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Nutritional ecology of essential fatty acids: an evolutionary perspective

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Abstract

There are four types of fatty acids but only two types are essential nutritional requirements for many animals. These are the omega-6 polyunsaturated fatty acids (n-6 PUFA) and the omega-3 polyunsaturated fatty acids (n-3 PUFA) and because they cannot be converted to one another they are separate essential dietary requirements. They are only required in small amounts in the diet and their biological importance stems largely from their role as constituents of membrane lipids. They are synthesised by plants and, as a generalisation, green leaves are the source of n-3 PUFA while seeds are the source of n-6 PUFA in the food chain. While the fatty acid composition of storage fats (triglycerides) is strongly influenced by dietary fatty acid composition, this is not the case for membrane fats. The fatty acid composition of membrane lipids is relatively unresponsive to dietary fatty acid composition, although n-3 PUFA and n-6 PUFA can substitute for each in membrane lipids to some extent. Membrane fatty acid composition appears to be regulated and specific for different species. The role of essential fats in the diet of animals on (1) basal metabolic rate, (2) thermoregulation, (3) maximum longevity, and (4) exercise performance is discussed.

Nutritional ecology of essential fatty acids:
an evolutionary perspective

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Abstract.

There are four types of fatty acids but only two types are essential nutritional requirements for many animals. These are the omega-6 polyunsaturated fatty acids (n-6 PUFA) and the omega-3 polyunsaturated fatty acids (n-3 PUFA) and because they cannot be converted to one another they are separate essential dietary requirements. They are only required in small amounts in the diet and their biological importance stems largely from their role as constituents of membrane lipids. They are synthesised by plants and as a generalization, green leaves are the source of n-3 PUFA while seeds are the source of n-6 PUFA in the food chain. While the fatty acid composition of storage fats (triglycerides) is strongly influenced by diet fatty acid composition, this is not the case for membrane fats. The fatty acid composition of membrane lipids is relatively unresponsive to diet fatty acid composition, although n-3 PUFA and n-6 PUFA can substitute for each in membrane lipids to some extent. Membrane fatty acid composition appears to be regulated and specific for different species. The role of essential fats in the diet of animals on (i) basal metabolic rate, (ii) thermoregulation, (iii) maximum longevity, and (iv) exercise performance is discussed.

Introduction

Fats are essential for life; not because they are a source of energy but rather because they are essential components of membranes. All living cells are separated from the environment by a lipid-based cellular membrane. Within the three major domains of life – Archaea, Bacteria and Eucarya – the Archaea differ markedly from both the Bacteria and Eucarya in the fats that make up their membranes. While the core of archaeal cell membranes consists of isoprenoid chains both bacterial and eukaryotic membranes are based on lipids containing fatty acid chains. The isoprenoid chains that constitute the membrane lipids of the Archaea provide a "toughness" to their membranes that allows them to make their living in some of the most hostile environments on our planet (see Hochachka and Somero 2002; Valentine and Valentine 2010). In this brief review, we will only concern ourselves with the fatty acids that constitute the bacterial and eucaryotic membranes.

We have commenced this contribution with a broad evolutionary perspective to counteract the common emphasis on energy provision and storage when considering the biology of fats. In our opinion, this is an unfortunate consequence of the most common approach to teaching lipid biochemistry, which is generally anthropocentric rather than evolution-centric. When teaching lipids, generally triacylglycerols (and energy-storage) are covered first and the more complex phospholipids (and membrane structure) are considered later. Yet from an evolutionary perspective phospholipids precede triacylglycerols. Our basic biochemistry (including lipogenesis) was evolved by the bacteria before the evolution of eucaryotes and from an evolutionary perspective, the processes of lipogenesis (the synthesis of fatty acids) can be interpreted as an important process to manufacture the essential membranes of life rather than the synthesis of an energy store. Bacteria do not store their energy as triacylglycerol molecules and the evolution of triacylglycerols as an efficient form of energy storage can be considered as a (eukaryotic) modification of the basic processes (evolved by prokaryotes) to make membrane lipids. Indeed, in their cellular synthesis, triacylglycerols are made by modification of a phospholipid (phosphatidic acid).

Like they have DNA, all living organisms also have lipid membranes and just as DNA has been described as an 'eternal molecule', so lipid membranes can be considered an 'eternal structure' as membranes are made from pre-existing membranes. Bacteria and plants are capable of making all their membrane lipids 'de novo' from non-lipid sources and thus fats are not essential nutrients for them. However, this is not the case for higher animals

which although they can synthesise both saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) from non-lipid sources, they are incapable of 'de novo' synthesis of the essential polyunsaturated fatty acids (PUFA). Consequently, neither SFA nor MUFA are essential in the diet of animals. However, both types of polyunsaturates, the omega-3 PUFA and the omega-6 PUFA, are essential requirements in the diet of higher animals. Furthermore, although higher animals are able to elongate and further desaturate both omega-3 PUFA and omega-6 PUFA they are unable to inter-convert these two types of PUFA and thus both omega-3 and omega-6 PUFA are *independent* and *separate* essential components of the diet of higher animals. We use the vague phrase 'higher animals' in this context because although *C. elegans* have the genes for the necessary enzymes they are not present in the *Drosophila* genome.

The physiological reason for the dietary essentiality of omega-3 and omega-6 PUFA relates to their essential role in membranes and hormone precursors. All diet fatty acids, (whether they are SFA, MUFA or both types of PUFA) can be catabolized as a source of energy and all provide approximately the same amount of energy per unit mass. As far as the provision of metabolic energy is concerned neither the length of the fatty acid molecule nor its degree of saturation/unsaturation is significant. Indeed, because energy can be obtained from other molecules (such as carbohydrates and proteins), although fatty acids are common sources of energy, they are not essential sources of energy in the diet of animals. However, this is not the case for the manufacture of cellular membranes and it is for this reason that some fatty acids are essential dietary requirements.

The fatty acid chains that comprise membranes of animal cells are generally limited to chain lengths of between 16 carbons and 22 carbons. The fact that the biochemical process of lipogenesis generally produces palmitic acid (a 16-carbon saturated fatty acid) is likely a reflection of its initial importance in the manufacture of bacterial membrane lipids. In bacteria, plants and animals 'elongase' enzymes are responsible for producing longer chain fatty acids (i.e. >16 C) from this 16-carbon product of lipogenesis and a series of different 'desaturase' enzymes are responsible for the insertion of double bonds into specific positions these long-chain (i.e. 16-22 carbon) fatty acids. Figure 1 provides an outline of the basic structure of fatty acids as well as the main steps involved in the synthesis of a variety of fatty acids in plants and animals.

(Fig 1 here).

Sources of essential fatty acids in the diet: leaves, seeds and meat

The evolutionary explanation as to why both omega-3 PUFA and omega-6 PUFA became essential components of the diet of animals is similar to the explanation as to (i) why ascorbic acid is also known as vitamin C for some (but not all) mammals and (ii) why some amino acids are also essential in the diet of animals.

Most mammals can synthesise ascorbic acid (either in the liver or kidney, or both) and thus it is not a vitamin (i.e. an essential dietary micronutrient) for them. However, humans and some other species cannot synthesise ascorbic acid and for these species it is a vitamin. The evolutionary interpretation of this situation for humans is that one of our recent ancestors 'lost' the enzymatic ability to synthesise enough ascorbic acid for normal function. Of course, this loss doesn't need to be complete, but it can be due to mutational changes in one or more of the enzymes responsible such that the enzymes either become incapable or less capable of ascorbic acid synthesis. However, if the diet of the animal concerned already contains enough ascorbic acid to satisfy requirements, there is no selective disadvantage associated with this mutational change and this is the time when the ascorbic acid became vitamin C. This is thought to have been the case during the evolution of humans and such a proposal is compatible with the high degree of frugivory in primate diets (e.g. see Chivers 1992). Similarly ascorbic acid is a vitamin for a number bat species including fruit bats (Jenness *et al*. 1980).

