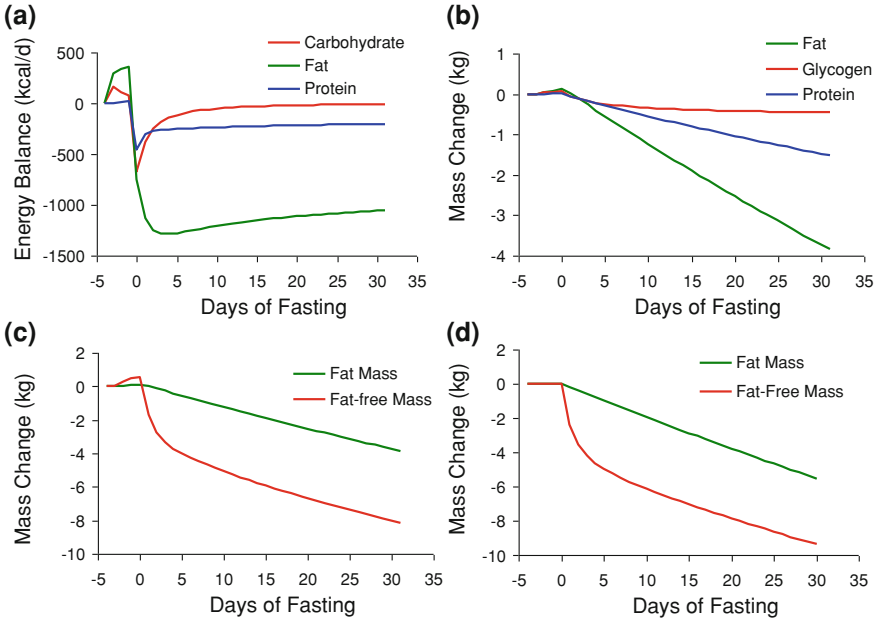

Fig. 22.6 **a** Daily net macronutrient imbalances simulated by the computational model in Benedict's starving lean subject. **b** Simulated changes of stored body glycogen, protein, and fat in Benedict's subject. **c** Simulated changes of body fat and fat-free masses in Benedict's subject. **d** Simulated body composition changes in the average obese subject during starvation

was lost in the obese subjects compared to Benedict's lean subject requiring an additional cumulative energy imbalance of 17,000 kcal. This amounts to more than 500 kcal/d corresponding to the increased energy expenditure of the obese men.

22.8 Limits of Prolonged Starvation

Of course, starvation cannot proceed indefinitely without severe physiological repercussions. The survivable length of starvation has been attributed primarily to the body fat reserves and the ability of these reserves to spare functionally important body protein. For a period of time in the 1960s and 1970s, starvation became a popular treatment for obesity and regularly involved fasts of many weeks (Drenick et al. 1964; Thomson et al. 1966). The longest known period of therapeutic fasting was published in 1973 when a 27-year-old man weighing 207 kg fasted continuously for a period of 382 days losing 125 kg (Stewart and Fleming 1973). Clearly, such a prolonged fast would be impossible for a lean subject such as Levanzin since his body contained insufficient energy stores. As body fat becomes depleted, the only remaining fuel source is the functionally important

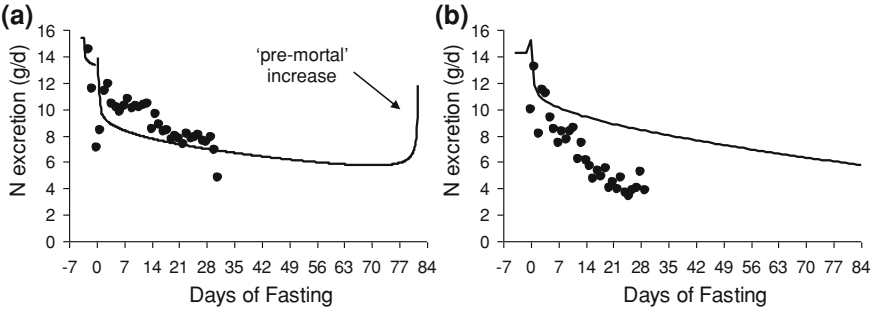

Fig. 22.7 **a** Measured nitrogen excretion rate (●) during starvation in Benedict's lean subject. Model simulation of an extended 82-day period of starvation reveals a predicted premortal increase of nitrogen excretion (*curve*) indicating the physiological limits of starvation. **b** Average nitrogen excretion during starvation in obese men (●) illustrating greater nitrogen sparing compared to the model simulations (*curve*) with no indication of a premortal increase of protein catabolism after 82 days

body protein, whose mobilization results in a classic tell-tale sign of impending death—the 'premortal rise' of nitrogen excretion (Lusk 1976).

Figure 22.7a depicts the simulated nitrogen excretion in Levanzin's fast showing a reasonable agreement with the measurements. Extending the simulated fast to 82 days when the body fat becomes depleted resulted in a premortal rise of nitrogen excretion coinciding with a rapid increase in body protein catabolism to meet energy needs. This simulation suggests that 82 days was the upper limit of a survivable fast for such a lean subject (Fig. 22.7a). Of course, many other factors could claim such a person's life well before this upper limit, and people engaged in hunger strikes have certainly died on a more abbreviated time frame (Leiter and Marliss 1982). Figure 22.7b illustrates a simulated 82-day fast in obese men and shows that nitrogen excretion remained low since body fat was still far from being depleted in these men. Interestingly, the measured average nitrogen excretion over the 30-day fast was significantly lower than the model simulations, suggesting that the current computational model does not completely simulate the nitrogen sparing mechanisms observed in obese subjects (see also Harlow, Chap. 17). Addressing this apparent model deficiency will be a subject of future work.

22.9 Summary

The computational model of human macronutrient metabolism highlighted in this chapter simulates the myriad of metabolic adaptations that occur in response to starvation, including the rapid switch from predominantly carbohydrate oxidation toward fat oxidation and a concomitant increase of ketone production to supply the brain with its energy needs. The model also illustrates how metabolic rate slows

out of proportion to weight loss during starvation and thereby reduces the body's overall energy needs—an adaptation that works to oppose the weight loss that is so often a desired in an increasingly obese world.

