Michael E. Symonds *Editor*

Adipose Tissue Biology

Second Edition

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Contents

Chapter 1
The Evolution of Mammalian Adipose Tissues

Caroline M. Pond

Abstract Anatomical organization, genes and metabolic pathways in white, beige and brown adipose tissues are traced from their invertebrate origins through lower vertebrates to mammals and birds. Invertebrate storage organs and adipose tissues of lower vertebrates are also metabolic regulators. In large turtles, some depots are thermogenic or insulators. Reptilian, avian and mammalian adipocytes sort fatty acids, especially essential polyunsaturates. All mammals have numerous adipose depots, many with site-specific properties including thermoregulation, structural roles or paracrine interactions with contiguous tissues. Paracrine provisioning of lymph nodes with fatty acid sorting optimizes cellular nutrition during fasting or on deficient or imbalanced diets, averts competition with other tissues and utilizes scarce resources efficiently. The mechanisms may be defective in HIV/AIDS and Crohn's disease and some obesity-related diseases. Thermogenesis by shivering and non-shivering mechanisms in muscle occurs in some lower vertebrates and, in birds, is as effective as mammalian brown adipocytes. Facultative thermogenesis emerged gradually in birds and mammals, utilizing genes of reptilian ancestors, including some resembling uncoupling proteins. Mammalian thermogenic tissue evolved from muscle that lost contractile functions and expanded its mitochondria and lipid-storage capacity, thus generating confusing resemblances to white adipocytes. As well as storage and endocrine functions, adipose tissues' capacities for paracrine interactions, fatty acid sorting and thermogenesis supported the evolution of mammalian heterothermy (i.e. diet-induced thermogenesis, torpor and hibernation), lactation and their ability to exploit nutritionally imbalanced diets. These features probably appeared early in mammalian evolution enabling rapid colonization of new habitats, including efficient utilization of poorer quality diets, and metabolic support of lactation that enables fast-growing young to delay maturation of specialised dentitions. The contribution of 'grandmothers' to their descendants' evolutionary fitness drove selection for post-menopausal longevity, aided by larger lower-body superficial depots that protect cardiovascular and metabolic health. Sex differences in human adipose tissue distribution evolved under such sexual selection plus

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adaptations to heat dissipation. Natural obesity without metabolic impairment found in some arctic mammals evolved by numerous genetic modifications over at least a million years, much longer than human adjustments to modern diet, cooking, heating and clothing.

Keywords Comparative • Reptiles • Mammals • Birds • Primates • Apes • Bears • Paracrine interactions • Immune system • Fatty acids • Perinodal • Crohn's disease • Colitis • Lipid-soluble toxins • Hibernation • Diet-induced thermogenesis • Herbivory • Lactation • Thrifty genes • Sex differences • Cold adaptation

1.1 Introduction

For many centuries, comparative biology and medicine advanced in parallel, with many practitioners making important and mutually beneficial contributions to both fields. Increasing specialization in the twentieth century forced them apart until the rise of molecular phylogeny, medical genomics and developmental biology in the 1990s reunited the estranged partners. Adipose tissues have been one of the most spectacular beneficiaries of this rapprochement: comparative and medical biologists now recognise that their findings are as mutually supportive to each others' progress as they have even been. This chapter is a three-way synthesis of comparative concepts from wild animals in natural systems, experimental data from laboratory animals & *ex vivo* cultures and human studies to elucidate the normal functions and pathologies of adipose tissues.

Although research involving adipose tissues has expanded enormously during the past 50 years (Rosen and Spiegelman 2014), evolutionary and comparative studies lagged behind metabolism, endocrinology and human epidemiology. Both white (WAT) and brown (BAT) adipose tissues have been largely omitted from genetic and developmental investigations into the origins and evolution of tissues and cell types that complement the long-established discipline of comparative anatomy, functionality and adaptation because they appear too variable, too closely linked to diet and body condition to reveal any general principles determining their site-specific properties and anatomical distribution or phylogenetic relationships to 'lean' tissues.

Interest in its origins and evolution was stimulated by recognition of WAT's endocrine and paracrine relationships, its role in metabolic regulation and its value as a source of stem cells and in reconstructive surgery as well as lipid storage and recently accelerated by the study of the uniquely mammalian tissues BAT and beige or brite adipocytes (Cohen and Spiegelman 2015). Understanding of adipose tissues has progressed from its dismissal by comparative anatomists to its recognition as central to the evolution of the skin, immune system, thermoregulation, mammalian lactation and the metabolic control that underpins these systems. This chapter outlines the origins and evolution of the anatomy, physiology and many functions and

specializations of adipose tissues and their relevance to medical sciences; the evolution of the genes involved is left to experts (Caesar et al. 2010).

1.1.1 Comparative Perspectives on Obesity and Diabetes

Obesity and adipose tissues, almost synonymous in the mid-twentieth century, drifted apart as the focus of the former shifted to appetite control and inheritance and of the latter to adipokines (Dodson et al. 2014; Sanchez-Gurmaches and Guertin 2014), development (Chau et al. 2014; Sanchez-Gurmaches and Guertin 2014) and involvement in inflammation and immunity (Exley et al. 2014; Mraz and Haluzik 2014; Couturier et al. 2015).

Of several recent attempts to account for the evolution of obesity in humans, some hardly mention current understanding of the organisation and basic properties of adipose tissues (Power and Schulkin 2009; Isler 2014), while others recognise their central, distinctive roles in human appearance, social and sexual behaviour and metabolism (Wells 2006, 2010).

Obesity is unusual among human diseases in that very similar conditions are integral and essential components of the habits and life history of certain wild animals. Natural obesity, like pathological obesity, arises from 'overeating', periods in which animals become hyperphagic, in some cases aided by sedentary habits. But in wild animals, obesity is always transient and controlled: hyperphagia and fat deposition are followed by periods of anorexia and/or intensive exercise, leading to weight loss (Pond 1998). Adaptive obesity is never a direct cause of diabetes, cardiovascular disease or reproductive dysfunction. The study of natural obesity can reveal much about the 'ideal' structure, composition and anatomical distribution of adipose tissue, the neural and endocrine control of blood composition, appetite and energy expenditure and about the causal relationships between high levels of stored lipid and the adverse metabolic changes that are so frequently associated with obesity in humans.

The origins and incidence of Type 2 diabetes have been explored in many dimensions from metabolism, molecular signalling and immunity to human evolution, ecology and social behaviour (Watve 2012). A general theory of macronutrient nutrition, food selection and foraging (Simpson and Raubenheimer 2012) integrates the scattered and fragmentary information about wild species with human nutritional problems, including obesity. Validated by observations and experiments on organisms ranging from fungi and flies (Solon-Biet et al. 2015) to bears (Erlenbach et al. 2014), its tenets unite nutrition with an impressive range of topics in ecology, cell biology, physiology, immunology, psychology and lifespan (Simpson et al. 2015), though not yet with gross anatomy and the contributions of different organs and tissues. The evolution of adipose tissues, their gross anatomy and relations with other tissues, and, at the microscopic level, adipocytes and the many other cell types they incorporate, have until recently received little attention.

Comparative physiology and genomics during the past 20 years have demonstrated remarkable similarities in the relationships between diet, metabolic control, energy storage and key life history parameters including longevity and fecundity (Fontana et al. 2010). Concepts developed from the study of insects (*Sophophora*, formerly *Drosophila*), nematode worms (*Caenorhabditis*) and other 'lower' organisms have entered medical thinking (Blüher 2008) and the search for new drugs (Hofbauer and Huppertz 2002). Therefore, it is appropriate to begin with an evolutionary and comparative perspective on the structure and functions of adipose tissues.

1.2 Storage Tissues

Tissues and physiological control systems that enable animals to survive long periods of fasting, during which body fabric is depleted and metabolism adjusted, arose early in evolution, so many similarities, but also some important contrasts, are found among living phyla.