The same principle applies to other essential components of the diet of animals, such as certain amino acids. Really, it is not protein per se that is an essential component in the diet of animals, but rather it is a number of amino acids that are essential. It is because amino acids are normally obtained by animals in the form of proteins (i.e. combined amino acids) that we regard protein as dietary essential. The same principle applies to the polyunsaturated fats. Not all fats are essential, only the two types of polyunsaturates. It is of interest that after it was discovered that certain fats were essential in the diet of rats, it was initially suggested that they should be classified as 'vitamins'. This is because the amount required was quite small and more consistent with the vitamin requirements than energy requirements. This situation emphasises the importance of fats as essential components of cellular membranes rather than essential source of energy. Fats are not essential dietary requirements for all animals. For example, although *Drosophila melanogaster* and other higher animals lack the

enzymes to synthesise n-6 PUFA and n-3 PUFA, this is not the case for the simpler diploblastic nematode, *Caenorhabditis elegans* (e.g. see Shmookler Reis *et al.* 2011).

In the natural environment, animals generally eat other organisms (or parts of them). Thus they eat the membranes of other organisms and apart from some exceptional cases, there is no such thing as a 'fat-free' diet for animals in the wild. Although, there may be no visible fat in an animal's food, it is almost always present in the form of cellular membranes. Nectar feeders (such as hummingbirds, honeybees, etc., as well as some mammals) obtain their energy overwhelmingly as dietary carbohydrate. However they cannot live on sugar alone and generally must also consume some cellular components. Thus many nectar-feeders also consume insects or other parts of the plant, notably pollen. Often this is described as necessary for their 'nitrogen' requirements (more properly, it should be said that it is necessary for their essential amino acid requirements) but of course it is also necessary for obtaining essential fats and vitamins. For many years, it was thought that because intact pollen was a major component of the faeces of some pollen-eaters that such animals were unable to use the nutrients that pollen contain. However, it for the small mammal *Cercatetus nanus*, pollen is an excellent source of protein with pollen nitrogen having a greater biological value than insect nitrogen (van Tets and Hulbert 1999). Thus obviously pollen is capable of being digested and, although we know of no specific studies, it is almost certainly a significant source of essential fats for some species. This is supported by the high relative abundance of both types of PUFA in pollen which together can constitute nearly 60% of the fatty acids in pollen fats (e.g. Manning 2001; Haddad *et al.* 2007).

Plants are generally the original source of both types of PUFA in the food chain. Although both types of essential fats come from plants, as a generalisation it can be said that omega-3 PUFA dominate in plant leaves while omega-6 PUFA dominate in seeds. Indeed it appears that omega-3 PUFA may be essential components of chloroplast membranes. The relative abundance of these two types of essential fats can be quantified as *PUFA Balance* (= omega-3 PUFA as percent of total PUFA) and Figure 2 presents the PUFA balance of several representative foods. As can be seen from this figure, "leaves" have considerably more omega-3 PUFA than they have omega-6 PUFA (i.e. a *high PUFA Balance*) while "seeds" have more omega-6 than omega-3 PUFA (i.e. a *low PUFA Balance*).

(Fig 2 here)

Because animals can neither synthesise nor inter-convert omega-3 and omega-6 PUFA, their PUFA Balance of their own fats is often influenced by diet. For example, marine/aquatic food is relatively high in omega-3 PUFA; i.e. it has a high PUFA Balance value (see Fig 2). The basic reason for this is that the plants in marine ecosystems are predominantly "leaves" and do not make "seeds". Even for terrestrial animals, the nature of their food will influence their PUFA Balance. For example, the meat (muscle) and fat from 'grass-fed' animals have higher PUFA Balance values than those from 'grain-fed' animals (e.g. Fig 2). The phrase "you are what you eat" comes to mind but a more accurate phrase would be "you are influenced by what you eat". This is because the fats of food are total fats, i.e. the combination of storage fats (triglycerides) and membrane fats (phospholipids), and the fatty acid composition of these two different types of fats respond differently to dietary fatty acid composition. This will be considered in more detail in the next section.

There is another general difference between the essential fats obtained from plant food and animal food sources. The PUFA (both omega-3 and omega-6) from plant foods are generally 18 carbons long, whereas those from animal foods include 20-carbon and 22-carbon as well as the 18-carbon fatty acids. Indeed, some animal-based fats (e.g. fish) overwhelmingly contain 20- and 22-carbon PUFA and negligible 18-carbon PUFA. These 20- and 22-carbon PUFA are not only longer but are also generally more polyunsaturated than the plant-based PUFA (see Fig 1). If they have not been obtained preformed in the diet, they are all made by modification of 18-carbon PUFA in diet via the action of the elongase and desaturase enzymes as outlined in Figure 1. Animals that eat other animals will obtain these 20- and 22-carbon PUFA already preformed in their diet. Cats (and possibly felids in general) have lost the enzymatic ability to desaturate the 18-carbon PUFA and are thus unable to make 20- and 22-carbon PUFA (Rivers *et al.* 1975). Cats are thus *obligate* carnivores.

Interestingly, this may not be restricted to felids as recent studies suggest the adults of the blowfly *Calliphora stygia* are also unable to synthesise 20- and 22-carbon PUFA from 18-C PUFA in their diet (Hulbert, unpublished observations). Although in the lab adults can be maintained and lay eggs when fed only sugar and yeast, larval blowflies likely have an essential requirement for preformed long-chain PUFA in their diet. Like lions and tigers, blowflies may also be *obligate* carnivores!

Little is known about the specific nutritional requirements of most species and this is especially true of the essential fatty acid requirements of different species. As with vitamins, in some species gut microbes maybe an important source of newly synthesised PUFA. As

outlined above, PUFA are essential in the diet to manufacture membrane lipids and not to provide energy (although they can be catabolized with similar efficiencies as the non-essential fats) and consequently the actual amounts required in the diet are relatively small. For example, for humans it is suggested that omega-6 PUFA should constitute ~2%, and omega-3 PUFA ~1% of dietary energy intake (ISSFAL - International Society for Study of Lipids and Fatty Acids).

The simplified account of the synthesis and biochemical modification of fatty acids presented here is largely based on knowledge from terrestrial plants and vertebrate animals (predominantly mammals). The importance of membranes in living organisms has resulted in much diversity in the biochemical processes for both synthesis and modification of fatty acids in marine organisms, especially marine microbes. This has resulted in distinctive fatty acid patterns ("signatures") in the lipids of some organisms. When combined with the limited biochemical modification, large reservoirs of storage fats in some animals as well as other characteristics, has led to the recent development of "quantitative fatty acid signature analysis" of storage fats as a method to estimate predator diets and for trophic analysis. Consideration of this area is beyond this manuscript and the interested reader is referred to other sources (e.g. Iverson et al. 2004; Nichols & Nichols 2008; Thiemann et al., 2008; Williams & Buck 2010).

Dietary fats affect storage fats but have only small effect on membrane fats

The diet of humans can be extremely variable between individuals as well as over time. For other species, there is normally much less variation between individuals but the diet of animals can vary considerably over time both because of seasonal and geographic availability of food. Thus the fatty acid composition of the diet can sometimes vary both for a single species and between species. Indeed as mentioned at the end of the previous section this is used for trophic analysis, especially of marine ecosystems.