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Chapter 23
Alternate Day Fasting: Effects on Body Weight and Chronic Disease Risk in Humans and Animals

Krista A. Varady

23.1 Introduction

Excess adiposity, particularly visceral adiposity, appears to be a key factor for the development of certain chronic diseases, such as cardiovascular disease and type 2 diabetes (Mangge et al. 2010; Matsuzawa 2008). Excess visceral fat is generally accompanied by elevated blood pressure, increased triglycerides, reduced HDL cholesterol, and/or elevated fasting plasma glucose concentrations (Kishida et al. 2011). Dietary restriction regimens are often recommended as a first line of therapy for the reduction of body weight and visceral fat mass. The most common form of dietary restriction employed is traditional calorie restriction (CR) (Fontana 2008). CR regimens generally involve reducing energy intake by 15–40% of needs daily. However, since many obese individuals find it difficult to restrict calories everyday, an alternative diet strategy, termed alternate day fasting (ADF), was created. ADF regimens generally involve a 24 h period of complete or partial fasting (termed the 'fast day') alternated with a 24 h period of ad libitum feeding (termed the "feed day"). Since the ADF approach allows individuals to eat ad libitum every other day, adherence to these protocols is significantly increased when compared to CR (Varady et al. 2011a, b, c). The effects of ADF on body weight and chronic disease risk indicators have yet to be summarized. Accordingly, the present review examines the ability of ADF regimens to facilitate weight loss in human and animal models. The ability of this fasting regimen to reduce the risk of certain chronic diseases, such as cardiovascular disease and type 2 diabetes will also be discussed.

K. A. Varady (✉)
Department of Kinesiology and Nutrition,
University of Illinois at Chicago, Chicago, IL, USA
e-mail: varady@uic.edu

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23.2 Effect of ADF on Body Weight and Visceral Fat Mass

23.2.1 Animal Studies

Only two animal studies (Varady et al. 2009a, b, 2010) have examined changes in both body weight and visceral fat mass by ADF (Table 23.1). Both of these studies (Varady et al. 2009a, b, 2010) demonstrate significant reductions in body weight after only 4 weeks of diet. In the study by Varady et al. (2010), the degree of weight loss by ADF was similar to that of CR, suggesting that these regimens may produce comparable degrees of weight loss in mice. In the other study by Varady et al. (2009a, b), similar degrees of weight loss were attained in mice consuming an ADF-high fat diet when compared to mice consuming an ADF-low fat diet. As such, ADF may improve body weight over a wide range of percent fat intake, which (if translatable to humans) might enhance subject tolerability and compliance with this fasting regimen (Varady et al. 2009a, b). Reductions in visceral fat mass were also noted in both of these studies (Varady et al. 2009a, b, 2010; Table 23.1). Interestingly, these decreases in visceral fat were accompanied by marked increases in subcutaneous fat (Varady et al. 2009a, b, 2010). Thus, ADF may result in a preferential redistribution of body fat from visceral to subcutaneous compartments (Varady et al. 2009a, b, 2010). Whether these effects can be reproduced in humans still requires confirmation, however. It will also be useful to determine whether these quantitative changes in fat allocation are associated with qualitative changes in fat composition (see Price and Valencak, Chap. 15).

23.2.2 Human Trials

Six studies (Halberg et al. 2005; Heilbronn et al. 2005; Johnson et al. 2007, Varady et al. 2009a, b, 2011a, b, c) to date have examined the effect of ADF on body weight reduction in humans (Table 23.1). Findings from these trials reveal that this diet is able to produce significant reductions in body weight after relatively short trial durations (i.e., 2–12 weeks). For instance, after only 3 weeks of diet, adult subjects decreased their body weight by 4% in the study by Heilbronn et al. (2005). When treatment duration was extended to 8–12 weeks, further reductions in body weight were demonstrated (6–8% weight loss) (Johnson et al. 2007; Varady et al. 2009a, b, 2011a, b, c). These trials (Halberg et al. 2005; Heilbronn et al. 2005; Johnson et al. 2007; Varady et al. 2009a, b, 2011a, b, c) suggest that implementing ADF for short periods of time may help individuals lose significant amounts of weight. Future studies examining the long-term effects of this diet (>24 weeks) on body weight are well warranted. Moreover, trials that examine whether or not individuals are able to maintain weight loss by employing ADF will also be of great interest. As for changes in visceral fat mass, only three recent trials (Varady et al. 2009a, b, 2011a, b, c) have examined the impact of the diet on this body

Table 23.1 Studies examining the effect of alternate day fasting on body weight and visceral fat mass

Animal studies

Reference	Animal model	Trial length (weeks)	Intervention groups[b–d]	Effect of ADF on body weight (% change)	Effect of ADF on visceral fat mass (% change)
Varady, 2010 (Varady et al. 2010)	$n = 18$ C57BL6 mice Age 2 months	4	1. ADF ($n = 6$) 2. 25% CR ($n = 6$) 3. Control ($n = 6$)	↓[a]	↓[a]
Varady, 2009 (Varady et al. 2009a, b)	$n = 18$ C57BL6 mice Age 2 months	4	1. ADMF–High fat diet ($n = 6$) 2. ADMF–Low fat diet ($n = 6$) 3. Control ($n = 6$)	↓[a]	↓[a]

Human trials

	Subjects	Trial length (weeks)	Intervention groups[b–c]	Effect of ADF on body weight (% change)	Effect of ADF on visceral fat mass (% change)
Halberg, 2005 (Halberg et al. 2005)	$n = 8$, M Normal weight Age 25 ± 1 years	2	1. ADF ($n = 8$)	↓1	–
Heilbronn, 2005 (Heilbronn et al. 2005)	$n = 16$, MF Normal weight Age 32 ± 2 years	3	1. ADF ($n = 16$)	↓4[a]	–
Johnson, 2007 (Johnson et al. 2007)	$n = 10$, MF Obese Age not specified	8	1. ADMF ($n = 10$)	↓8[a]	–

(continued)

Table 23.1 (continued)

	Subjects	Trial length (weeks)	Intervention groups[b–c]	Effect of ADF on body weight (% change)	Effect of ADF on visceral fat mass (% change)
Varady, 2009 (Varady et al. 2009a, b)	$n = 16$, MF Obese Age 46 ± 2 years	8	1. ADMF ($n = 16$)	↓6[a]	↓4[a]
Varady, 2010 (Varady et al. 2010)	$n = 14$, MF Obese Age 40 ± 2 years	12	1. ADMF ($n = 14$)	↓8[a]	↓10[a]
Varady, 2010 (Varady et al. 2011a, b, c)	$n = 11$, MF Normal weight Age 52 ± 2 years	12	1. ADMF ($n = 11$)	↓7[a]	↓8[a]