1.2.1 Invertebrates

Many invertebrates, especially those that undergo diapause or metamorphosis, have specialised liver-like tissues involved in whole-body metabolic regulation and energy storage. The most thoroughly studied is the insect 'fat body'. This irregularly shaped, sometimes relatively large, structure develops in the abdomen, an anatomical position that maximises contact with the haemolymph and permits large changes in volume with minimal impact on other organs. Its most abundant cell type, called 'adipocytes' by some authors, store glycogen and acylglycerols, releasing the breakdown products in response to metabolic demand from other tissues (Arrese and Soulages 2010). The basic mechanisms of fatty acid uptake and transport, lipogenesis and lipolysis are essentially similar in insect 'adipocytes' and vertebrate white adipose tissue.

The insect fat body also secretes several peptide metabolic regulators (Slaidina et al. 2009) that, at least in drosophila (Arthropoda, Insecta, Diptera), function remarkably like insulin-like growth factors in vertebrates (Okamoto et al. 2009). Neuropeptide Y belongs to an ancient family of peptides that mediate signals between storage cells and the nervous system in various invertebrates (de Jong-Brink et al. 2001; McVeigh et al. 2005).

Insulin is another ancient signal molecule known in *Caenorhabditis elegans* (Nematoda) (Michaelson et al. 2010) and in drosophila (DiAngelo and Birnbaum 2009) as well as all vertebrates. In lower vertebrates such as teleost fish, cells other than pancreatic β cells may be competent to secrete insulin (Roy et al. 2003).

Genes coding for and regulating these messenger molecules and their receptors are among the many gene families that diversified in early vertebrate evolution

(Larsson et al. 2008). Most of the signals and receptors shown to be regulators of appetite and energy storage in mammals are known in the sea squirt *Ciona* (Ascidia, Chordata), an invertebrate chordate (Kawada et al. 2010). The appetite-suppressing hormone leptin seems to be specific to vertebrates, probably appearing early in the evolution of fish (Gorissen et al. 2009), thus long preceding the evolution of adipocytes that are its major producers in higher vertebrates. Insects have analogous peptides that signal peripheral energy stores to the nervous system (Al-Anzi et al. 2009).

1.2.2 Vertebrate Adipose Tissues

Most animal cells contain small quantities of triacylglycerols that serve as energy reserves. Triacylglycerols spontaneously form homogeneous compartments in an aqueous environment. In most tissues that store substantial quantities (brown adipocytes, angiosperm seeds, etc.), the lipids form droplets a few microns in diameter, or around 1–10 fL (10^{-14}–10^{-15} L) in volume (Cinti 2007). Extending the interface between triacylglycerols and lipolytic enzymes may facilitate rapid mobilisation of the lipid stores that supports abrupt transitions between dormancy and vigorous activity. The evolution from yeasts to mammals has been traced for intracellular lipid droplets (Ottaviani et al. 2011) and wider aspects of the biochemistry of lipid storage and its metabolic control (Birsoy et al. 2013).

Single large lipid droplets, usually 0.1–1 nL (10^{-8}–10^{-9} L) in volume, are a special feature of vertebrate white adipocytes. The unusual arrangement is mediated by adipose-specific protein 27 (FSP27) (known in humans as cell death-inducing DFF45-like effector C (CIDEC)) that promotes lipid uptake and coalescence of droplets while reducing the maximum rate of lipolysis (Puri et al. 2007). Experimental reduction of CIDEC in isolated adipocytes increases lipolysis (Ito et al. 2010). The protein probably functions in conjunction with perilipin forming the interface between lipids and proteins (Brasaemle et al. 2000; Shen et al. 2009). FSP27/CIDEC is unique to vertebrates though structurally similar proteins are found in several invertebrate groups (Wu et al. 2008).

From a comparative perspective, these findings suggest that white adipose tissue evolved as a readily deposited, slowly mobilised lipid store suitable both for taking up circulating fatty acids following large, rich meals and for supporting prolonged fasts with low rates of energy expenditure. The evolution of jaws equipped early gnathostome vertebrates as top predators that probably ate relatively large, nutrient-dense prey irregularly and sometimes infrequently (Janvier 2009). The special features of white adipose tissue compared to invertebrate storage tissues exemplify its role as protection for other tissues against lipotoxicity due to excessive lipid accumulation as well as long-term storage (Unger 2002; Unger and Scherer 2010). White adipocytes may be among the novel cell types to appear during early vertebrate evolution, alongside diversification of cell types in the immune system such as mast cells (Crivellato and Ribatti 2010). Advances of vertebrate over invertebrate storage tissues include protection for other tissues against lipotoxicity due to

excessive lipid accumulation as well as long-term storage (Unger 2002; Unger and Scherer 2010) and its metabolic support of cellular immunity (van Niekerk and Engelbrecht 2015).

1.2.3 Fish, Amphibians and Reptiles

Many extant fish, especially the primitive groups, store large quantities of triacylglycerols in the liver and/or skeletal muscle as well as adipose tissue. Quite closely related species show distinct patterns of deposition and mobilization of lipids from the various depots (Weil et al. 2013) but the functions and mechanisms involved are poorly understood.

Almost all adipokines known from mammals have been identified in bony fish (Nishio et al. 2008; Murashita et al. 2009; Ronnestad et al. 2010). Rainbow trout (*Oncorhynchus mykiss*) migrate long distances, fuelled almost entirely by fatty acids that are stored in adipose tissue and transported to muscles by extremely efficient lipoproteins (Weber 2009). Under the highly artificial conditions of fish farms, salmon adipocytes display some of the pathological changes known in obese mammals (Todorčević et al. 2010), but there are no reports of similar effects in wild fish. Transgenic manipulation of the zebra fish (*Danio rerio*) has developed a teleost model of obesity that is remarkably similar to the mouse (Song and Cone 2007; Holtta-Vuori et al. 2010). Messenger molecules with some resemblance to mammalian leptin can be detected in this fish, of which one may have some involvement in energy metabolism (Gorissen et al. 2009), but in a related teleost, its main source is the liver, not adipocytes (Huising et al. 2006).

Most adult amphibians hibernate (or aestivate) for long periods supported by fat accumulated during (often brief) periods of food abundance. Much of the triacylglycerols are stored in paired fat-bodies that are loosely suspended in the abdomen, much like those of insects, and in some species, in and under the thin, distensible skin (Wygoda 1987). In these sites, expansion and shrinkage of the storage tissue avoid distorting adjacent organs.

Blood pressure is higher in reptiles and their body shape is more constrained by tougher, less distensible skin so adipose tissue is more compact and its anatomical arrangement is more varied. Most snakes and lizards have a few large depots but in Testudines (tortoises and turtles), adipose tissue is partitioned into numerous small depots that superficially resemble those of mammals (Pond and Mattacks 1984), an arrangement that may maximise storage capacity while minimising distortion of contiguous tissues.

In the enormous leatherback turtle (*Dermochelys coriacea*), the anatomical distribution and chemical composition of adipose depots seem to be specialized to thermal insulation (Davenport et al. 1990), perhaps extending the range of these partially endothermic reptiles to cooler seas. As well as ‘blubber’ under the carapace and around the viscera and muscles, the abundant adipose tissue in the turtle’s head and neck suggest that it insulates key neural, glandular and vascular structures

from the surrounding water and from the oesophagus, cooled by ingestion of large volumes of low-nutrient food (Davenport et al. 2009).

Very low rates of energy expenditure interspersed with brief periods of much higher metabolic rate are fundamental strategies in nearly all extant reptiles (Secor and Diamond 1997, 1999). They fatten readily and can withstand and recover completely from very prolonged fasts (McCue 2010). However, reptiles are nutritionally fragile, with poor capacity to rebalance dietary minerals and other micronutrients (Frye 1981; Allen and Ullrey 2004). Nutritionally imbalanced diets are a major cause of morbidity in captive reptiles, including severe obesity (Frye 1981). Adipose tissue triacylglycerols are particularly important for provisioning yolk-rich eggs (Warner et al. 2008) so female reptiles are often fatter than conspecific males just before the breeding season and more dependent upon accessing suitable diets.