The influence of a particular environmental parameter on an organism can be quantified by analysing in detail the relationship between the same variable in the environment and the organism. This has been previously carried out for a variety of physical variables (such as temperature, osmotic pressure, etc.,) but not so commonly for chemical variables (such fatty acid composition). When the body temperature of an animal is constant despite environmental temperature variation (i.e. the slope of the relationship = 0), the animal

is called a "thermoregulator". At the other extreme, when body temperature varies in response to environmental temperature, such that essentially body temperature is the same as environmental temperature (i.e. the slope of the relationship = 1), the animal is called a "thermoconformer". Similarly, species can be categorised as "osmoregulators" or "osmoconformers". Such categories are not completely exclusive. For example, (i) the same animal could be an "osmoregulator" over a certain range of saline environments but be an "osmoconformer" over a different range of environmental salinities; or (ii) an animal could partly but not completely compensate body fluid concentration in response to changes in environmental salinity (e.g. say the slope of the animal/environment relationship = 0.5). In this latter case, the animal is partly a "conformer" and partly a "regulator".

This approach was used by Hulbert *et al*. (2005) to quantify the relationship between diet fatty acid composition and membrane fatty acid composition. Recently, it has been used to compare the diet influence on both membrane lipids (i.e. phospholipids) and storage fats (i.e. triglycerides) for an outbred laboratory strain of the rat (*Rattus norvegicus*) fed a moderate fat (25% energy) diet. The results of this study for skeletal muscle membrane lipids have been published (Abbott *et al.* 2010) while those for membrane lipids of other tissues as well as storage fats are currently in review. This study used twelve diets that were nutritionally complete and identical except for fatty acid composition. Some of its findings are presented in Figure 3.

(Fig 3   here)

As can be seen from Figure 3, membrane fats and storage fats differ in their relationship to diet fatty acid composition. While storage lipids were quite responsive to diet fatty acid composition this was not the case for membrane lipids, which were relatively unresponsive. We can use the slope of the relationships to quantify the 'responsiveness' to the composition of the diet. A slope of '0' indicates no response (i.e. perfect regulation irrespective of diet composition) while a value of '1' indicates strong response (i.e. perfect conformity with diet composition). For SFA storage fats had an average slope of 0.27, while membrane lipids had an average slope was 0.01. With respect to MUFA, the average slopes were 0.53 and 0.06 respectively for storage fats and membrane lipids. Similarly for both classes of essential fatty acids, the corresponding average slopes for the n-6 PUFA were 0.62

and 0.18, while for the n-3 PUFA they were 0.44 and 0.11 respectively for storage fats compared to membrane lipids.

In summary, this study on rats showed that adipose tissue triglycerides demonstrated the greatest responsiveness to diet, plasma triglycerides were the next most responsive to diet fatty acid composition. The fatty acid composition of brain membrane lipids was almost completely unresponsive to diet, and while membrane lipids for the other three tissues (liver, heart and skeletal muscle) were slightly more responsive than brain, they were also relatively unresponsive. If we compare the different types of fats, we find that, for both membrane lipids and storage fat, diet n-6 PUFA content had the greatest influence while diet SFA content had the least influence.

Although Figure 3 shows that a moderate-fat diet has limited influence on the membrane composition (at least in the rat), the conclusion that diet has little influence on membrane composition needs to include the proviso: "as long as the diet is not devoid of either, or both of the essential fats". Many studies of laboratory animals have shown that if the diet is deficient of these essential fatty acids, then membrane fatty acid composition is modified such that there is generally an elevated MUFA content as well as the appearance of the unusual mead acid (20:3 n-9). This 20-carbon fatty acid is an omega-9 PUFA that is synthesised by elongation and desaturation of oleic acid (18:1 n-9), the most common MUFA in membranes. Mead acid is normally not observed in membrane lipids from animals fed diets with sufficient omega-6 PUFA and omega-3 PUFA. It is nowadays used as a clinical marker of essential fatty acid deficiency.

The influence of membrane fatty acid composition on animal function

Relatively little is known about the mechanisms that regulate fatty acid composition of membranes but the fact that membrane fatty acid composition varies among mammal and bird species in a systematic manner suggest that it is ultimately under genetic control. There is rapid turnover of fatty acids in membrane lipids and it is likely that it is the specific characteristics of the membrane-located enzymes responsible for the cycles of deacylation-reacylation of membrane lipids that determine the fatty acid composition of membranes. It is likely that it is a physical property of membrane bilayers (such as membrane fluidity/viscosity) that is actually regulated. For example it is known that at least one of these enzymes, an acyl transferase, is controlled by the fluidity of the surrounding membrane

bilayer (Fryst *et al.* 1996). Another piece of evidence of genetic control is that different wild-derived mice strains differ in membrane fatty acid composition (Hulbert *et al.* 2006) despite being kept in the same environment and fed identical diets for their entire lives (Miller *et al.* 2002).

It has long been known that the pace of metabolism varies with body mass of mammals and bird species (e.g. Kleiber 1961) and that this has substantial ecological implications for endotherms (e.g. Peters 1983). Investigation of the mechanistic basis of this body-size variation in metabolic activity showed that it was made up of two main factors: (i) smaller endotherms tend to have relatively larger internal organs, and (ii) that the cellular metabolic rates are greater in small compared to larger endotherms (see Hulbert and Else 1999; 2000). Furthermore, most of the energy consumed during resting cellular metabolic rate of endotherms is associated with membrane-associated activities. For example, the high cellular metabolic activity of small mammals compared to large mammals was associated with 'leakier' membranes and consequently high levels of ion pumping by the membrane-associated Na^+ pumps (Couture and Hulbert 1995a). Similarly, the high level of cellular metabolic activity of endotherms compared to similar-sized ectotherms were also associated with greater activity of the membrane-associated Na^+ pumps in cells from endotherms (Hulbert and Else 1990). These findings emphasised the importance of membranes in cell metabolic activity.

Following up the observation by Gudbjarnason *et al.* (1978), relating heart rates of mammals (ranging in size from mice to whales) to fatty acid composition (specifically 22:6n-3 content) of their cardiac phospholipids (i.e. their cardiac cellular membranes), it was discovered that such a relationship was not restricted to the heart but was also present in a number of other mammalian tissues (Couture and Hulbert 1995b; Hulbert *et al.* 2002b). Since this initial finding, correlations between membrane fatty acid composition and species body size have been reported for mitochondrial membrane phospholipids as well as total tissue phospholipids, for several tissues and for birds as well as mammals (e.g. Porter *et al.* 1996; Hulbert *et al.* 2002a; Brand *et al.* 2003). Although the relationship differs between mammals and birds, and is different between tissues, there is a consistent pattern among the various studies whereby the membrane lipids from fast-metabolism small species have a higher unsaturation index (i.e. a greater density "double-bonded carbons" in the membrane bilayer) than slow-metabolism large species. The dominant contributor to these trends were the omega-3 PUFA and especially docosahexaenoic acid (22:6 n-3), which is the most highly

polyunsaturated fatty acid commonly found in natural membranes. A similar association of membrane fatty acid composition with cell metabolic rate has been observed in comparisons of endotherms and ectotherms of the same-size and with the same body temperature.

A brief comment should be made concerning a recent study of muscle membrane fatty acid in mammals ranging from mice to elephants (Valencak and Ruf 2007). Although the title of this study concluded "n-3 polyunsaturated fatty acids … have no role for metabolism", it should be noted that their method of analysis 'corrected' for body mass trends. In other words, it statistically factored out any variation in basal metabolic rate between the different species that was also associated with variation in their body mass. A more accurate conclusion from their analysis would have been that their data demonstrated no statistically significant relationship between the n-3 PUFA content of muscle phospholipids with the variation in basal metabolic rate that is <u>NOT</u> associated with variation in body mass. Conventional analysis of their data (provided in supplementary tables) show similar body-size relationships as those described by the previous studies cited above.