ADF alternate day fasting, *ADMF* alternate day modified fasting, *CR* calorie restriction, *F* female, *M* male

[a] Posttreatment values significantly different ($P < 0.05$) from baseline values within the ADF group

[b] Alternate day fasting (ADF) regimen: Fed ad libitum for 24 h on the feed day, alternated with 24 h fast on the fast day

[c] Alternate day modified fasting (ADMF) regimen: Fed ad libitum for 24 h on the feed day, alternated with 75% energy restriction on the fast day

[d] Control group: Fed ad libitum daily

composition parameter (Table 23.1). In all of the three aforementioned studies subjects were calorie restricted by 75% of their needs on the fast day, and were permitted to eat ad libitum on the feed day. After 8–12 weeks of diet, visceral fat mass was reduced by 4–10% from baseline, and decreases in visceral fat were positively associated with body weight loss (Varady et al. 2009a, b, 2011a, b, c). For example, the most potent reduction in visceral fat mass (10%) was attained in the trial that achieved the greatest amount of weight loss (8%) (Varady et al. 2011a, b, c), whereas the smallest reduction in visceral fat (4%) was noted for the study with the least amount of weight loss (6%) (Varady et al. 2009a, b). A key limitation of these studies is that each one implemented dual-emission X-ray absorptiometry (DXA) to assess visceral (trunk) fat mass. The DXA technique is frequently employed in clinical trials to measure abdominal fat mass because it is cost-effective and easy to perform. However, this method only provides an estimate of fat contained in the trunk, and does not allow for an accurate quantification of actual visceral fat mass (Park et al. 2002). Therefore, more accurate techniques (i.e., magnetic resonance imaging; MRI) will need to be employed in future trials of ADF to assess the true impact of this diet on changes in regional fat distribution.

23.3 Effect of ADF on Cardiovascular Disease Risk

23.3.1 Animal Studies

To date, five studies (Ahmet et al. 2005, 2010; Krizova and Simek 1996; Mager et al. 2006; Wan et al. 2003) have been performed that examine the impact of ADF on cardiovascular health in animal models (Table 23.2). In the study by Mager et al. (Mager et al. 2006), reductions in heart rate were observed after 16 weeks of ADF in Sprague–Dawley rats. Similar effects on heart rate were also shown by Wan et al. (2003) after 24 weeks of treatment. In both of these studies, treatment-induced decreases in systolic and diastolic blood pressure were observed after 4 weeks, and persisted throughout the remainder of the trial (Mager et al. 2006; Wan et al. 2003). Improvements in circulating lipid levels have also been demonstrated with ADF diets in rodent models (Krizova and Simek 1996). After 8 weeks of ADF, both total cholesterol and triglyceride levels decreased in mice (Krizova and Simek 1996). Cardiac myocyte response to myocardial infarction (MI) induction has been studied by Ahmet et al. (2005). MI was induced by coronary artery ligation after 12 weeks of ADF or control ad libitum diet in Sprague–Dawley rats (Ahmet et al. 2005). At 24 h after MI induction, the number of apoptotic myocytes in the affected area was 4-fold reduced, and the size of the MI was 2-fold smaller in the ADF group when compared to the ad libitum fed controls (Ahmet et al. 2005). A distinct reduction in neutrophil infiltration was also demonstrated, suggesting a decrease in inflammatory response (Ahmet et al. 2005). While the majority of animal studies report cardio-protective findings for ADF,

Table 23.2 Studies examining the effect of alternate day fasting on cardiovascular disease risk

Animal studies

Reference	Animal model	Trial length (weeks)	Intervention groups[c, e]	Body weight	Effect of ADF on CVD risk parameters
Krizova, 1996 (Krizova and Simek 1996)	$n = 30$ C17/B1-10 mice Age 2 months	8	1. ADF–Low fat diet ($n = 10$) 2. ADF–High fat diet ($n = 10$) 3. Control ($n = 10$)	↑[a] (both groups)	Total cholesterol: ↓[a] (both groups) Triglycerides: ↓[a] (both groups)
Ahmet, 2005 (Ahmet al. 2005)	$n = 60$ SD rats Age 2 months	12	1. ADF ($n = 30$) 2. Control ($n = 30$)	↓[b]	Myocardial infarction induced (week 12) 24 h after MI: Myocardial infarction size: 2 × smaller[b] Apoptotic myocytes number 4 × less[b] Inflammatory response after MI: ↓[b]
Mager, 2006 (Mager et al. 2006)	$n = 12$ SD rats Age 2 months	16	1. ADF ($n = 6$) 2. 40% CR ($n = 6$)	∅	Heart rate: ↓[a] Blood pressure: ↓[a]
Ahmet, 2010 (Ahmet et al. 2010)	$n = 40$ SD rats Age 4 months	24	1. ADF ($n = 20$) 2. Control ($n = 20$)	↓[b]	Diastolic dysfunction: ↑[a] Cardiac reserve: ↓[a]
Wan, 2003 (Wan et al. 2003)	$n = 24$ SD rats Age 3 months	24	1. ADF ($n = 8$) 2. 2DG suppl ($n = 8$) 3. Control ($n = 8$)	↓[b]	Heart rate: ↓[b] Blood pressure: ↓[b]

(continued)

Table 23.2 (continued)

Animal studies

Reference	Animal model	Trial length (weeks)	Intervention groups[c, e]	Body weight	Effect of ADF on CVD risk parameters
Heilbronn, 2005 (Heilbronn et al. 2005)	$n = 16$, MF Normal weight Age 32 ± 2 years	3	1. ADF ($n = 16$)	↓4[a]	Blood pressure: ∅ HDL cholesterol: ↑[a] Triglycerides: ↓[a]
Varady, 2011 (Varady et al. 2011a, b, c)	$n = 16$, MF Obese Age 46 ± 2 years	8	1. ADMF ($n = 16$)	↓6[a]	LDL peak particle size: ↑[a] Proportion of small LDL particles: ↓[a] Proportion of large LDL particles: ↑[a]
Bhutani, 2010 (Bhutani et al. 2010)	$n = 16$, MF Obese Age 46 ± 2 years	8	1. ADMF ($n = 16$)	↓6[a]	Blood pressure: ↓[a] LDL cholesterol: ↓[a] Triglycerides: ↓[a] C-reactive protein: ∅ Homocysteine: ∅

∅ No effect, *2DG suppl* supplementation with 2-deoxy-D-glucose, *ADF* alternate day fasting, *ADMF* alternate day modified fasting, *CR* calorie restricted, *CVD* cardiovascular disease, *F* female, *M* male, *SD* sprague–Dawley