1.3 White Adipose Tissue in Mammals and Birds

White adipose tissue was presumably named from post-mortem observations on wild insectivores and piscivores or young domestic livestock. It appears yellow to brown in older herbivores and their predators, and in human consumers of dairy products, as accurately illustrated in Rembrandt's 1632 masterpiece *The Anatomy Lesson of Dr. Nicolaes Tulp*. The colour arises from passive (i.e. non-enzymatic and probably non-functional) accumulation of carotenes and any other lipid-soluble residues, including synthetic toxins ingested with food (Polischuk et al. 2002). Thus sequestered, they are mostly harmless until released into the circulation during prolonged fasting or exercise, lactation, egg production or cachexia (Yordy et al. 2010; Fang et al. 2015). Their presence in mobilized and secreted lipids constitutes a major hazard to wildlife, especially during reproduction (De Andres et al. 2016), and in humans is implicated in infant health (Lignell et al. 2011), cardiovascular disease (Bergkvist et al. 2015), cancer (Irigaray et al. 2007) and dementia (Kim et al. 2015).

Dissectible WAT comprises >0.5–50% of the live body mass of free-ranging wild mammals, with an average of about 7% (Pond and Mattacks 1985c). Tissue from wild species generally contains less lipid and more protein, especially collagen, than homologous samples from people and laboratory and domesticated livestock (Pond and Mattacks 1989). Regardless of fatness, the white adipose tissue of large species is composed of fewer, relatively larger adipocytes than that of smaller species of similar dietary habits in both mammals (Pond and Mattacks 1985c) and birds (Pond and Mattacks 1985b). In this respect, adipocytes resemble neurons and contrast with most other cell types in mammals (Savage et al. 2007). Lipid droplet volume, the principal determinant of adipocyte size, is itself related to lipolysis (Ito et al. 2010). By controlling the rates of mobilisation of stored fatty acids and clearance of excess energy absorbed from the diet, white adipocytes are central to metabolic rate during feasting as well as fasting. This scaling of adipocyte volume to body size may reflect the complex and very controversial relationship between

body mass and basal metabolic rate (Kolokotrones et al. 2010). The topic has not been thoroughly investigated in reptiles or any other lower vertebrates.

Comparative biology shows that some functions of the liver in lower vertebrates take place in adipose tissue in mammals. Leptin was first described as a secretion from mammalian adipose tissue, the archetypal adipokine (Caro et al. 1996). Adipose tissue is its main source in all extant mammals including the most primitive (Doyon et al. 2001). Very similar molecules that regulate appetite and energy metabolism are known in all the major classes of vertebrates (Dridi et al. 2004). Although adipose tissue is present, sometimes in substantial quantities, the liver is the main source of leptin in teleost fish (Huising et al. 2006) and in birds (Taouis et al. 2001). Comparative data are too sparse to establish how many other hepatic functions have been 'taken over' by adipose tissue in mammals.

As well as its central role in lipid storage and metabolism, mammalian adipose tissue also participates in amino acid metabolism, particularly that of the non-protein, energy-supplying amino acid, glutamine (Curthoys and Watford 1995; Kowalski et al. 1997). Site-specific differences in glutamine synthesis and turnover suggest depot specialization comparable to that of fatty acid metabolism (Digby and Pond 1995; Digby 1998). Many years after these studies, the role of glutamine as a precursor to fatty acid synthesis (Crown et al. 2015) and in adipocyte differentiation and maturation (Green et al. 2016) are now being investigated.

White adipose tissue of mammals (Pond and Mattacks 1985b), and to a lesser extent that of birds (Pond and Mattacks 1985a), is partitioned into a few large and numerous small depots that merge only when greatly expanded. White adipose tissue metabolism and its neural and endocrinological controls are similar in both groups (Price et al. 2008) as are its involvement in immune function (see Sect. 1.6.1). Avian adipocytes mature much earlier in embryonic development, where they manage yolk lipids, directing appropriate fatty acids into structural lipids and others to oxidation (Speake et al. 1998).

1.3.1 Anatomical Distribution and Site-Specific Properties

In all mammals, white adipose tissue is distributed to a common pattern, though with substantial differences in relative mass between (Pond 1998), and to a lesser extent within species (Pond et al. 1995).

Depots were characterized first by site-specific differences in relative adipocyte volume and various biochemical features (Pond 1992, 1998). Then within-depot differences were shown to enable functionally important paracrine relationship with embedded lymphoid structures (Pond and Mattacks 1995, 1998, 2003; Pond 2007). Site-specific differences in human adipose tissues, until recently regarded as irrelevant, are now identified by a widening range of genetic, developmental and functional properties, many of significance to medicine (Sbarbati et al. 2010; Macotela et al. 2012; Pinnick et al. 2014; Sanchez-Gurmaches and Guertin 2014; Gil-Ortega et al. 2015; Karpe and Pinnick 2015) and livestock production (Dodson et al. 2014).

The largest depots in mammals are found inside the abdomen and between the skin and superficial musculature. Intra-abdominal depots include the mesentery and the omentum, a uniquely mammalian structure, and small quantities associated with the gonads. The adipocytes in these depots plus those surrounding the heart share common developmental origins distinct from that of the superficial sites (Chau et al. 2014). The epididymal depots are exceptionally large and easily dissected out in murid rodents (rats, mice & hamsters) and for this reason alone have been intensively studied. In other mammals, the depots on the inner walls of the abdomen extending around the kidneys and into the pelvis are usually bigger. Detailed study of adipose depots in domestic livestock reveals their cellular compositions and metabolism to be complex and often variable (Dodson et al. 2014); the same may also be true of humans.

The cellular composition of superficial adipose tissues is complex and diverse with functions other than lipid storage (Alexander et al. 2015). Comparison of mammals of body mass 0.1–500 kg and similar proportions of adipose tissue shows that the superficial depots are both thicker and more extensive in larger specimens than in smaller ones because the ratio of surface area to volume is lower (Pond and Ramsay 1992). The resulting confluence of depots that appear discrete in smaller species can impede identifying homologous depots with larger ones, including humans. Abdominal volume and body surface area decrease relative to body mass with increasing size, so superficial adipose tissue can be impressively thick in large mammals, creating the impression they are ‘fatter’. Total dissection is essential to establish body composition.

One of the largest such depots, the inguinal depot on the anterior thigh and abdominal wall (often just called ‘subcutaneous’ in lab rodents and ‘femoral’ in humans), is also the most consistently present in mammals (and birds) (Pond and Mattacks 1986b; Pond 1998). Genetic, physiological and epidemiological studies in humans (Karpe and Pinnick 2015) suggest an explanation: inguinal adipose tissue can accommodate additional lipid stores without promoting inflammation and increased risk of cardiovascular and metabolic disease. In other words, these specialized depots support rapid fattening without diminishing fitness in endothermic animals of high metabolic rate, a fundamental capability for mammalian reproduction (see Sect. 1.7.3).

Many birds and mammals become transiently obese during migration, breeding, moulting or before seasonal food shortages but most remain ambulatory and some perform prolonged, strenuous exercise. Some species of knot (small seabirds, Charadriiformes) carry relatively enormous fuel loads for long-distance migration by selective atrophy of non-essential organs and appropriate redistribution of adipose tissue (Piersma et al. 1999; Battley et al. 2000). In such ‘adaptively obese’ in animals, the additional body mass imposes surprisingly low, sometimes undetectable, additional energetic costs in flight and, perhaps even more surprisingly, in walking. For example, locomotion is unusually efficient in camels, partly through replacement of some limb muscles by non-energy consuming tendons (Alexander et al. 1982). Locomotory efficiency is unimpaired by adipose tissue that can reach 32% body mass in Svalbard rock ptarmigans (*Lagopus muta hyperborea*) (Lees et al. 2010).

After decades of confusion, the tangled relationship between adipose tissues and thermoregulation, both thermogenesis (Sect. 1.4.1) and thermal insulation, is becoming clearer. Many large, naturally obese mammals occur in areas that are seasonally cold, giving rise to the long-standing and widely disseminated belief that adipose tissue accumulates between the skin and underlying body muscles an adaptation to thermal insulation. However, comparative data on the partitioning of white adipose tissue between superficial and internal depots in the mammalian order Carnivora of similar body conformation but widely different sizes do not support this theory (Pond and Ramsay 1992). The superficial depots are simply the most convenient repository for large quantities of lipid regardless of habits and habitats.