While most previous comparative studies show correlations (or associations) between membrane composition and metabolic activity of different species, and that species with membranes that have relatively high amounts of n-3 PUFA also have faster membrane-associated activities, they do not provide experimental proof of a connection between membrane composition and cellular metabolic activity of different species. Such experimental support of the importance of membrane lipids for cellular metabolic rate comes from 'species-crossover' studies. In these studies it has been experimentally demonstrated that the molecular activity of individual Na^+ pumps from endotherms decrease their activity when their surrounding membrane lipids are replaced with membrane lipids from ectotherms and furthermore that the converse also occurs, namely ectotherm pumps increase their molecular activity when surrounded by endotherm membrane lipids (Else and Wu 1999; Wu *et al.* 2004). There is evidence that this effect is likely due to the physical properties (such as their compressibility, lateral pressure, fluidity etc.,) imparted to membrane bilayers by their distinctive fatty acid compositions (Wu *et al.* 2002).

The physical properties (e.g. fluidity, viscosity) of membrane bilayers are strongly influenced by temperature such that as temperature is lowered membranes bilayers become more viscous. To compensate for this effect, many animals alter the fatty acid composition of membrane lipids when kept at low temperatures. This response to low temperature often involves the increased incorporation of both MUFA and PUFA and has been called

"homeoviscous" adaptation. It was first described for bacteria but is also manifest in vertebrates, being most studied in fish (e.g. see Hazel 1995).

Membrane PUFAs are also very important membrane constitutents because of certain chemical properties. In this respect, they are in strong contrast to membrane SFA and MUFA. For example, arachidonic acid chains (20:4 n-6) attached to phospholipids are the source of important chemical messengers. Both eicosanoids (inflammation mediators) and endocannabinoids (appetite regulators) are manufactured by enzymatic modification of membrane lipids containing this fatty acid. A huge amount of research has been carried out on these processes with respect to physiological and medical implications but, to our knowledge, almost none concerning nutritional ecology. Another distinctive property of membrane PUFA is that they are capable of undergoing lipid peroxidation. In this respect, both omega-3 and omega-6 PUFA in membranes are distinctly different to SFA and MUFA. Furthermore, the more polyunsaturated a membrane PUFA molecule is, the more prone it is to peroxidative damage. In light of this, the systematic patterns of membrane composition observed among mammals and birds have been related to their distinctive maximum longevities. Species with highly polyunsaturated membranes tend to be short-living while long-living species tend to have membrane lipids that are less polyunsaturated but contain more MUFA. This has been called the "membrane pacemaker theory of aging" (reviewed by Hulbert *et al.* 2007).

Influence of dietary fats on animal function and ecology

Most experimental research on the influence of dietary fat has been carried out with a medical, health or agricultural perspective and has been performed using either humans, laboratory rodents or agricultural animals. Relatively little has been carried out with the ecological implications as the emphasis. We will limit this very brief review to consideration of diet fat effects on (i) basal metabolic rate, (ii) thermoregulation, (iii) maximum lifespan and (iv) exercise performance of animals.

Basal metabolic rate

If membrane fatty acid composition is an important factor influencing basal metabolic rate (BMR) but diet fatty acid composition has only a very small influence on membrane fatty acid composition then we can conclude that manipulation of diet fatty acid

composition should have only minimal influence on basal metabolism. This seems to be the case, although there are reports of small changes in metabolic rate (generally not BMR) in response to changes in diet fatty acid composition in some animals. A lack of substantial effect of diet fatty acid composition on BMR is supported by the fact that the fatty acid composition of the diet fed to species prior to their fasting (for the determination BMR) is ignored by different laboratories, although they often report similar values for the same species. Indeed, in many cases the fatty acid composition of diet fed to species in captivity will differ to that of their normal diet in the wild. In the study of rats fed the twelve moderate-fat complete diets that differed only in fatty acid composition referred to previously (see Fig 3), there were minimal effects of diet fat composition on their minimal metabolic rate measured at room temperature 22-24°C (Abbott and Hulbert, ms in preparation). This metabolic rate is related to, but not the same as BMR.

The conclusion that diet fatty acid composition will likely have minimal influence on a species BMR is based on two assumptions; (i) that the diet contains enough essential fats (i.e. PUFA) to satisfy the minimal requirements of the particular species, and (ii) that the influence of diet on membrane composition in the particular species is similar to that observed for the rat (see figure 3). Two studies report feeding rats a high PUFA diet increased metabolic rate compared to a saturated fat diet however neither measured metabolic rate under BMR conditions (Shimomura *et al.* 1990; Takeuchi *et al*. 1995). Similarly, both baboons (Savage and Goldstone 1965) and fowl (Newman et al. 2002) have a higher metabolic rates when fed diets with high PUFA content compared to being fed a saturated fat diet, however in both cases metabolic rate was not measured under BMR conditions. Similarly, Pierce et al. (2005) report that diet fatty acid composition significantly affected peak metabolic rate but not the resting metabolic rate of the migratory songbird *Vireo olivaceous*.

Although it is not evidence of a direct effect of diet on BMR, it is of interest, in view of the high omega-3 PUFA content of their food, that both fish-eating seals (Hurley and Costa 2001) and fish-eating seabirds (Ellis 1984) have BMRs higher-than-predicted for their body mass. Similarly, in view of the low PUFA and high MUFA content of termites (see Faulkenstein *et al.* 2001), it is of interest that termite-eating mammals have a lower-than-predicted BMR (McNab 1986).

Thermoregulation

Ectothermic vertebrates behaviourally thermoregulate around a "preferred" body temperature. Almost twenty years ago Geiser and colleagues reported the fascinating finding that diet fatty acid composition influenced the preferred body temperature that lizards select and this was later confirmed by other investigators (Simandle *et al*. 2001). All species investigated selected a lower preferred body temperature when fed a PUFA-enriched diet than when fed an SFA-enriched diet (Geiser *et al*. 1992; 1994). Since the mechanisms whereby a specific "preferred" body temperature is 'chosen' or regulated are unknown, it is also not known how diet fatty acid composition may change the preferred body temperature. Alterations in eicosanoid production and metabolism, as well as changes in membrane composition are possible mechanisms.

Changes in membrane fatty acid composition were implicated early in study of hibernation physiology (Raison and Lyons 1971) and although some of the remodelling of cellular membrane lipids prior to the hibernating season is independent of diet (e.g. Arnold et al. 2011) dietary changes may also be important. Several laboratory studies have shown PUFA-enriched diets result in longer hibernation bouts and lower body temperatures during hibernation (e.g. Geiser and Kenagy 1987, Frank 1992; Florant 1998). This influence of dietary PUFA has also been reported for lab studies on mammals that exhibit shallow daily torpor (Geiser 1991) as well as for birds (Ben-Hamo et al. 2011). It is not restricted to laboratory studies, as measurements on free-ranging arctic ground squirrels have shown diet PUFA levels influence subsequent torpor patterns (Frank et al. 2008). Although the membrane composition of homeothermic mammals appears relatively resistant to diet-induced changes, this may not the case for hibernating mammals. For example, Geiser (1990) was able to change (by 20%) the unsaturation index of mitochondrial membrane lipids of the chipmunk (*Eutamias amoenus*) by diet manipulation.

These studies of diet PUFA effects on thermoregulation have generally used vegetable oils as the PUFA source for diet-enrichment. Consequently, because the PUFA in vegetable oils are overwhelmingly omega-6 PUFA, the effects of diet PUFA obtained may be restricted to these essential fatty acids and may not be due to omega-3 PUFA, the other type of essential fatty acids. In this respect it is interesting that in a study examining the influence of diet-enrichment with omega-3 PUFA on hibernation in the marmot (*Marmota flaviventris*) very different effects were observed (Hill and Florant 2000). These investigators found that marmots fed a diet enriched with the omega-3 PUFA, linolenic acid (18:3 n-3), did not

hibernate at all, while marmots fed a control diet with plenty of the omega-6 PUFA, linoleic acid (18:2 n-6) exhibited normal hibernation behaviour. This is interesting for two reasons. Firstly, linoleic acid (18:2 n-6) is the dominant PUFA found in seeds while linolenic (18:3 n-3) is the dominant PUFA of green leaves and consequently implicates the type of food eaten by hibernators prior to their hibernating season as possibly being important. The second reason why it is interesting is that it suggests that the effect of diet PUFA on hibernation/torpor patterns may be mediated by mechanisms associated with eicosanoids as omega-6 and omega-3 PUFA have opposing effects on eicosanoid metabolism.