[a] Posttreatment values significantly different ($P < 0.05$) from baseline values within the ADF group

[b] Posttreatment values of ADF group significantly different ($P < 0.05$) from posttreatment values of control group

[c] Alternate day fasting (ADF) regimen: Fed ad libitum for 24 h on the feed day, alternated with 24 h fast on the fast day

[d] Alternate day modified fasting (ADMF) regimen: Fed ad libitum for 24 h on the feed day, alternated with 75% energy restriction on the fast day

[e] Control group: Fed ad libitum daily

one recent study (Ahmet et al. 2010) suggests that ADF may have deleterious effects on heart health. In this study by Ahmet et al. (2010), young rats were fed an ADF diet for 6 months. Chronic ADF resulted in a reduction of cardiomyocyte size and augmented myocardial fibrosis (Ahmet et al. 2010). The fibrotic ADF hearts manifested diastolic dysfunction at rest, and a diminished diastolic and systolic reserve capacity (Ahmet et al. 2010). Although the mechanisms responsible for the deleterious effects of ADF on cardiac function are not clear, these data provide a cautionary note to the adoption of long-term ADF.

23.3.2 Human Trials

Only three trials (Bhutani et al. 2010; Heilbronn et al. 2005; Varady et al. 2011a, b, c) have been performed that examine the cardio-protective effects of ADF in human subjects (Table 23.2). Although these studies (Bhutani et al. 2010; Heilbronn et al. 2005; Varady et al. 2011a, b, c) all have short trial lengths, these preliminary data suggest that this diet regimen may be an effective means of preventing cardiovascular diseases in human subjects. For instance, in the study by Heilbronn et al. (Heilbronn et al. 2005), 3 weeks of ADF resulted in significant reductions in triglycerides, accompanied by increases in HDL cholesterol, in normal weight subjects. No change in blood pressure was observed, however (Heilbronn et al. 2005). Similar improvements in lipid profile were also demonstrated in the study by Bhutani et al. (Bhutani et al. 2010). Following 8 weeks of ADF, obese men and women experienced reductions in both LDL cholesterol (25% decrease) and triglyceride levels (32% decrease) (Bhutani et al. 2010). Significant decreases in systolic blood pressure were also observed after this longer intervention period (8 weeks) (Bhutani et al. 2010). No changes, however, were noted for C-reactive protein or homocysteine concentrations (Bhutani et al. 2010). The impact of this diet on LDL particle size has also been evaluated (Varady et al. 2011a, b, c). An increase in the proportion of small, dense LDL particles is an independent risk factor for the development of vascular diseases (Ip et al. 2009; Krauss 2010). The mechanisms linking small LDL particles to atherogenesis include: increased oxidizability, augmented permeability through the endothelial barrier, and longer residence time in plasma (Griffin 1999). Interestingly, in the study by Varady et al. 2011a, b, c, LDL peak particle size increased after 8 weeks of ADF in obese participants. Likewise, the proportion of small LDL particles was shown to decrease, while the proportion of large particles was shown to increase (Varady et al. 2011a, b, c). Given that a reduction in the proportion of small LDL particles may be cardio-protective (Ip et al. 2009; Krauss 2010), these preliminary findings suggest that ADF may help reduce the risk of cardiovascular diseases in humans.

23.4 Effect of ADF on Type 2 Diabetes Risk

23.4.1 Animal Studies

Five studies (Anson et al. 2003; Krizova and Simek 1996; Mager et al. 2006; Pedersen et al. 1999; Wan et al. 2003) employing animal models have examined the effect of ADF on type 2 diabetes risk (Table 23.3). Fasting glucose levels have generally been reported to decrease in response to ADF in animal models. For instance, three studies (Anson et al. 2003; Pedersen et al. 1999; Wan et al. 2003) demonstrated reductions in circulating glucose after 20–24 weeks of ADF, while one study reported no effect after 16 weeks of treatment (Mager et al. 2006). As for insulin concentrations, consistent reductions were noted after 20 weeks (Anson et al. 2003) and 24 weeks (Wan et al. 2003) of diet. In the study of Anson et al. (2003), both glucose and insulin were reduced to a similar extent in the ADF and 40% CR groups. Thus, it is possible that these two diets may have similar effects on glucose and insulin levels in animals. Increases in fat oxidation in liver and muscle have also been observed after relatively short periods (8 weeks) of ADF (Krizova and Simek 1996). Impaired fat oxidation can contribute to ectopic accumulation of intracellular lipid and the development of insulin resistance (Heilbronn et al. 2004). As such, these increases in fat oxidation by ADF may increase insulin sensitivity.

23.4.2 Human Trials

Type 2 diabetes risk parameters have been measured in two published human studies of ADF (Halberg et al. 2005; Heilbronn et al. 2005; Table 23.3). Evidence from these trials suggests that ADF has no effect on fasting glucose, yet may beneficially modulate other indices of diabetes risk, such as insulin sensitivity (Halberg et al. 2005; Heilbronn et al. 2005). For instance, Halberg et al. (2005) show that insulin-mediated glucose uptake increased after 2 weeks of ADF, based on the euglycemic-hyperinsulinemic clamp technique. These findings are similar to those of Heilbronn and colleagues (Heilbronn et al. 2005) who showed that, after 3 weeks of ADF, insulin response to a test meal was reduced, implying improved insulin sensitivity. Interestingly, this effect on insulin sensitivity only occurred in male subjects (Heilbronn et al. 2005). Another diabetes risk parameter that has shown a sex-specific effect is glucose tolerance. After 3 weeks of ADF, women but not men exhibited an increase in the area under the glucose curve (Heilbronn et al. 2005). This unfavorable effect on glucose tolerance, accompanied by an apparent lack of an effect on insulin sensitivity, in women suggests that short-term ADF may be more beneficial in men than women for reducing type 2 diabetes risk. As for insulin levels, Halberg et al. (2005) demonstrated that 2 weeks of ADF had no effect on fasting insulin, while (Heilbronn et al. 2005)

Table 23.3 Studies examining the effect of alternate day fasting on type 2 diabetes risk