The contributions of fur and superficial adipose tissue to body insulation have been studied in marine mammals (Cetacea, Pinnipedia, Sirenia). In those such as fur seals that retain body hair, its main function is energy storage as in Carnivora, but in whales and others with reduced hair, the outer layer is specialized to adjustable thermal insulation mainly by efficient control of blood flow, and the inner layer to storage (Liwanag et al. 2012). UCP1 has been detected in the inner layer of blubber of porpoises and other small cetaceans, suggesting it may thermogenic as well as insulatory (Hashimoto et al. 2015).

The recent identification in laboratory mice of dermal adipose tissue, a small (only a few adipocytes thick) layer distinct from the often more massive subcutaneous layer (Alexander et al. 2015) is consistent with these findings in aquatic mammals and with the site-specific differences identified in layers of subcutaneous adipocytes in pigs (Hausman et al. 2007; Klein et al. 2007) and humans (Ardilouze et al. 2004). Murine dermal adipocytes serve as an insulating sleeve that thickens up to fourfold following prolonged exposure to cold. Those around hair follicles support hair growth, have antimicrobial roles and contribute to wound healing (Alexander et al. 2015; Zhang et al. 2015). The possibility that they also detect cooling (Ye et al. 2013) should be investigated. Thermal insulation in endothermic mammals must be adjustable because the metabolic rate of small mammals is high and during energetically demanding activities such as lactation, dissipation of heat generated as a by-product of digestion and metabolism, is limiting (Król et al. 2007). In experimentally overfed mice, too much superficial adipose tissue decreases skin thickness and elasticity (Ezure and Amano 2010). Additional superficial adipose tissue would exacerbate these problems so in wild mammals, its abundance and distribution must be well controlled.

1.3.2 Cellular Structure of Adipose Tissue

The total number of white adipocytes scales to (Body Mass)$^{0.75}$, and they range in volume from 0.01 nL in bats and shrews, to up to 4 nL in well-fed baleen whales (Pond and Mattacks 1985c). Carnivorous mammals and ruminants have about four times more adipocytes than non-ruminant herbivores (whose energy metabolism is based mainly on glucose) of the same body mass but are not on average fatter,

because the adipocytes are smaller. By coincidence, the adipocytes of rats and mice, small non-ruminant herbivores, are about the same size (0.1–1 nL) as those of humans, large omnivores who these days eat a high-fat diet.

Wild mammals that naturally become obese have up to 5 times, usually only 2–3 times, more adipocytes than would be expected in comparable non-obese species. Western adults have at least ten times more adipocytes in proportion to their body mass than would be expected from the comparison with wild mammals (Pond 1998). The limited information on other primates suggests that their adipocyte complements can also become disproportionately large (Pond and Mattacks 1987; Pereira and Pond 1995). Thorough studies of wild mammals always reveal much inter-individual variation in the total number of adipocytes that cannot be attributed to age, sex or any obvious feature of dietary history, particularly in carnivores (Pond et al. 1995). The number of adipocytes does not seem to be a major determinant of the capacity for fattening even in naturally obese species. In these respects, humans (van Harmelen et al. 2003; Spalding et al. 2008) are similar to other mammals.

1.3.3 Structural Adipose Tissue

Small depots, consisting of large quantities of extracellular material enclosing pockets of metabolically inert adipocytes are found in all tetrapod vertebrates. The firm, resilient tissue absorbs impact forces during locomotion and distributes weight in the feet, especially those of large terrestrial mammals such as elephants (Weissengruber et al. 2006). Fatty tendons around the knee of emus and other large running birds may have a similar role (Regnault et al. 2014). The fetal development (Shaw et al. 2008) and adult functions (Theobald et al. 2006) of Kager's fat pads in the human heel and around the Achilles tendon have been studied in detail. As well as acting as shock absorbers, the adipose tissue protects blood vessels and facilitates movement (Theobald et al. 2006). Injury or atrophy of structural adipose depots in the extremities lead to pain and debilitation that can be exacerbated by diabetes (Chatzistergos et al. 2014). So the study of these tissues using modern biomechanical concepts (Mihai et al. 2015) and techniques (Payne et al. 2015) is timely.

Several small structural depots help shape the face in humans (Kahn et al. 2000), other primates and certain large birds (Pond 1998). The buccal (Bichat's) fat pads are particularly large in human and other higher primates where they contribute substantially to facial appearance from infancy to old age (Yousuf et al. 2010), and, for reasons that remain unclear, sometimes regress in HIV infection (Agarwal 2014).

The white adipose tissue in the orbit behind and around the eye is also primarily structural (Wolfram-Gabel and Kahn 2002) but it may be less metabolically inert and more like 'typical' depots than had been supposed. Adipocyte volume differs consistently in different parts of the orbit and the cell sizes of both samples scale to body mass in mammals ranging in size from whales to voles (Pond and Mattacks 1986a) as in the more abundant metabolically active depots (Pond and Mattacks

1985c). In adult guinea-pigs, total adipocyte complement in the intra-orbital depots correlates with that of the rest of the adipose mass, with corresponding differences in mean volume that enable the depot to occupy a constant space (Mattacks and Pond 1985). Lymph vessels permeate the tissue in certain chronic inflammatory conditions of the eye (Fogt et al. 2004) in which inflammatory cytokines and prostaglandins can be detected (Schäffler et al. 2006). Infiltration of immune cells and the formation of additional adipocytes in the intra-orbital depots are characteristic of Graves' ophthalmopathy (Heufelder 2001; Schäffler and Büchler 2007). Most innate and acquired lipodystrophies involve facial and intra-orbital depots (Garg 2000).

The use of such material, both whole tissue and the stem cells derived from it, for reconstructive and cosmetic surgery (Clauser et al. 2008; Stillaert et al. 2010) has reinvigorated the study of previously neglected tissues structural depots in the human face (Yousuf et al. 2010) and limbs (Panettiere et al. 2011) are being re-examined.

1.4 Brown and Beige Adipose Tissue

Brown adipose tissue (though not non-shivering thermogenesis) are unique to mammals (Cannon and Nedergaard 2004). The comparative anatomy and histology of white adipose tissue were studied in detail (Hoggan and Hoggan 1879) 40 years before similar investigation in brown 'adipose tissue' began (Rasmussen 1922, 1923). The similarities between the names of these tissues and their contrasting but apparently complementary contributions to obesity prompted biologists to emphasise their resemblances, an attitude that recent molecular and developmental findings reveal to be misleading.

The pattern of gene transcription in stem cells differentiating into brown adipocytes resembles that of muscle more closely than that of white adipocytes (Timmons et al. 2007). Brown adipocyte precursors can be detected in skeletal muscle (Crisan et al. 2008) and muscle-specific microRNAs can be found in such cells in tissue culture (Walden et al. 2009). Both muscle and brown adipose tissue have numerous mitochondria, rich blood perfusion and high capacity for uptake and oxidation of fatty acids, some of which may be stored as triacylglycerols in small droplets. In a further similarity to adipose tissue, skeletal muscle is now believed to secrete 'myokines' especially when strenuously active (Pedersen 2011). The resemblances between brown and white adipose tissue arose convergently and long-established histological methods emphasise their similarities more than their contrasts.

The situation is further complicated by the identification of beige or brite adipocytes, that arise from, and in intimate association with, white adipocytes (Wu et al. 2012) and occur in traditional 'brown' adipose depots (Lidell et al. 2013). Under beta-adrenergic stimulation, beige adipocytes may acquire thermogenic, energy dissipating properties similar to those of brown adipose tissue (Wu et al. 2013; McMillan and White 2015), though at rates well below those of brown adipose

tissue (Shabalina et al. 2015). Their presence in many intra-abdominal and superficial depots may contribute to the relationship between body fat patterning and metabolism (Sanchez-Gurmaches and Guertin 2014). Beige adipocytes may be the basis for tissues in laboratory rodents that appear to be mixtures of interconvertible brown and white adipocytes (Giordano et al. 2014). The presence of beige adipocytes may also explain the observations that 'white' adipose tissue of free-living wild mammals, particularly arctic species, contains a greater proportion of protein, even in obese specimens, than the corresponding depots of laboratory rodents or humans (Pond and Mattacks 1989).