Maximum Longevity

Among and between species, the lifespan of individual animals can vary dramatically but the maximum possible lifespan (MLSP) is characteristic for each species and as noted previously has been associated with membrane fatty acid composition distinctive for the particular species (see Hulbert *et al.* 2007). As with the consideration of dietary fatty acid composition and BMR above, if membrane fatty acid composition is an important determinant of maximum longevity but diet fatty acid composition has only a very small influence on membrane fatty acid composition then we can conclude that manipulation of diet fatty acid composition should have only minimal influence on maximum longevity. As with BMR, from the limited available evidence this appears to be generally the case. As with BMR, such a conclusion assumes (i) the diet is not deficient in essential fats, and (ii) that the relationship between diet and membrane composition is similar to that observed for the rat (see figure 3).

Anecdotal evidence supports such a conclusion. For example, the echidna (*Tachyglossus aculeatus)* is a very long-living mammal species and has a membrane composition (low PUFA, high MUFA) that is consistent with this exceptional MLSP (Hulbert *et al.* 2008). From its body mass the predicted MLSP for an echidna is ~14y, yet the maximum longevity recorded for the echidna is ~50y in captivity and a free-living individual has been observed over 45y (see Augee *et al.* 2006). In the wild, echidnas are termite/ant eaters and the lipids that make up these insects are low in PUFA and high in MUFA (Faulkenstein *et al.* 2001). Yet the longevity record for the captive echidna is from Philadelphia Zoo and this animal was fed "half a pint of milk and a raw egg daily"! (see p125 of Augee *et al.* 2006). Although we do not know the membrane composition of this echidna,

it is a reasonable conclusion that a diet with a higher PUFA content than their natural diet does not lessen the MLSP of echidnas.

Maximum longevity also varies among different genetic strains of some species. For example, wild-derived mice strains have a different membrane fatty acid composition compared to lab strains (Hulbert et al. 2006) and have extended longevity even when maintained in the same environment and fed the same diet for their entire life (Miller et al. 2002). When a mice strain that exhibits accelerated-senescence was fed fish oil, there was no change in maximum longevity although average longevity was decreased (Tsuduki et al. 2011). Similarly, Valencak and Ruf (2011) report no significant effects on longevity of mice fed PUFA-enriched diets.

Although such cases of diet manipulation do not change MLSP, there is a case where the absence of PUFA in the diet has been suggested to be responsible for extended longevity. This case concerns the honeybee *Apis mellifera*. Female honeybees can be either workers or queens, depending on what they are fed as larvae. Although genetically identical, workers and queens differ in their maximum longevity (Winston 1987). Queen honeybees can live for years while workers only live for weeks. Honeybee worker larvae have membranes high in MUFA but low in PUFA and in the first week after emergence adult worker honeybees accumulate PUFA in their membrane lipids (Haddad et al. 2007). During this period, they commence eating pollen for the first time and because pollen is rich in PUFA, it is likely that pollen is the source of this elevated membrane PUFA. However, queen honeybees do not eat pollen as adults and are fed "mouth to mouth" by workers for their entire life as adults. The membrane lipids of queen honeybees, unlike those of workers, do not accumulate PUFA and remain highly monounsaturated throughout their adult life. It has been suggested that this "mouth-to-mouth" feeding is a means of keeping the cell membranes of queen honeybees low in PUFA and is in turn responsible for their extended longevity compared to workers (Haddad et al. 2007).

Exercise Performance

As can be seen from Figure 3, although muscle membranes from the homeothermic mammal (rat) are relatively unresponsive to dietary changes in SFA and MUFA, they are more responsive to changes in dietary omega-3 PUFA and omega-6 PUFA. In the rat, dietary enrichment with either omega-3 PUFA or omega-6 PUFA will not change the total PUFA

content of muscle membranes but will result in a greater muscle content of the enriched PUFA type at the expense of the other type of PUFA (Ayre and Hulbert 1996; Peoples and McLennan 2010). In other words dietary omega-3 PUFA will replace omega-6 PUFA in muscle membrane lipids of the rat and vice versa. Although such dietary replacement has negligible effects on the 'in vitro' performance of isolated muscles (Ayre and Hulbert 1996b), it has recently been demonstrated that dietary fish oil results in greater fatigue resistance and contractile recovery in muscles when measured 'in vivo' compared to an omega-6 PUFA diet (Peoples and McLennan 2010). Thus, it is possible that the two different types of essential fats will differently influence the exercise performance of animals. Surprisingly, when the effects on whole animal performance of diet enrichment with omega-3 PUFA or omega-6 PUFA were compared using rats, it was observed that there was no effect on strength (Ayre and Hulbert unpublished results) but that omega-6 PUFA enrichment increased treadmill running endurance compared to diet omega-3 enrichment (Ayre and Hulbert 1997). This was a surprising result and the mechanism underlying the effect is unknown. In light of the individual muscle results cited above, it seems unlikely that this is due to effects on muscle contractile performance. It may be related to effects on the supply of energy (via either enhanced intramuscular energy stores or enhanced circulatory supply of energy) or it could be due changes in the response to the stimulus used to encourage continuance of treadmill (which were short blasts of compressed air on the rump of the rat).

Recently, there has been interest in potential role of diet essential fatty acids in the exercise performance of migratory birds. For example, semipalmated sandpipers (*Calidris pusilla*) migrate from the Arctic to South America and early in their migration they stop-over in Canada where they feed on an amphipod with high omega-3 PUFA content. During this stop-over they double in body mass, and it has been hypothesised that this increase in dietary omega-3 PUFA is a form of "natural doping" to prepare their flight muscles for the non-stop flight to South America (Maillet and Weber 2006). This hypothesis has been investigated by examining the effects of dietary enrichment with omega-3 PUFA on the sedentary bobwhite quail (*Colinus virginianus*). In support of this "natural doping" hypothesis, dietary omega-3 PUFA enrichment resulted in large increases in the activity of oxidative enzymes in the quail and that these increases were of the same degree as previously documented in other species following extreme regimes of endurance training (Nagahuedi *et al.* 2009). Seasonal and pre-migratory changes in the fatty acid composition of (i) muscle phospholipids, (ii) muscle triglycerides, and (iii) adipose tissue triglycerides have also been observed in a different

migratory bird species, the white-throated sparrow (*Zonotrichia albicollis)* and these changes have been attributed to diet (Klaiman *et al.* 2009). However, in this migratory bird there is an increase in omega-6 PUFA at the expense of omega-3 PUFA, which is unlike the situation in sandpipers. Experimental manipulation of the fatty acid composition of adipose tissue triglycerides in this species suggests it was likely changes in fatty acid composition of triglycerides (rather than muscle membrane fatty acid composition) played a more important role in exercise performance (Price and Guglielmo 2009).

Conclusion

The two types of polyunsaturated fatty acids; namely the omega-3 PUFA and the omega-6 PUFA are essential in the diet in higher animals. Little is known of precise amounts required by species apart from laboratory animals. These essential fats are important constituents of membrane lipids and have other important roles in the body, especially as precursors to important chemical signals. Their relative abundance varies between different foods and their relative abundance in the food of animals may have important consequences for animal performance. Diet variation has little influence on membrane composition (as long as the diet is not devoid of these essential fats) but does influence the composition of storage fats. There is emerging evidence that natural variation in diet fatty acid composition may have an influence on some aspects of animal performance (e.g. thermoregulatory behaviour, exercise performance), but not others (e.g. BMR, maximal lifespan). Relatively little is known of the nutritional ecology of these essential fats, which scientific research into it being only at an early stage.