Animal studies

Reference	Animal model	Trial length (weeks)	Intervention groups[c, d]	Body weight	Effect of ADF on DM risk parameters
Krizova, 1996 (Krizova and Simek 1996)	$n = 30$ C17/B1-10 mice Age 2 months	8	1. ADF–Low fat diet ($n = 10$) 2. ADF–High fat diet ($n = 10$) 3. Control ($n = 10$)	↑[a] (both groups)	Liver fat oxidation: ↑[a] Muscle fat oxidation: ↑[a] (both groups)
Mager, 2006 (Mager et al. 2006)	$n = 12$ SD rats Age 2 months	16	1. ADF ($n = 6$) 2. 40% CR ($n = 6$)	∅	Fasting glucose: ∅
Anson, 2003 (Anson et al. 2003)	$n = 24$ C57BL6 mice Age 2 months	20	1. ADF ($n = 8$) 2. 40% CR ($n = 8$) 3. Control ($n = 8$)	∅	Fasting glucose: ↓[b] Fasting insulin: ↓[b]
Pedersen, 1999 (Pedersen et al. 1999)	$n = 161$ BB rats Age 3 months	20	1. ADF ($n = 44$) 2. Fast for 24 h 2x/week ($n = 40$) 3. Control ($n = 77$)	↓[b]	Fasting glucose: ↓[b]
Wan, 2003 (Wan et al. 2003)	$n = 24$ SD rats Age 3 months	24	1. ADF ($n = 8$) 2. 2DG suppl ($n = 8$) 3. Control ($n = 8$)	↓[b]	Fasting glucose: ↓[b] Fasting insulin: ↓[b]

(continued)

Table 23.3 (continued)

Human trials

Reference	Subjects	Trial length (weeks)	Intervention groups[c, d]	Body weight (% change)	Effect of ADF on DM risk parameters
Halberg, 2005 (Halberg et al. 2005)	$n = 8$, M Normal weight Age 25 ± 1 years	2	1. ADF ($n = 8$)	↓1	Insulin-mediated glucose uptake: ↑[a] Insulin-induced lipolysis inhibition: ↑[a] Fasting glucose: ∅ Fasting insulin: ∅
Heilbronn, 2005 (Heilbronn et al. 2005)	$n = 16$, MF Normal weight Age 32 ± 2 years	3	1. ADF ($n = 16$)	↓4[a]	Glucose clearance: ↓[a] (women), ∅ (men) Insulin sensitivity: ∅ (women), ↑[a] (men) Fasting glucose: ∅ Fasting insulin after 32 h fast: ↓[a]

∅ No effect, *2DG suppl* supplementation with 2-deoxy-D-glucose, *ADF* alternate day fasting, *CR* calorie restricted, *DM* diabetes mellitus, *SD* sprague–dawley

[a] Posttreatment values significantly different ($P < 0.05$) from baseline values within the ADF group

[b] Posttreatment values of ADF group significantly different ($P < 0.05$) from post-treatment values of control groups

[c] Alternate day fasting (ADF) regimen: Fed ad libitum for 24 h on the feed day, alternated with 24 h fast on the fast day

[d] Control group: Fed ad libitum daily

show that 3 weeks of diet decreased insulin levels, but only after a 32 h fast. Further research examining the time-course effects of ADF on fasting insulin and insulin sensitivity could help clarify this matter.

23.5 Summary of Findings

Findings from both animal and human studies indicate that ADF may be an effective means of losing weight. More specifically, results from short-term human trials (3–12 weeks) demonstrate body weight reductions of 4–8% from baseline by ADF in normal weight and obese individuals. In view of these promising preliminary data, it will be of great interest to evaluate the degree of weight loss that can be achieved with ADF after longer periods of treatment (>24 weeks). Decreases in visceral fat mass were also noted in both human and animal models. Reductions in visceral fat mass in humans ranged 4–10% from baseline, after 12 weeks of diet. In rodents, decreases in visceral fat were accompanied by increases in subcutaneous fat mass. Thus, it is possible that ADF may result in a preferential redistribution of body fat from visceral to subcutaneous compartments. Studies examining whether these effects can be reproduced in humans is unquestionably well warranted.

The effects of ADF on vascular health are equivocal. The majority of animal and human data indicate that this diet is an effective means of lowering heart rate, blood pressure, LDL cholesterol, and triglyceride concentrations. However, more recent findings in rodents demonstrate that long-term ADF (6 months of treatment) results in the development of diastolic dysfunction with diminished cardiac reserve. These conflicting results indicate that additional studies of ADF in humans, particularly long-term studies, are necessary before any solid conclusions can be reached.

As for the effects of ADF on type 2 diabetes risk, data from human trials have been inconsistent, while animal findings suggest beneficial alterations. Fasting glucose, for instance, has been shown consistently to decrease in animal models after 20 weeks of treatment, but is unaltered in humans after 2–3 weeks of diet. Longer intervention periods may need to be implemented in human studies to see an effect on this parameter. Fasting insulin, on the other hand, is lowered in animal models, while equivocal findings have been demonstrated in humans. Although neither glucose nor insulin levels were affected in short-term human trials, interestingly, increases in insulin-mediated glucose uptake have been reported. Such findings would imply improved insulin sensitivity by ADF diets. In sum, the beneficial effects noted in animal studies indicate that prolonged ADF is a beneficial means of lowering type 2 diabetes risk. Results from human studies, however, are less clear. Although it seems reasonable to expect that ADF will improve insulin sensitivity in humans, the conflicting findings make it difficult to be certain.

23.6 Conclusion

Preliminary findings from both animal and human studies demonstrate that ADF may be an effective means of decreasing body weight and visceral fat mass. Whether these beneficial alterations in weight and body composition translate into decreased vascular disease and type 2 diabetes risk in human subjects is still debatable, however. It is important to note that the human studies included in this review were all limited in that they all lacked control groups and implemented short treatment durations. For this reason, future studies that employ longer trial lengths and include control groups will be required to answer these important questions relating to the efficacy of ADF for the treatment of chronic diseases.

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Chapter 24
Horizons in Starvation Research

Marshall D. McCue

24.1 Introduction

The challenge of food limitation is an inherent component of a heterotrophic lifestyle. Starvation was experienced by the earliest metazoans and continues to pose a risk for modern animals. The preceding reviews of fasting and starvation physiology in animals ranging from rotifers to humans reveal that animals have evolved a diversity of behavioral and physiological strategies to cope with periods of food limitation. Just as diverse as the different starvation strategies that animals have evolved are the different tolerances animals have to survive starvation. For example, some species are only able to tolerate several hours without eating, but others can survive several years without eating. Perhaps, the only common denominator among animals is that starvation cannot be endured indefinitely.