The anatomical distribution of beige adipocytes is yet to be studied as thoroughly as that of white or brown and preliminary reports suggest that their physiological roles may extend beyond thermogenesis. Gene activation in beige adipocytes that accumulate around chronic rotator cuff tears indicate that they also promote muscle repair (Meyer et al. 2015).

1.4.1 Origins of Thermogenic Mechanisms

Various tissues and metabolic pathways contribute to whole-body metabolic rate and facultative thermogenesis in lower vertebrates, many of them with common endocrine control (Silva 2006). A recent synthesis of the evolution of thermogenesis in vertebrates (Rowland et al. 2015) concluded that most ancient form of heat generation shivering in skeletal muscles was supplemented in teleost fish by non-shivering thermogenesis 'futile' cycles of calcium ion transport across the sarcoplasmic reticulum. At least two lineages of fish have evolved specialised 'heater organs', derived from skeletal muscle with greatly reduced contractile proteins and extensive, often folded, sarcoplasmic reticulum (Rowland et al. 2015). Some fish, especially large deep-water species, are functionally endothermic (Wegner et al. 2015) with white adipose tissue insulating the brain (Runcie et al. 2009).

Proteins resembling mammalian uncoupling proteins are also expressed in a reptile (the common green lizard, *Lacerta vivipara*) (Rey et al. 2008) and teleost fish (Jastroch et al. 2005) but reptiles, including dinosaurs (Grady et al. 2014) and their descendent groups (including prototherian and metatherian mammals) generate heat (that incubates eggs and other functions) in their extensive musculature by shivering and non-shivering biochemical cycles similar to those of fish (Rowland et al. 2015). Beige adipocytes have been proposed as an intermediate stage in the evolution of brown adipose tissue in eutherian mammals (Li et al. 2014).

The internal body temperature of almost all adult birds is slightly higher than that of euthermic mammals (Schleucher 2004) and in both groups, endothermy uses energy at 5–10 times the rates measured in ectotherms of similar body mass (Hulbert and Else 2000). Many birds, including some very small species, live in polar climates and/or swim in very cold water and, although feather insulation is as good or better than that provided by hair, endogenous thermogenesis is likely during sleep

and other periods of inactivity. Many nestling birds, and adults of a few species, become torpid at night or during periods of fasting and re-warm themselves with a mixture of shivering and non-shivering thermogenesis (Schleucher 2004; Geiser 2008). In spite of much wishful thinking and fruitless searching (Oliphant 1983; Saarela et al. 1989), brown adipose tissue cannot be demonstrated in birds (Mezentseva et al. 2008). Nonetheless, birds do have an uncoupling protein (UCP) that is structurally similar to UCP1, the key component of thermogenesis in mammalian brown adipose tissue (Raimbault et al. 2001; Emre et al. 2007).

Birds' relatively massive muscles are the principal source of thermogenesis, not adipose tissue. As well as shivering muscle mitochondria are uncoupled by membrane protein, adenine nucleotide translocase (ANT) not UCP, increased Na^+/K^+-ATPase activity on the plasma membrane (Walter and Seebacher 2009). Thermogenic substrate cycle involving the Ca^{2+}-ATPase pump on internal membranes regulated by sarcolipin also occur in mammalian skeletal muscle (Bal et al. 2012). The decline in activity from the maxima in neonates can be delayed by cold exposure (Pant et al. 2015). Such cycles of calcium ion transport across the sarcoplasmic reticulum are also found in their poikilothermic ancestors (Rowland et al. 2015).

Substrate cycles ('futile' cycles) in liver, muscle and white adipose tissue were described more than 30 years ago as mechanisms of metabolic regulation and thermogenesis (Newsholme et al. 1984). In small hamsters, rates of adipose tissue cycles of triacylglycerol lipolysis and fatty acid re-esterification differ between adipose depots, highest in small intermuscular sites, and respond to exercise (Mattacks and Pond 1988). Such cycles continue using significant amounts of energy even during starvation in rabbits suggesting that they are fundamentally important (Weber and Reidy 2012). With new findings in brown adipose tissue, interest in non-UCP dependent thermogenesis in mammalian adipose tissues waned, until recently revived (Flachs et al. 2013).

UCP1-based thermogenesis in adipose tissues evolved first in eutherian (placental) mammals probably closely linked to reproduction (Oelkrug et al. 2015). Thus the current hypothesis is that UCP is an ancient protein that in mammals evolved to the new role of thermogenesis by uncoupling the mitochondrial respiratory chain (Hughes and Criscuolo 2008). Facultative thermogenesis in skeletal muscle became so important that the contractile components disappeared, though the very small, rapidly mobilisable lipid droplets remained, ATP synthesis was much reduced though mitochondria became numerous, thus diverting myogenic pathways to form brown adipose tissue (Timmons et al. 2007; Mezentseva et al. 2008). Gene transcription studies reveal similarities between beige adipocytes and smooth muscle (Long et al. 2014) suggesting parallel evolution from contractility to thermogenesis (Rowland et al. 2015). Muscle-derived tissue is the primary source of non-shivering thermogenesis as well as shivering in mammals, as it is in birds. Both inherited this fundamental role for muscle from their reptilian ancestors. The mammalian tissue's confusing resemblances to white adipose tissue arise from its specialisation to thermogenesis fuelled by locally stored lipids at the expense of contractility.

This evolutionary perspective on recent molecular and developmental findings reveals the name 'brown adipose tissue', chosen after careful consideration of a

wide range of evidence from wild animals as well as humans (Rasmussen 1923), to be inappropriate leading to decades of the mistaken belief in its close resemblance to white adipose tissue, and later confusion with beige adipose tissue. A new name, perhaps 'thermogenic tissue', reflecting function regardless of developmental origin, would clarify the situation.

1.5 The Specificity of Fatty Acids

Since leptin was discovered in the early 1990s, the secretion and reception of adipokines has been centre stage in adipose tissue research, emphasising its similarities to other tissues of the immune and endocrine systems (Fantuzzi and Mazzone 2007; Galic et al. 2010). Nonetheless, improvements in equipment and techniques for separating, characterizing and quantifying lipids have greatly advanced understanding of adipose tissue's specialised roles in the sequestration, sorting and selective management of fatty acids and triacylglycerols.

1.5.1 Structural Lipids

All living cells are bounded by fatty membranes and most can oxidise fatty acids or their derivatives. After many years focussed on heritable information and protein synthesis, lipid membranes as barriers and in cell proliferation are now well recognized as central to the evolution of cellular life (Szostak et al. 2001; Stano and Luisi 2010).

Plants and algae synthesise fatty acids from primary photosynthetic products as and when they need them but animals obtain most of theirs from food. In vertebrates, most fatty acids are derived from the diet, with only minor metabolic modifications. For most animals most of the time, *de novo* synthesis contributes only a little, the main exceptions being those that fatten rapidly on a low-fat diet, often prior to reproduction, migration, diapause, hibernation or other prolonged fast.

Membrane fluidity is closely linked to the cells' capacity to support channels and receptors and to deform during movement. Failures in these processes are the principal mechanism of death during hypothermia in mammals such as humans that cannot hibernate (Boutilier 2001). Temperature modulation of membrane fluidity is determined mainly by fatty acid composition of the phospholipids, though the exact relationships are complex (Hayward et al. 2007). Several essentially similar mechanisms that adjust the fatty acid composition of membrane lipids to temperature are found in microbes, plants and animals (Guschina and Harwood 2006). Heterothermic animals most clearly demonstrate the relationships of dietary lipids and their metabolic modifications and anatomical organisation to physiological capacities. For example, the diurnal desert iguana, *Dipsosaurus dorsalis*, can tolerate a wide range of body temperatures (<5 to >40 °C); feeding experiments demonstrate that the fatty

acid composition of dietary lipids determines the temperature at which the lizards choose to rest (Simandle et al. 2001). The effects develop over several weeks and presumably involve alterations in the fatty acid composition of lipid membranes, though the neural links between diet, membrane composition and behaviour are unknown.

Structural lipids are also becoming more important in biomedical sciences. The fatty acid composition of membrane lipids has been implicated as a determinant of natural longevity in several lineages (Hulbert et al. 2014; Galván et al. 2015) and dietary fats correlate with certain psychiatric conditions including long-term cognitive impairment among elderly humans (Solfrizzi et al. 2010).