Acknowledgements

References

Abbott, S.K., Else, P.L., and Hulbert A.J. (2010). Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 polyunsaturated fatty acids. *British Journal of Nutrition* **103**, 522-529.

Arnold, W., Ruf, T., Frey-Roos, F., and Bruns, U. (2011). Diet-independent remodelling of cellular membranes precedes seasonally changing body temperature in a hibernator. *PLOS One* **6**, e18641

Augee, M.L., Gooden, B., and Musser, A. (2006). Echidna: Extraordinary Egg-laying Mammal. CSIRO Publishing, Melbourne, Vic.

Ayre, K.J., and Hulbert A.J. (1996a). Dietary fatty acid profile influences composition of skeletal muscle phospholipids in rats. *Journal of Nutrition* **126**, 653-662

Ayre, K.J., and Hulbert A.J. (1996b). Effects of changes in dietary fatty acids on isolated skeletal muscle function in rats. *Journal of Applied Physiology* **80**, 464-471

Ayre, K.J. and Hulbert A.J. (1997). Dietary fatty acid profile affects endurance in rats. *Lipids* **32**, 1265-1270

Ben-Hamo, M., McCue, M.D., McWilliams, S.R., and Pinshow, B. (2011). Dietary fatty acid composition influences tissue lipid profiles and regulation of body temperature in Japanese quail. *Journal of Comparative Physiology B* **181**, 807-816

Brand, M.D., Turner, N., Ocloo, A., Else, P.L., and Hulbert, A.J. (2003). Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. *Biochemical Journal* **376**, 741-48

Chivers, D. J. (1992). Diets and guts. In 'Cambridge Encyclopaedia of Human Evolution' (Eds. S. Jones, R. Martin and D. Pilbeam.) pp 60-64. (Cambridge University Press: Cambridge.)

Couture, P., and Hulbert, A.J. (1995a). On the relationship between body mass, tissue metabolic rate and sodium pump activity in mammalian liver and kidney. *American Journal of Physiology* **268**, R641-50.

Couture, P., and Hulbert, A.J. (1995b). Membrane fatty acid composition is related to body mass in mammals. *Journal of Membrane Biology* **148**, 27-39

Ellis, H.I. (1984). Energetics of free-ranging seabirds. In 'Seabird Energetics' (Eds. Whittow, G.C., and Rahn, H.) pp. 203-234 (Plenum Press: New York.)

Else, P.L., and Wu, B.J., (1999). What role for membranes in determining the higher sodium pump molecular activity of mammals compared to ectotherms. *Journal of Comparative Physiology B* **169**, 296-302.

Falkenstein, F., Kortner, G., Watson, K., and Geiser, F. (2001). Dietary fats and body lipid composition in relation to hibernation in free-ranging echidnas. *Journal of Comparative Physiology B* **171**, 189-194.

Florant, G.L. (1998). Lipid metabolism in hibernators: the importance of essential fatty acids. *American Zoologist* **38**, 331-340

Frank, C.L. (1992). The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiological Zoology* **65**, 906-920

Frank, C.L., Karpovich, S., and Barnes, B.M. (2008). Dietary fatty acid composition and the hibernation patterns of free-ranging arctic ground squirrels. *Physiological and Biochemical Zoology* **81**, 486-495

Fryst, H., Pham, D.V., Lubin, B.H., and Kuypers, F.A. (1996). Formation of vesicles by the action of acyl-CoA:1-acyllysophosphatidylcholine acyltransferase from rat liver microsomes: optimal solubilization conditions and analysis of lipid composition and enzyme activity. *Biochemistry* **35**, 2644-2650

Geiser, F. (1990). Influence of polyunsaturated and saturated dietary lipids on adipose tissue, brain and mitochondrial membrane fatty acid composition of a mammalian hibernator. *Biochimica et Biophysica Acta* **1046**, 159-166

Geiser, F. (1991). The effect of unsaturated and saturated dietary lipids on the pattern of daily torpor and the fatty-acid composition of tissues and membranes of the deer mouse *Peromyscus maniculatus*. *Journal of Comparative Physiology B* **161**, 590-597

Geiser, F., Firth, B.T., and Seymour, R. (1992). Polyunsaturated dietary lipids lower the selected body-temperature of a lizard. *Journal of Comparative Physiology B* **162**, 1-4

Geiser, F. and Kenagy, G.J. (1987). Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *American Journal of Physiology* **252**, R897-R901

Geiser, F., and Learmonth, R.P. (1994). Dietary fats, selected body-temperature and tissue fatty-acid composition of agamid lizards (*Amphibolurus nuchalis*). *Journal of Comparative Physiology B* **164**, 55-61

Gudbjarnason, S., Doell, B., and Oskardottir, G. (1978). Docosahexaenoic acid in cardiac metabolism and function. *Acta Biologica et Medica Germanica* **37**, 777-84

Haddad, L., Kelbert, L., and Hulbert, A.J. (2007). Extended longevity of queen honeybees compared to workers is associated with peroxidation-resistant membranes. *Experimental Gerontology* **42**, 601-9

Hazel, J.R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology* **57**, 19-42.

Hill, V.L., and Florant, G.L. (2000). The effect of a linseed oil diet on hibernation in yellow-bellied marmots (*Marmota flaviventris*). *Physiology and Behavior* **68**, 431-437

Hochachka, P.W., and Somero, G.N. (2002). Biochemical Adaptation. Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford

Hulbert, A.J., Beard, L., and Grigg, G.C. (2008). The exceptional longevity of an egg-laying mammal, the short-beaked echidna (*Tachyglossus aculeatus*) is associated with peroxidation-resistant membrane composition. *Experimental Gerontology* **43**, 729-33.

Hulbert, A.J., and Else, P.L. (1990). The cellular basis of endothermic metabolism: A role for "leaky" membranes? *News in Physiological Science* **5**, 25-28.

Hulbert, A.J., and Else, P.L. (1999). Membranes as possible pacemakers of metabolism. *Journal of Theoretical Biology* **199**, 257-274

Hulbert, A.J., and Else, P.L. (2000). Mechanisms underlying the cost of living in animals. *Annual Review of Physiology* **62**, 207-35.

Hulbert, A.J., Faulks, S.C., Buttemer, W.A., and Else, P.L. (2002a). Acyl composition of muscle membranes varies with body size in birds. *Journal of Experimental Biology* **205**, 3561-9.

Hulbert, A.J., Faulks, S.C., Harper, J.M., Miller, R.A., and Buffenstein, R. (2006). Extended longevity of wild-derived mice is associated with peroxidation-resistant membranes. *Mechanisms in Ageing and Development* **127**, 653-7.

Hulbert, A.J., Pamplona, R., Buffenstein, R., and Buttemer, W.A. (2007). Life and death: metabolic rate, membrane composition and life span of animals. *Physiological Reviews* **87**, 1175-213

Hulbert, A.J., Rana, T. and Couture P. (2002b). The acyl composition of mammalian phospholipids: an allometric analysis. *Comparative Biochemistry and Physiology B* **132**, 515-527

Hulbert, A.J., N. Turner, L.H. Storlien and P.L. Else (2005). Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews* **80**, 155-169.

Hurley, J.A., and Costa, D.P. (2001). Standard metabolic rate at the surface and during trained submersions in adult California sea (*Zalophus californicus*). *Journal of Experimental Biology* **204**, 3273-3281.

ISSFAL (International Society for the Study of Fatty Acids and Lipids) Recommendations for dietary intake of polyunsatiurated fatty acids. (the following website was accessed on 21/10/11   http://www.issfal.org/statements/pufa-recommendations

Iverson, S.J., Field, C., Bowen, W.D., and Blanchard, W. (2004). Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* **74**, 211-235

Jenness, R., Birney, E., and Ayaz, K. (1980). "Variation of l-gulonolactone oxidase activity in placental mammals". *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **67**, 195–204

Klaiman, J.M., Price, E.R., and Guglielmo, C.G. (2009). Fatty acid composition of pectoralis muscle membrane, intracellular fat stores and adipose tissue of migrant and wintering white-throated sparrows (*Zonotrichia albicollis*). *Journal of Experimental Biology* **212**, 3865-3872

Kleiber, M. (1961). The Fire of Life. John Wiley and Son, New York.