The individual organism is the fundamental unit of natural selection, and a particular individual will either survive or succumb to a given period of starvation; this response is fairly easy to quantify. However, to fully appreciate the biology of starvation one must consider the repercussions of starvation beyond the individual organism. In fact, the effects of starvation transcend traditional hierarchal levels from atoms (see Hatch, Chap. 20) to communities (see Kirk, Chap. 3).

A common phrase around my laboratory is that "good research often leads to as many questions as it answers." The progress we have made investigating fasting and starvation over the past century supports this thesis and exposes a broad research horizon. In this chapter, I highlight novel research directions and pose questions that may complement our current knowledge about starvation and fasting. Given the integrative nature of many of these questions, meaningful

M. D. McCue (✉)
Department of Biological Sciences, St. Mary's University,
One Camino Santa Maria, San Antonio, TX 78228, USA
e-mail: mmccue1@stmarytx.edu

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progress toward this horizon will require the combined expertise of scientists from many areas (see Zhang et al., Chap. 13).

24.2 Paleoecology and Climate Change

Many of the Earth's major extinctions were related to mass starvation events resulting chiefly from diminished primary production (Chin 2007; Rhodes and Thayer 1991) (see McCue, Chap. 1). Can geological and fossil records be used to quantify the intensity of such events of mass starvation (e.g. Miller 2003)? Is it possible to identify signs of nutritional stress among fossilized animal tissues (e.g. Ambrose 1991; Cormie and Schwarcz 1996; Koch et al. 2009; Tutken et al. 2004)? How might starvation tolerance in extant species, whose progenitors survived mass extinctions, compare with those lineages that did not survive (e.g. Jeffery 2001; Russell 1965)?

Climate change models consistently predict changes in temperature and precipitation patterns that will no doubt alter the biogeographic distribution of food sources (Harrison and Pearce 2000; Porter et al. 2002), and ultimately increase the likelihood of starvation in some communities. Therefore, any intelligent analysis of the impending effects of climate change must consider which species are most susceptible to food limitation driven by climate change (e.g. McKinney and Lockwood 1999). Will migratory species be able to alter their migratory schedules and routes to avoid starvation (e.g. Walther et al. 2002)? Will species that do not currently migrate eventually evolve migratory strategies to cope with local food limitation? To what extent are different animals capable of shifting their diets to prevent starvation-induced mortality (e.g. Chin 2007)?

24.3 Monitoring Starvation in Wild Populations

Many wild animal populations are constrained by food resources. To better understand the frequency and severity of local starvation events we will need to improve how we document this phenomenon (e.g. Hine 1957; Tracy et al. 2006). Noninvasive measurements of body condition (e.g. Lourdais et al. 2002a, b; Korine et al. 2004; Stevenson and van Tets 2008; Totzke and Bairlein 1998) and semi-invasive measures of blood or urinary metabolites are routinely used to identify nutritional stress in some species, (e.g., Cabanac et al. 2005) but these techniques need validation for other types of animals. Moreover, it will be prudent to identify certain species that can be used as indicators of the nutritional status' of entire ecological communities (Beaupre and Douglas 2009).

Captive animals may not respond the same way to starvation as those in the wild. Animals in the wild have a potentially different set of behavioral options to animals observed in the laboratory. How do these responses differ between the wild and the laboratory (e.g. Costa and Sinervo 2004; Goldstein and Pinshow 2006)? Furthermore, in the wild instances of complete starvation are difficult to confirm and "starving"

animals may actually eat when unobserved. Such instances preclude accurate estimates of the nutritional state of wild animals if responses are based only on laboratory studies of complete starvation. To better interpret natural scenarios carefully designed experiments should be developed that more realistically reflect environmental variability (e.g. thermal, structural, etc.) of natural conditions and mimic situations where complete starvation is not necessarily occurring (e.g., Cabanac et al. 2005).

24.4 Starvation and Gastrointestinal Symbionts

Most animals harbor populations of symbiotic microflora and microfauna in their gastrointestinal tracts. Many of these, most notably foregut fermenters such as ruminants (see Ullrey, Chap. 18) and some birds (Clench and Mathias 1995; Downs et al. 2000; Pacheco et al. 2004) and hindgut fermenters such as ungulates (see Harlow, Chap. 17), rodents, and lagomorphs (Breazile 1971), rely extensively on symbiotic microbes to convert otherwise indigestible materials (e.g. cellulose and pectin) into molecules (e.g. amino acids, lactic acid, and volatile fatty acids) that can be readily oxidized to synthesize ATP and essential materials (e.g. polyunsaturated fatty acids, vitamins, and indispensable amino acids) (Breazile 1971; Stevens and Hume 1995). In the nourished state these symbionts may be localized to particular regions of the gastrointestinal tract, and their numbers are regulated by intraspecific and interspecific competition (Mackie 2002; Savage 1986). Starvation invariably reduces the supply of nutrients to these microbes causing a microscopic "energy crisis." This energy shortage is usually coupled with a "housing crisis" caused by the remodeling of gastrointestinal tissues of the host tissue (see Lignot, Chap. 14). What physiological responses do gut microbes have to these starvation-induced challenges? Which types of microbes are most susceptible to host starvation (e.g. Byrne and Dankert 1979; Gossling et al. 1982; Tannock and Savage 1974) and which species flourish? If species or entire communities of microflora become locally extinct, what processes are responsible for their reintroduction in the host gut (e.g. Mackie et al. 2004; Troyer 1982)? This question is analogous to those posed by early ecologists investigating island biogeography (Simberloff 1974) and metapopulaton theory (Hanski and Gilpin 1991) and could be just as fruitful. Recently developed genomic approaches may permit researchers to model the changes in microbial ecology along the gastrointestinal tract over the course of starvation (e.g. Costello et al. 2010; Mackie 2002; McCue 2010).

24.5 Prioritization of Mass and Energy Reserves

When an animal fasts, its constituent organs, tissues, and cells also face resource limitation; however, these compartments do not necessarily share this burden equally (see Bauchinger and McWilliams, Chap. 12). The specific physiological

rules that regulate how endogenous resources are mobilized and where they are allocated during starvation have been fine-tuned over evolutionary time, yet they remain far from being fully understood even in the simplest animals. The physiological prioritization of different tissues during starvation have been known for nearly a century (see Lignot and Le Maho, Chap. 2), but we still lack basic understanding of the signals that permit atrophy of some tissues while others (e.g. neural tissues) remain virtually unchanged (Munoz-Garcia et al. 2012).