Although it is generally assumed that some, perhaps many, of the fatty acids in an animal's structural lipids have been components of its own or its mother's storage lipids, trafficking between neutral lipids and phospholipids has been little studied. An exception is the demonstration of the resemblance between the compositions of fatty acids in newly formed lymphoid cells and the triacylglycerols in contiguous adipocytes, suggesting that specialised adipocytes supply fatty acids to adjacent immune cells (Pond and Mattacks 2003; Mattacks et al. 2004a; Pond 2009).

1.5.2 Storage Lipids as Fuels

As well as providing fatty acids appropriate to structural lipids in various kinds of cells operating under various physiological conditions, the composition of triacylglycerols is important to their role as energy stores during strenuous exercise, immune responses and thermogenesis. Biomechanical and metabolic studies show that human running is not very efficient compared to that of animals adapted to fast long-distance travel (Alexander 2004). However, exercise physiologists recognize that comparative studies can offer tips on improving athletic performance.

Long-distance migration in birds, especially small species, is among the most metabolic demanding of all activities, fuelled almost entirely by fast, sustained mobilisation of storage lipids (Weber 2009). Sandpipers (*Calidris pusilla*) demonstrated selective incorporation of dietary fatty acids into structural or storage lipids and evidence for adaptive desaturation that maximises energy density and efficient mobilisation of the storage lipids during prolonged flight (Maillet and Weber 2006). However, studies of another species of sandpiper (*Philomachus pugnax*) produced no evidence for similar selectivity of fatty acids mobilised during shivering elicited by prolonged exposure to cold (Vaillancourt and Weber 2007). This comparison suggests that active lipid management entails some physiological cost: the process is essential preparation for migration (Weber 2009) which requires precise coordination between muscles during flight but is dispensable for shivering, a more chaotic activity. Similar investigations on mammals have not yet been performed.

1.5.3 Fatty Acid Sorting

In mammals including humans, selective deployment and transport of fatty acids begins as dietary lipids are absorbed from the gut (Hodson et al. 2009; Hodson and Fielding 2010). Both brown and white adipose tissue can harbour triacylglycerols of a wide range of compositions and various lipid-soluble substances, including potentially toxic contaminants and metabolic waste products. As well as storing and mobilizing metabolically useful lipids and glutamine, adipose tissue is a repository for such unexcretable end-products, especially in elderly.

The capacity of rat adipocytes for selective release or retention of fatty acids that differ in chain length and degree of saturation was identified more than 20 years ago (Raclot and Groscolas 1993). The process has been demonstrated in several mammals including humans and the cellular mechanisms are now well understood (Raclot 2003). Fatty acids released from adipocytes into the circulation contain more highly unsaturated fatty acids and fewer long-chain saturated and monounsaturated fatty acids than the triacylglycerols from which they are derived. Raclot (2003) concludes that 'the observation that the molecular structure of fatty acids seems to govern their release does not support the idea of a particular demand of the body for specific fatty acids.' Comparative studies in a broader context reveal this conclusion to be unduly pessimistic. When supplemented by fatty acid synthesis and modification, dietary choice and selective intake, these mechanisms contribute to lipid deployment and storage appropriate to temperature and other conditions.

This important biochemical mechanism has been little studied in other vertebrates. Experimental starvation of diamondback rattlesnakes (anatomically advanced, physiologically versatile snakes) kept at temperatures at which they would normally feed found some evidence for selective retention of essential polyunsaturated fatty acids in whole-body homogenates (McCue 2007). Studies of egg formation and embryonic development in the viviparous lizard *Pseudemoia entrecasteauxii* also reveal some capacity for fatty acid sorting in reptiles (Speake et al. 1999).

The process is much more specific and efficient in birds (Speake and Thompson 1999). Avian embryos oxidise mostly carbohydrate in the early stages of development, later switching to lipids. In domestic chickens, the cells lining the embryonic gut start 'eating' droplets of yolk around the twelfth day of incubation and pass its lipids into the blood as lipoproteins. At the same time, mature white adipocytes appear (early compared with mammalian fetuses) and take up the yolk-derived lipids. The adipocytes and the lipoproteins manage the embryo's irreplaceable lipid provisions, incorporating appropriate fatty acids into structural lipids while others are oxidized (Speake et al. 1998). For example, most polyunsaturated fatty acids in the yolk lipoproteins of king penguin eggs are preferentially incorporated into structural lipids in the brain and eyes, while the more abundant saturates are used in energy production (Groscolas et al. 2003). The composition of yolk lipids is similar in several species of penguin in contrasting habitats (Polito et al. 2012).

This capacity for fatty acid sorting is one of the major advances of avian embryos over their reptilian ancestors and is essential to the growth and maturation of the large complex brain and eyes (Speake and Thompson 1999). For example, only 0.24% of the key neural polyunsaturate, docosahexaenoic acid (22:6*n*-3), in the egg yolk of water pythons ends up in the structural lipids of the hatchlings' brains compared to nearly 20% in bird embryos (Speake et al. 2003).

By adjusting the relationship between diet and egg composition, fatty acid sorting facilitates utilization of new foods and extension of range, including breeding in captivity. The avian capacity for fatty acid sorting may be retained into adult life, contributing to selective incorporation of certain polyunsaturated fatty acids into adipocyte triacylglycerols and muscle membranes during the fattening period that precedes long-distance migration, thereby improving the efficiency of prolonged, strenuous exercise (Maillet and Weber 2006; Weber 2009). The fact that fatty acid sorting by adipose tissue has been investigated thoroughly only recently, more than 100 years after its role as a lipid repository was recognised, reflects the progress of scientific concepts and instrumentation.

1.6 Paracrine Interactions with Adipose Tissue

Functional interpretation of the anatomy of brown adipose depots was established long ago: its thermogenesis warms essential organs by direct conduction into contiguous tissues and by convection via the blood (Heaton 1972; Rothwell and Stock 1984; Cannon and Nedergaard 2004). But attempts to interpret the anatomy of the many minor depots of white adipose tissue that are intimately associated with the vasculature, skeletal and cardiac muscle, skin and the immune system have lagged far behind.

Until the 1990s, physiological studies of white adipocytes concentrated heavily on the large depots, especially epididymal and perirenal, which provide enough 'pure' adipose tissue for most biochemical analyses. Adipocytes in the small and large depots are histologically similar, so were assumed to be physiologically and functionally similar as well. Doubts raised by the observation that lymph nodes (in neonatal guinea-pigs) are firmly attached to the surrounding adipose tissue were ignored (Gyllensten 1950). The anatomical arrangement attracted little interest until site-specific properties indicating paracrine interactions between minor adipose depots and contiguous tissues were demonstrated, first in perinodal adipose tissue about lymph nodes (Pond and Mattacks 1995), then in 'adventitious' perivascular tissue around blood (Löhn et al. 2002) and lymph vessels (Dixon 2010).

The concept of 'paracrine' was originally, and largely still is, associated with control systems rather than cellular nutrition (Grossman 1979), reflecting the emphasis on informational mechanisms that has prevailed since the 1960s. Evidence for 'paracrine' interactions between mature adipocytes and other tissues was presented in the mid-1990s (Pond and Mattacks 1995, 1998) but the universality of the mechanism was not recognised until the late 1990s (Trayhurn and Beattie 2001).

These days, the paracrine relationships involving white adipose tissue are mainstream (Rosen and Spiegelman 2014) and are investigated as routes for drug delivery (Trevaskis et al. 2015).

The best understood are with muscle, lymphatics and blood vessels, but in mammals, 'yellow' bone marrow adipocytes secrete several adipokines and may interact locally with osteocytes (Hardouin et al. 2014; Devlin and Rosen 2015). The adipose tissue surrounding the prostate may also modulate its properties (Sacca et al. 2012). Even the epididymal depot of murid rodents, so widely studied as 'archetypal' white adipose tissue that it seemed to have evolved for scientists' convenience, has been recognised as essential to spermatogenesis in the contiguous testes (Chu et al. 2010). Recently, beige adipocytes have been implicated in paracrine mechanisms of tissue repair (Meyer et al. 2015).