Maillet, D., and Weber, J-M. (2006). Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. *Journal of Experimental Biology* **209**, 2686-2695

Manning, R. (2001). Fatty acids in pollen: a review of their importance for honey bees. *Bee World* **82**, 60-75

McNab, B.K. (1986). The influence of food habits on the energetics of eutherian mammals. *Ecological Monographs* **56**, 1-19.

Miller, R.A., Harper, J.M., Dysko, R.C., Durkee, S.J., and Austad, S.N. (2002). Longer life spans and delayed maturation in wild-derived mice. *Experimental Biology and Medicine* **227**, 500-8.

Nagahuedi, S., Popesku, J.T., Trudeau, V.L., and Weber, J-M. (2009). Mimicking the natural doping of migrant sandpipers in sedentary quails: effects of dietary n-3 fatty acids on

muscle membranes and PPAR expression. *Journal of Experimental Biology* **212**, 1106-1114

Newman, R.E., Bryden, W.L., Fleck, E., Ashes, J.R., Buttemer, W.A., Storlien, L.H., and Downing, J.A. (2002). Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. *British Journal of Nutrition* **88**, 11-18.

Nichols, P.D., and Nichols, C.A.M. (2008). Microbial signature lipid profiling and exopolysaccharides: experiences initiated with Professor David C white and transported to Tasmania, Australia. *Journal of Microbiological Methods* **74**, 33-46

Peoples, G.E., and McLennan, P.L. (2010). Dietary fish oil reduces skeletal muscle oxygen consumption, provides fatigue resistance and improves contractile recovery in the rat in vivo hindlimb. *British Journal of Nutrition* **104**, 1771-1779

Raison, J.K., and Lyons J.M. (1971). Hibernation: alteration of mitochondrial membranes as a requisite for metabolism at low temperature. *Proceedings of the National Academy of Science, U.S.A.* **68**, 2092-2094

Rivers, J.P.W., Sinclair, A.J., and Crawford, M.A. (1975). Inability of the cat to desaturate essential fatty acids. *Nature* **258**, 171-173

Peters, R.H. (1983). The Ecological Implications of Body Size. Cambridge University Press, Cambridge.

Pierce B.J., McWilliams, S.R., O'Connor, T.P., Place, A.R., and Guiglielmo, C.G. (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *Journal of Experimental Biology* **208**, 1277-1285.

Porter, R.K., Hulbert, A.J., and Brand, M.D. (1996). Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. *American Journal of Physiology* **271**, R1550-60.

Price, E.R., and Guglielmo, C.G. (2009). The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis*). *American Journal of Physiology* **297**, R775-R782

Savage, N., and Goldstone, B.W. (1965). Effect of different dietary fats on oxygen consumption and serum lipid levels in the baboon (*Papio ursinus*). *British Journal of Nutrition* **19**, 459-467.

Shimomura, Y., Tamura, T., and Suzuki, M. (1990). Less body fat accumulation in rats fed a safflower oil diet than rats fed a beef tallow diet. *Journal of Nutrition* **120**, 1291-1296.

Shmookler Reis, R.J., Xu, L., Lee, H., Chae, M., Thaden, J.J., Bharill, P., Tazearslan, C., Siegel, E., Alla, R., Zimniak, P., and Ayyadevara, S. (2011). Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging* **3**, 125-147

Simandle, E.T., Espinoza, R.E., Nussear, K.E., and Tracy, C.R. (2001). Lizrds, lipids and dietary links to animal function. *Physiological and Biochemical Zoology* **74**, 625-640

Takeuchi, H., Matsuo, T., Tokuyama, K., Shimomura, Y., and Suzuki, M. (1995). Diet-induced thermogenesis is lower in rats fed a lard diet than those fed a high oleic acid safflower oil diet, a safflower oil diet, or a linseed oil diet. *Journal of Nutrition* **125**, 920-925.

Thiemann, G.W., Iverson, S.J., and Stirling, I. (2008). Polar bear diets and arctic marine food webs: insights from fatty acid analysis. *Ecological Monographs* **78**, 591-613

Tsuduki, T., Honma, T., Nakagawa, K., Ikeda, I., and Miyazawa, T. (2011). Long-term intake of fish oil increases oxidative stress and decreases lifespan in senescence-accelerated mice. *Nutrition* **27**, 334-337

Valencak, T.G., and Ruf, T. (2011). Feeding into old age: long-term effects of dietary fatty acid supplementation on tissue composition and life span in mice. *Journal of Comparative Physiology B* **181**, 289-298

Valencak, T.G., and Ruf, T. (2007). N-3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. *Aging Cell* **6**, 15-25

Valentine, R. and Valentine, D. (2010). Omega-3 Fatty Acids and the DHA Principle. CRC Press Boca Raton, FL, USA

van Tets, I.G., and Hulbert A.J. (1999). A comparison of the nitrogen requirements of the eastern pygmy possum, *Cercartetus nanus*, on a pollen and on a mealworm diet. *Physiological and Biochemical Zoology* **72**, 127-137

Williams, C.T., and Buck, C.L. (2010). Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. *Journal of Ornithology* **151**, 531-543

Wu, B.J., Hulbert, A.J., Storlien L.H. and Else P.L. (2001). Molecular activity of Na$^+$,K$^+$-ATPase from different sources is related to packing of membrane lipids. *Journal of Experimental Biology* **204**, 4271-4280.

Wu, B.J., Hulbert, A.J., Storlien L.H. and Else P.L. (2004). Membrane lipids and sodium pumps of cattle and crocodiles: an experimental test of the membrane pacemaker theory of metabolism. *American Journal of Physiology* **287**, R633-R641.

Figure legends:

Figure 1. An outline of the metabolic pathways involved in the synthesis of membrane fatty acids. Individual fatty acids are identified by number of carbons : number of double bonds (and position of most terminal double bond). The most common fatty acids are in black text while those in grey text are least common. SFA refers to saturated fatty acids, n-9 UFA includes the main monounsaturated fatty acid and unusual omega-9 polyunsaturates. The omega-6 and omega-3 polyunsaturated fatty acids are respectively designated by n-6 PUFA and n-3 PUFA. E refers to elongase enzyme, while Δ12-D, Δ15-D, Δ9-D, Δ6-D, and Δ5-D refer to specific desaturase enzymes. Black solid arrows represent reactions occurring in the endoplasmic reticulum, black dashed arrows indicate partial β-oxidation occurring in the peroxisomes, and blue broken arrows denote the desaturase enzymes lacking in higher animals.

Figure 2. Omega-3 PUFA and omega-6 PUFA content of a variety of foods. In each pie chart the dark sector represents the n-3 PUFA and the light sector represents the n-6 PUFA. Beside each pie-chart are shown PUFA balance (n-3 PUFA as % of total PUFA) value and total PUFA content (per 100g food). The data are from the FSANZ website.