Basal metabolic rate (BMR) and standard metabolic rate (SMR) are among nearly a dozen ways of expressing standardized measures of animal metabolism. BMR and SMR are considered to be the lowest measurable rates of metabolism for nonhibernating endotherms and ectotherms, respectively (IUPS 2001). Interestingly, many studies have reported clear evidence for starvation-induced hypometabolism (i.e. where metabolic rates fall below BMR or SMR) that appear to be distinct from estivation and independent of body temperature (see McCue et al., Chap. 8; Overgaard and Wang Chap. 5). Despite growing evidence that starvation can reduce BMR and SMR the concept of starvation-induced hypometabolism remains unrecognized by the IUPS Thermal Commission. Progress in having this unique metabolic response formally recognized should occur on two fronts. First, additional studies must rigorously document this phenomenon in more species. Second, researchers must identify the regulatory mechanisms that permit metabolic rates to fall to new steady-state levels during starvation.

24.6 Lipids and Fatty Acids

Because of their high energy content, lipids, particularly triacylglycerols stored in adipose tissues that have low metabolic requirements, are the most important fuel for fasting animals. Several studies have reported that fatty acids are differentially mobilized from adipose tissue at rates that depend on their respective length and degree of unsaturation (Babayan 1987; Connor et al. 1996; Mustonen et al. 2009; Nieminen et al. 2006; Raclot and Groscolas 1993; Wheatley et al. 2008); however, the rates at which different fatty acids are β-oxidized in the mitochondria do not necessarily reflect their abundances in the blood (see Price and Valencak, Chap. 15). What are the relative impacts of each step (e.g. mobilization, circulatory transport, membrane transport, and oxidation; McWilliams et al. 2004; Price et al. 2008) on the differential changes in fatty acid composition routinely observed in fasted individuals (Ben-Hamo et al. 2011; Austin 1993; McCue 2008; Nieminen and Mustonen 2007; Tandler et al. 1989)?

The levels of *trans* fatty acids can be particularly high in the tissues of animals that rely on bacterial symbionts for digestion (e.g. ruminants; Hartman and Shorland 1959). Although naturally low in humans, increased levels of *trans* fatty acids are being reported in humans that routinely consume the products of hydrogenated oils (Mozaffarian et al. 2006). Diets rich in these *trans* fatty acids are often correlated with pathophysiological conditions (e.g. coronary heart disease;

Hu et al. 2001). In contrast to what is known about how chain length and degree of unsaturation influences the fates of fatty acids, very little is known about how *cis*- and *trans*- isomers may be differentially used during fasting. Using $^{13}CO_2$ breath testing, DeLany et al. (2000) found that rats oxidized *trans* fatty acids more rapidly than *cis*-isomers. Can controlled fasting protocols (e.g. alternating feeding and fasting days; see Varaday, Chap. 23) be a useful clinical treatment for the accumulation of *trans* fatty acids in humans? Additionally, can starvation-induced changes in ratios of *cis* and *trans* fatty acids in the blood be developed into a reliable indicator of nutritional stress as we have done with fatty acid chain length and degree of unsaturation (e.g. Afroz et al. 1971; Jezierska et al. 1982; McCue 2008; Pan and Storlein 1993; Smith et al. 2003; Zak et al. 2005)?

24.7 Starvation Training or Starvation Hardiness?

The ability to tolerate starvation is typically considered to be a characteristic of a species, or a function of a particular individual's level of endogenous nutrient reserves. Little research has focused on the sources of variability in starvation tolerance within conspecifics possessing similar nutrient reserves (see Gibbs and Reynolds, Chap. 4). The factors underlying intraindividual differences in starvation tolerance need to be explored (Gibbs 1999). The results of early studies suggest that humans and nonhuman animals may be able to improve their ability to tolerate starvation following repeated bouts of fasting. Are these improvements similar to the gradual improvement in performance following routine physical training (i.e. the training effect), or are they more closely related to acute exposure to extreme temperatures that leads to improved thermal tolerance (e.g. cold hardiness)? If starvation tolerance is plastic, then which particular responses are most affected by repeated exposure to fasting or starvation? What are the physiological mechanisms that establish and maintain the nutritional memory in individuals previously exposed to starvation? How long does it take to realize improvements in starvation tolerance and how long do the effects last? To what degree is this phenotypic plasticity correlated with the evolutionary history of a species? For example, do species that traditionally occupy habitats characterized by unpredictable food resources consistently exhibit stronger starvation training effects? If so, what tradeoffs are associated with these changes?

24.8 Starvation Life History Traits

Many animals, like zooplankton inhabiting ephemeral ponds, do not have the option of moving to a new foraging patch in the event of a shortage of food. Planktonic rotifers probably experience the highest incidence of starvation-induced mortality among the animals discussed in the volume. This group has apparently evolved divergent strategies to survive starvation. During complete

starvation some species decrease or altogether cease egg production, whereas other species actually increase egg production (Kirk 1997; Kirk et al. 1999; Weithoff 2007; Yoshinaga et al. 2003). The latter strategy hastens starvation-induced mortality of the individual; however, because eggs survive until food is again available this strategy likely improves the chances of species survival. To date, changes in reproductive output in response to starvation challenges have only been examined in a few species. Consequently, it remains unclear how these seemingly opposing strategies evolved among closely related groups. Answers to questions such as: which strategy is the most derived, have these strategies evolved repeatedly, and what environmental conditions favor one strategy over another, could be addressed by examining how additional rotifer species respond to starvation and analyzing these traits using ecologically informed phylogenetic comparisons (Garland et al. 2005). Recently, artificial selection for starvation resistance combined with genomic analyses like that developed for Drosophila models may offer unique insights into the evolution of starvation tolerance and how different life history traits are correlated with one another (see Gibbs and Reynolds, Chap. 4).

24.9 Characterizing the Progression of Starvation

The ability to sequentially switch among the three major classes of metabolic fuels (i.e. carbohydrates, lipids, and proteins—usually in that order) is a hallmark of species that are considered "adapted" to fasting (Castellini and Rea 1992); unfortunately, we know little about the extent to which species that are not considered to be "fasting-adapted" also employ this strategy. The timing of these fasting-induced switches in metabolic fuels is typically measured indirectly using various proxies like reductions body mass (see Hall, Chap. 22). A reduction in the rate of mass loss shortly after the onset of fasting can indicate the transition from carbohydrate to lipid oxidation, and an increased rate of mass loss as fasting progresses may indicate the transition from lipid to protein oxidation. While this qualitative observation is useful for studying animals that are tractable, easy to weigh, and able to fast for long periods, it is not useful for the study of aquatic animals (e.g. fishes), very large animals (e.g. hippopotami, elephant seals), very small animals (e.g. zooplankton, and marine and terrestrial arthropods), secretive animals (e.g. bats and snakes), or species that cannot tolerate fasting for long periods (e.g. many small mammals and birds). What about some aquatic species that do not exhibit starvation-induced reductions in mass (Comoglio et al. 2004; Hervant and Renault 2002; Moon 1983)? Is it possible to develop standardized methodologies for characterizing the progression in animals generally?