1.6.1 The Immune System

The involvement of adipose tissue in immune function was inferred 70 years ago from developmental and anatomical observations (Gyllensten 1950) but became widely recognised in the 1990s, with reports of localized interactions around lymph nodes (Pond and Mattacks 1995) and systemic effects (Grünfeld et al. 1996). Other chapters address the exchange of signal molecules and the role of macrophages in inflammation of adipose tissue in obesity. This section concerns the evolution of functional, non-pathological relationships between adipose tissue and immune structures.

According to a recent theory (van Niekerk and Engelbrecht 2015), the capacity of white adipose tissue to support the metabolic costs of the cellular responses to pathogens was more important for the evolution of adaptive immunity in early vertebrates (i.e. jawless and jawed fish) than gene evolution or selective pressures. Many invertebrate lineages have the necessary genes and are similarly exposed to pathogens (Downs et al. 2014), but inadequate metabolic scope prevented the evolution of adaptive immunity as efficient as that of vertebrates.

The evolution of relationships between adipose and immune tissues can be traced through fish and poikilothermic tetrapods, but has been most thoroughly studied in mammals. At all levels from gross anatomy to molecular complexity, both the immune system and adipose tissues are more elaborate and diverse in mammals than in reptiles. Mammalian lymphoid organs are more numerous and elaborate, and involve more genes, proteins and cell types than those of other vertebrates, and many components are efficiently deployed only in association with membranes of appropriate composition (Zapata and Amemiya 2000). Although anatomically complex lymph nodes widely distributed throughout the body were described long ago as a characteristic feature of eutherian (placental) mammals, immunologist and lymphologists took longer to recognise their functional relationships to adipose tissue (Harvey et al. 2005; Harvey 2008).

Comparative studies show that associations between the immune system and adipose tissue evolved early in mammalian evolution (Pond 2003b). In the echidna

(*Tachyglossus*), a primitive prototherian mammal that lays large eggs (but feeds its nestlings on secreted milk), tiny lymph nodules embedded in fatty tissue are present throughout the chest, neck and pelvic regions (Diener and Ealey 1965). The larger, more complex lymph nodes of Metatheria (marsupials) are surrounded by adipose tissue in adult kangaroos (Old and Deane 2001). Although the authors do not mention adipose tissue, their images of developing lymph nodes in another small metatherian, the quokka (*Setonix brachyurus*), reveal adipocytes surrounding lymphoid tissue by the age of 2 weeks (Ashman and Papadimitriou 1975).

Parallel advances in the anatomical, and probably physiological, relations between adipose and immune tissues also evolved in birds, endothermic descendents of a different group of reptiles. Lymph nodes in birds are smaller, simpler and less abundant than those of mammals, but are nonetheless associated with adipose tissue: 'The simplest [lymph nodes in birds] represent non-encapsulated lymphoid infiltrates embedded in the fat tissue' (Zapata and Amemiya 2000). In the more complex lymph nodes of domestic chickens, lymphoid cells are intimately associated with adipocytes in various ways (Oláh and Glick 1983). Thus close association between lymphoid and adipose tissues seems to be a fundamental feature of endothermic vertebrates.

1.6.2 Perinodal Adipose Tissue Around Lymph Nodes

Investigations into the adipose tissue surrounding lymph nodes were prompted by the observation that these small clumps of adipocytes retained their lipid content in very lean but otherwise healthy wild mammals in which most other adipose tissue—cardiac depots being another important exception—had been depleted to invisibility.

Apart from slightly smaller volume and more extracellular and vascular material, perinodal adipocytes are anatomically indistinguishable from those elsewhere in the same individual and are identified only by biochemical properties (Pond and Mattacks 1995; Pond 2005). All such properties are most pronounced in the adipose tissue nearest to nodes and diminish with distance from them. Perinodal adipose tissue is arbitrarily defined as within a radius of 10 mm around a lymph node. Many, possibly most, of the fatty acids incorporated into lipids in lymph node lymphoid cells that are newly formed in response to immune stimulation are derived from triacylglycerols in perinodal adipocytes (Pond and Mattacks 2003). *In vitro* studies demonstrate that adipose stromal cells migrate from perinodal adipose tissue into adjoining lymph nodes where they interact with indigenous cells (Gil-Ortega et al. 2013).

The adipocytes in depots containing lymph nodes, especially perinodal adipocytes, seem to be partially emancipated from supplying lipolytic products to more remote tissue. Although such adipocytes respond *in vitro* more strongly to maximal noradrenalin, *in vivo*, they contribute less lipolytic products to the circulation during fasting than those in depots containing few or no lymphoid structures (Mattacks and Pond 1999). The basal rate of lipolysis in perinodal adipocytes is slightly lower than

that of other adipocytes but significant increases can be detected within an hour of an experimentally elicited immune response (Pond and Mattacks 1998). Increased release of fatty acids from perinodal adipocytes around the lymph node(s) draining the site of the immune stimulus reaches a maximum after about 6 h and then wanes, disappearing totally after about 24 h, unless prolonged by further stimulation. With repeated immune stimulation, increased lipolysis and responses to interleukin-4 and tumour necrosis factor-α spread to adipocytes situated further from the simulated lymph node within 12 h and to perinodal adipocytes around other, remote, lymph nodes within 24 h (Pond and Mattacks 2002).

The appearance of more receptors for tumour necrosis factor-α on perinodal adipocytes follows a similar time course in response to mild immune stimulation (MacQueen and Pond 1998). Perinodal adipocytes respond much more strongly than those not anatomically contiguous to lymphoid structures to tumour necrosis factor-α, interleukin-4 and interleukin-6 and probably other cytokines (Mattacks and Pond 1999). These signal molecules may mediate the paracrine interactions between adipocytes and the lymphoid cells that they supply.

The popliteal perinodal adipose tissue is most frequently studied only because these depots are easily accessible and being paired facilitates experimental design. The responses of perinodal adipocytes around other lymph nodes are qualitatively similar but differ quantitatively. The largest and most sustained responses are consistently found in the mesentery and omentum of rodents (Pond and Mattacks 2002; Mattacks et al. 2004a; Sadler et al. 2005), and probably also in humans, in which the patterns of site-specific differences in adipocyte triacylglycerol composition (the property most easily measured in preserved samples) are similar (Westcott et al. 2005).

Many of the site-specific differences in gene expression in murine mesenteric adipose tissue compared to epididymal or inguinal (Caesar et al. 2010) can be explained as adaptations to interactions with lymphoid cells within or emanating from lymph nodes. Human visceral depots include more blood vessels, especially in obesity, and are more susceptible to inflammation than superficial adipose tissue (Villaret et al. 2010). The gene products mediating the relationship between lymph vessels and adjacent adipocytes have been identified (Harvey et al. 2005). Chronic inflammation and induced genetic defects in lymph vessel growth can stimulate adipose tissue formation in quantities amounting to obesity (Harvey 2008). Perilymphatic adipose tissue (PLAT) exchanges signal molecules with cells in the lymph vessels it surrounds (Souza-Smith et al. 2015).

1.6.3 Permeating Dendritic Cells

Dendritic cells interact with adjacent adipocytes. Those extracted from the adipose tissue stimulate lipolysis, while those from adjacent lymph nodes inhibit the process, though the effects are strong only in perinodal and milky spot-rich samples and minimal in the adipocytes extracted from adipose sites more than 10 mm from

lymph nodes (Mattacks et al. 2005). Inducing mild inflammation by injection of lipopolysaccharide amplifies these effects, suggesting that they are integral to immune responses. Switching from anti-lipolytic to pro-lipolytic secretions seems to be among the transformations that dendritic cells undergo as they migrate from the lymph nodes through the adjacent adipose tissue, and thus should be considered as part of the maturation process (Mattacks et al. 2005). The lymph vessels that permeate the perinodal adipose tissue facilitate the uptake of dendritic cells from among the adipocytes and return them to the nodes, where they contribute to the inflammatory responses (Kuan et al. 2015).