Figure 3. The relationships between the fatty acid composition of the diet and membrane lipids (left-hand graphs) and storage lipids (right-hand graphs) of tissues from the rat (*Rattus norvegicus*). In each figure the diagonal dashed line represents the line of perfect conformity to diet composition. SFA=saturated fatty acids; MUFA= monounsaturated fatty acids; n-6 PUFA= omega-6 polyunsaturated fatty acids and n-3 PUFA= omega-3 polyunsaturated fatty acids. Rats were fed one of 12 moderate-fat diets (25% energy) that differed only in fatty acid composition. Data for muscle are from Abbott et al. (2010) which should be consulted for experimental details. Data for all other tissues are from Abbott et al. (ms in review).
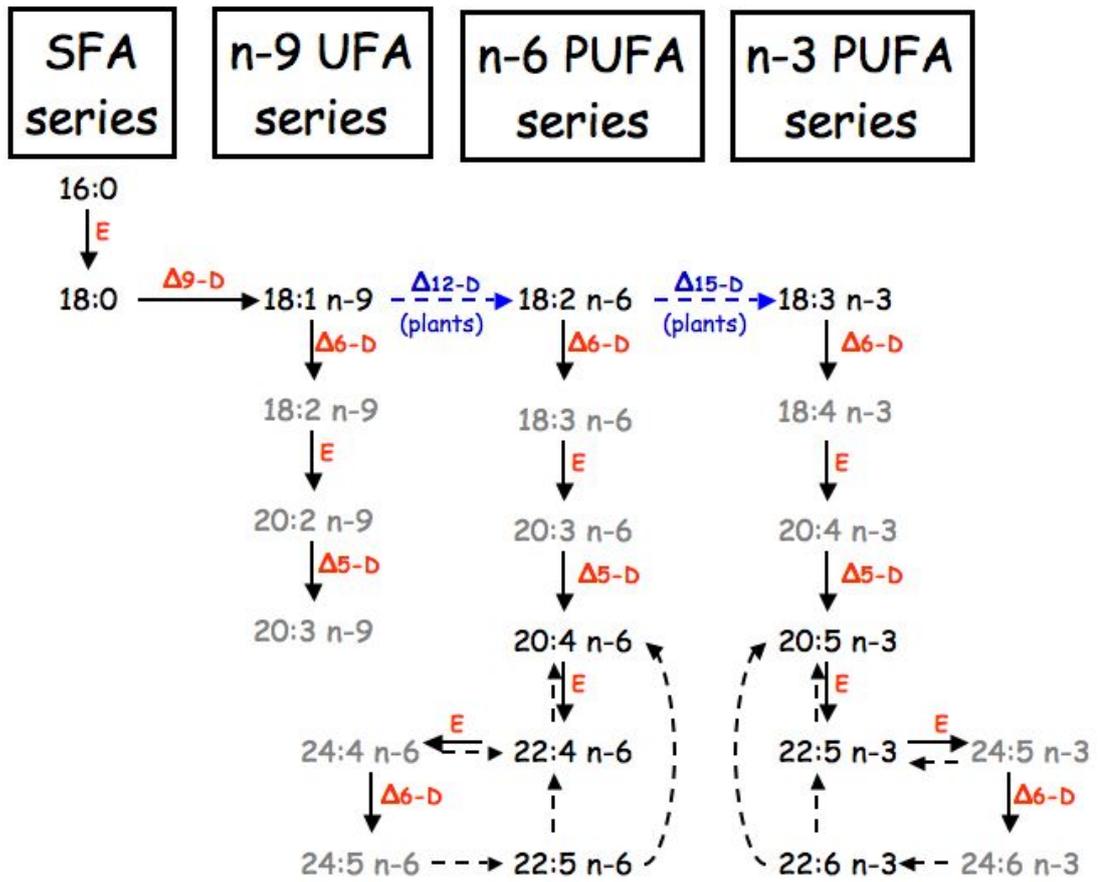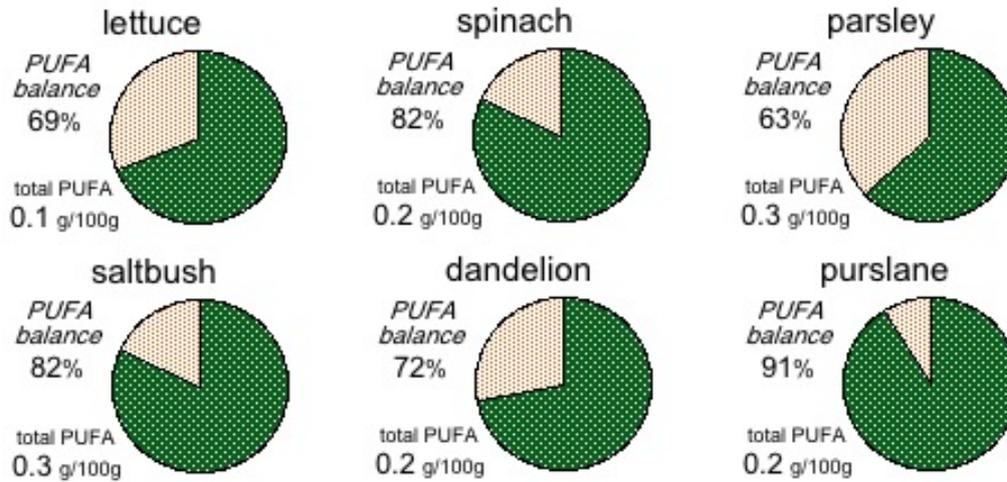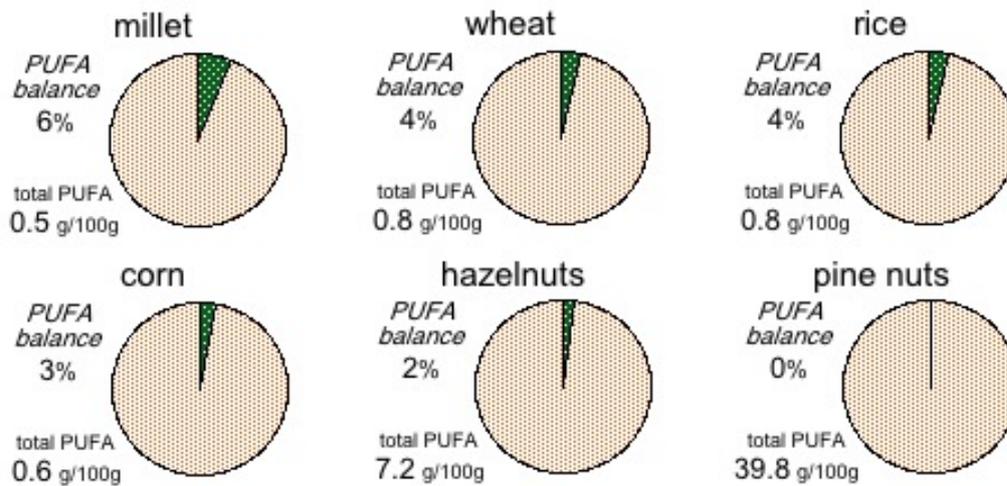
Fig 1

fig 2

GREEN LEAVES

lettuce
PUFA balance 69%
total PUFA 0.1 g/100g

spinach
PUFA balance 82%
total PUFA 0.2 g/100g

parsley
PUFA balance 63%
total PUFA 0.3 g/100g

saltbush
PUFA balance 82%
total PUFA 0.3 g/100g

dandelion
PUFA balance 72%
total PUFA 0.2 g/100g

purslane
PUFA balance 91%
total PUFA 0.2 g/100g

SEEDS, NUTS & GRAINS

millet
PUFA balance 6%
total PUFA 0.5 g/100g

wheat
PUFA balance 4%
total PUFA 0.8 g/100g

rice
PUFA balance 4%
total PUFA 0.8 g/100g

corn
PUFA balance 3%
total PUFA 0.6 g/100g

hazelnuts
PUFA balance 2%
total PUFA 7.2 g/100g

pine nuts
PUFA balance 0%
total PUFA 39.8 g/100g

ANIMAL MEATS etc.,

rabbit
PUFA balance 53%
total PUFA 1.0 g/100g

beef (grass fed)
PUFA balance 31%
total PUFA 1.0 g/100g

beef (grain fed)
PUFA balance 10%
total PUFA 0.7 g/100g

rainbow trout
PUFA balance 81%
total PUFA 2.0 g/100g

prawn
PUFA balance 86%
total PUFA 0.2 g/100g

egg (chicken)
PUFA balance 9%
total PUFA 1.4 g/100g

fig 3