All animals rely on some degree of protein oxidation as a source of cellular energy causing them to continually generate nitrogenous wastes. Changes in rates of nitrogen excretion are sometimes measured to indicate the transition in metabolic fuels since they can be monitored noninvasively. An increase in the rate of nitrogen excretion is considered particularly useful to identify the timepoint at

which an animal switches from lipid to protein oxidation (i.e. the switch from phase II to phase III of fasting; e.g. Cherel et al. 1992; Comoglio et al. 2004; Sartori et al. 1995). Unfortunately, like changes in mass loss, this approach is poorly suited for studies of animals that cannot fast for long periods or species capable of extensive nitrogen recycling (Balter et al. 2006; Nelson 1987). It is also problematic for studies of most reptiles that store nitrogenous wastes for days to months in their cloaca before voiding it (e.g. Lillywhite et al. 2002; McCue and Pollock 2008).

Starvation-induced changes in circulating metabolites (e.g. glucose, amino acids, fatty acids, lipids, proteins, urea, and ketones) can provide insight into the physiological transitions that take place during starvation, but only once they have been validated in a particular species (Jenni-Eiermann and Jenni 1998; Sanchez-Guzman et al. 2004). Like the two above-mentioned indirect measures, this approach is not conducive for studies of very small animals that cannot tolerate long periods of starvation and can be problematic when repeated blood sampling is likely to confound preexisting physiological stress. Most importantly, measurements of blood metabolites do not provide any information about whether the concentration of a particular metabolite (1) increases as the result of increased synthesis (or mobilization), or decreased uptake by tissues, or (2) decreases as the result of decreased synthesis or mobilization, or increased uptake by tissues (e.g. McCue 2010).

24.10 Measuring Endogenous Nutrient Oxidation Directly

Why are direct measurements of endogenous fuel oxidation during starvation not more common? Breath testing, the practice of monitoring the excretion of $^{13}CO_2$ in animals whose bodies have been previously enriched in particular ^{13}C-labeled nutrients, provides a direct measure of starvation-induced switches in metabolic fuels. Animals fed diets supplemented in ^{13}C-glucose will store a portion of this sugar as glycogen. On fasting, these animals convert the ^{13}C-labeled glycogen into $^{13}CO_2$ that can be simply collected from exhaled breath and measured using an isotope analyzer (e.g. McCue et al. 2010, 2011a, b). The method can also be modified for water-breathing animals (e.g. Ronnestad et al. 2001).

It is also possible to dose animals with a common ^{13}C-labeled fatty acid, such as palmitic acid, that will become integrated into triacylglyceride stores, and to a lesser extent into phospholipids (e.g. McCue et al. 2009). During starvation these tracers are eventually mobilized and oxidized, generating $^{13}CO_2$ that can be measured in a breath sample (Fig. 24.1). We assume animals to be in phase II of fasting when glycogen stores have diminished and lipid oxidation is maximal.

It is further possible to enrich an animal with a ^{13}C-labeled, nongluconeogenic, amino acid, such as leucine. Although some level of protein turnover is inevitable (see Bauchinger and McWilliams, Chap. 12) a sharp increase in amino acid-derived $^{13}CO_2$ can be collected in breath and used to indicate the onset of phase III

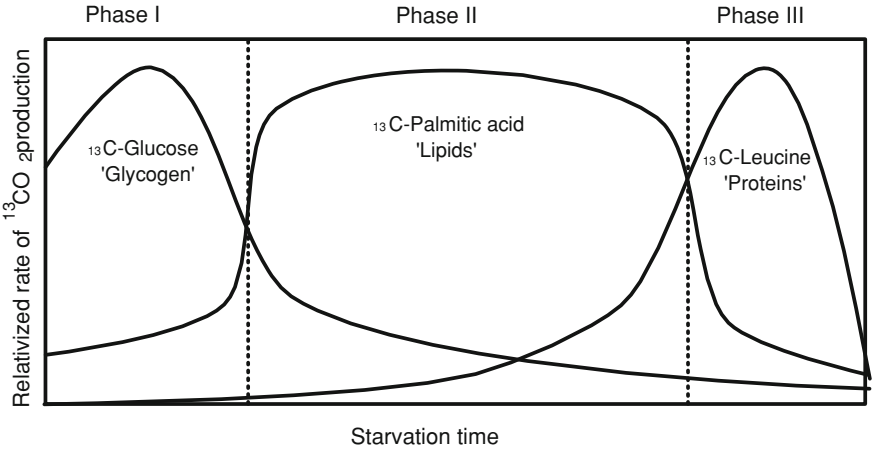

Fig. 24.1 The outcome of stable isotope breath testing during starvation. The three curvilinear responses represent hypothetical rates of $^{13}CO_2$ production and the timing of phase transitions in three individuals fed ^{13}C-labeled carbohydrates, fatty acids, or amino acids (figure modified from Khalilieh et al. in review)

of starvation (Fig. 24.1). Once the time course of metabolic fuel switching is well-characterized, researchers can begin the next step of identifying the regulatory pathways that underlie these coordinated systematic switches in metabolic fuels during starvation. Can this methodology be moved from the laboratory to the field (Goldstein and Pinshow 2006)?

24.11 Conclusion

Over the past century significant progress has been made in documenting how different animals respond to starvation, yet we still face many questions. To accelerate progress to this end we must strategically allocate our own limited resources. First, we need to broaden our existing knowledge base by using new methodological, analytical, and statistical techniques to examine starvation responses in model species. In doing this it will be important to emphasize that "model" species for starvation research are not necessarily the same species used in traditional physiological research. Second, we must explore the physiology of starvation in heretofore unexamined species. In doing this we must be cognizant of the need to explore starvation in species that are not necessarily considered to be well adapted to starvation. Such a comparative approach will allow us to better distinguish between unique adaptations and those shared by animals generally. Third, human population growth and climate change have led us to a precipice from which we can take a giant leap forward and use our knowledge to predict the role that starvation will have in the lives and deaths of future generations of heterotrophs.

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