The fatty acid compositions of lipids in intercalated dendritic cells closely resemble those of adjacent adipocytes (Mattacks et al. 2004a). Site-specific differences and experimental changes of the dietary lipids alter the fatty acid composition of both types of cells, but the similarities between cells that were contiguous *in vivo* remain. The simplest explanation for this resemblance is that maturing dendritic cells acquire fatty acids (and perhaps other precursors) from adjacent adipocytes, rather than from remote sources via the blood or lymph, as was previously assumed (Mattacks et al. 2004a). Structural lipids are the most easily traced, but those used for the production of signal molecules or ATP are probably of similar origin.

In all normal monogastric mammals that have been investigated, the triacylglycerols of adipocytes near to lymph nodes are disproportionately rich in polyunsaturated fatty acids, including the specific precursors of eicosanoid and docosanoid signal molecules that are integral to lymphoid cell function (Mattacks and Pond 1997; Pond 2003c). These differences in composition presumably arise by selective uptake and/or release of fatty acids that differ in chain length and degree of unsaturation (Raclot 2003). The site-specific differences in adipocyte-derived fatty acids thus conferred on intercalated dendritic cells add another source of structural, and perhaps also functional, diversity to these cells that hitherto have been classified by genes activated and proteins synthesised (Gehring et al. 2008).

In rats fed unaltered or sunflower oil-supplemented diets, prolonged experimental inflammation alters the composition of fatty acids in lipids of perinodal adipose tissue, and hence that of fatty acids incorporated into permeating dendritic cells (Mattacks et al. 2004a). But the fatty acid composition of phospholipids in such dendritic cells from unstimulated and immune-stimulated rats whose diet over the previous 6 weeks has been supplemented with fish oils are indistinguishable from those of immune-stimulated rats eating standard diets and hardly change under experimental inflammation. These data imply that diets enriched with fish oil create membrane compositions in dendritic cells that are ideal for supporting the immune response, thus eliminating the need for further adaptation in response to immune stimulation. Over a period of several weeks, the ratio of *n*-6/*n*-3 fatty acids in triacylglycerols in the perinodal adipose tissue surrounding the locally inflamed lymph node also changes, partially rectifying the composition imposed by dietary imbalances (Mattacks et al. 2004a). This mechanism may be among the ways that perinodal adipocytes minimise the impact of fluctuations in dietary lipids on whole-body immune function and may be physiologically important, especially during fasting and hibernation (Pond 2009).

The involvement of perinodal adipocytes in immune responses not only begins within minutes but can persist for months. In a rat experiment to explore recovery from simulated low-level chronic inflammation, the numbers of dendritic cells recovered from the locally stimulated lymph node and its perinodal adipose tissue were found to rise at least tenfold within 4 weeks of local subcutaneous injection of 20 μg of lipopolysaccharide three times a week and remained high for as long as this regime was applied (Sadler et al. 2005). Dendritic cell numbers were still significantly above baseline 12 weeks after termination of the regime of simulated low-level chronic inflammation. These effects were observed in node-associated adipose tissue remote from the site of stimulation as well as that adjacent to it with parts of the mesentery and omentum being among the most responsive. The mesenteric lymph nodes and their contents atrophy in mice made obese by a high-fat diet, apparently poisoned by high concentrations of fatty acids and lipoproteins (Kim et al. 2008). These findings have implications for slow, deleterious changes in both the immune system and adipose tissue induced by chronic stress and prolonged inflammation.

1.6.4 Adipose Tissue in Normal Immune Function

Immune cells of the innate and adaptive systems, including macrophages, neutrophils, B cells and T cells permeate adipose tissue at normal body composition and, in greater numbers, in obesity (Grant and Dixit 2015; Travers et al. 2015). But 'ordinary' subcutaneous white adipocytes respond to infections in adjoining skin by secreting antimicrobial peptides, supplemented by local proliferation and maturation of preadipocytes (Zhang et al. 2015). Inflammatory processes in metabolically active adipocytes are an integral component of adipose tissue's response to demand for increased fat storage (Asterholm et al. 2014). Although impaired interactions between the tissues are fundamental to obesity (Grant and Dixit 2015), the attitude that adipose tissue is controlled by the immune system (Brestoff and Artis 2015) is questionable.

Perinodal adipose tissue is specialized for more precise, localized paracrine interactions with the immune system, as summarized in Fig. 1.1. Many immunologically important fatty acids are dietary essentials, and hence can be limiting, especially during anorexia associated with major inflammatory diseases (Johnson 2002). By ensuring that the immune system has priority access to essential lipids, this mechanism complements sickness-induced anorexia, an ancient mechanism that has been demonstrated in arthropods (Adamo et al. 2010) and lower vertebrates as well as in mammals (Johnson 2002; Straub et al. 2010).

Without effective lipid management, key precursors may not be available when and where they are needed and could be squandered by increased oxidation of lipids during anorexia. By releasing appropriate fatty acids to lymphoid cells when and where they are required, the perinodal adipose tissue promotes efficient utilization of essential fatty acids and partially emancipates immune function from fluctuations

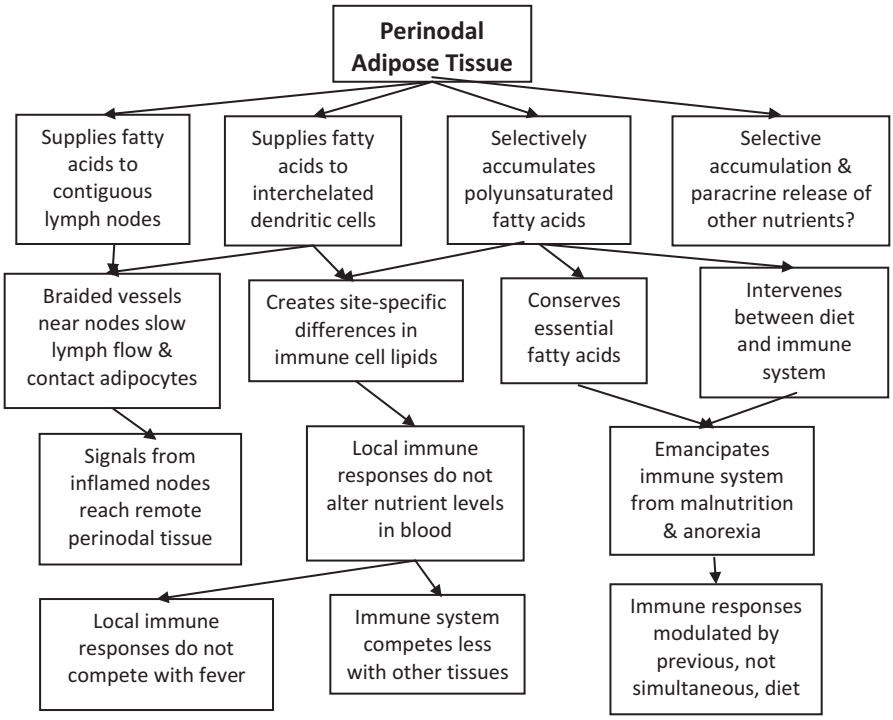

Fig. 1.1 Summary of the structure, properties and functions of mammalian perinodal adipose tissue and their roles in metabolism during immune responses

in the abundance and composition of dietary lipids (Pond 2003b). In rats, the selective accumulation of polyunsaturated fatty acids that generates the *n*-6/*n*-3 ratio appropriate for lymphoid cells is quite slow (Mattacks et al. 2004a) and can probably be overwhelmed by prolonged dietary deficiencies or excesses. Nothing is known about the extent to which the efficiency and robustness of these mechanisms differ between individuals or between species, thus making their immune systems more, or less, susceptible to impairment by dietary imbalance or insufficiency.

Paracrine control of lipolysis by lymphoid cells reduces competition with other tissues for specific, essential lipids, thus enabling fever and other energetically expensive defences against pathogens to take place simultaneously with proliferation, maturation and activation of lymphoid cells and with functions such as lactation and exercise, even during anorexia or starvation (Pond 2007). Under some circumstances, notably prolonged anorexia nervosa, immune function remains surprisingly efficient in spite of massive reduction in adipose tissue mass (Nova et al. 2002), less fever in response to infection (Birmingham et al. 2003) and altered plasma cytokines (Brichard et al. 2003). As long as local interactions between adipose and lymphoid tissues are unimpaired, the mammalian immune system can probably function over a wide range of body compositions. Obvious cachexia with extensive muscle