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COMMENTARY

The under-appreciated fats of life: the two types of polyunsaturated fats

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ABSTRACT

There are two types of polyunsaturated fatty acids (i.e. fats that contain multiple carbon-carbon double bonds) – omega-6 and omega-3. They are not interconvertible, and they contribute 'double-bonded carbons' to different depths in bilayer membranes, with different effects on membrane processes. This Commentary emphasises the importance of these fats for biological membrane function and examines their evolution and biochemistry. Omega-6 and omega-3 fatty acids are separately essential in the diet of animals, and they pass up the food chain largely from plants, with 'seeds' being a prevalent source of omega-6, and 'leaves' a prevalent source of omega-3. The dietary balance between these fatty acids has a strong influence on membrane composition. Although this aspect of diet has been little investigated outside of the biomedical field, emerging evidence shows it can alter important physiological capacities of animals (e.g. exercise endurance and adiposity), which has implications for activities such as avian migration and hibernation and torpor, as well as significant implications for human health. This Commentary will focus on the separate effects of omega-3 and omega-6 on membrane properties and will emphasise the importance of the balance between these two fatty acids in determining the function of biological membranes; I hope to convince the reader that fats should be considered first and foremost as the basic unit of biological membranes, and secondarily as a means of energy storage.

KEY WORDS: Arachidonic acid, Membrane composition, Omega-6 fatty acids, Omega-3 fatty acids

Introduction

Life as we know it requires movement of molecules, so they may collide, vibrate and interact. Furthermore, life requires a 'Goldilocks' level of movement (not too little, nor too much). This is manifest in that complex life generally occurs in a relatively narrow range of temperature (which represents the average kinetic energy and thus movement in a system) of 0–50°C. It is also significant that the two groups of animals (mammals and birds) that have evolved sophisticated physiological systems to maintain a constant body temperature despite fluctuations in environmental temperature, regulate body temperature over the narrower range of 32–42°C. Even a super-organismal living system, a honeybee hive, maintains a temperature of 34–37°C during the active season (Fahrenholz et al., 1989). This narrow temperature range is thought to allow the optimal level of molecular movement for biological processes.

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Movement in the aqueous cytoplasm of cells was obvious under the microscope for many years but it was not until 1970 that movement in the non-aqueous compartment of cells, their membranes, became apparent. This was first observed as the rapid intermixing of cell surface antigens following fusion of mouse and human cells, and lowering the temperature slowed the rate of movement of these cell surface proteins (Frye and Edidin, 1970). The 'fluid mosaic' model of cell membranes followed (Singer and Nicolson, 1972) and, since then, measurements have shown that membrane lipids, which constitute the basis of all cellular membranes, undergo high-speed lateral movement within the membrane such that an average lipid molecule in the membrane can circumnavigate a red blood cell in seconds (Nelson and Cox, 2005).

The general perception of 'fat' in biological systems is primarily as a source of energy. Here, I hope to convince you that this is a secondary (later-evolved) function. An evolutionary consideration of biochemistry shows that fats should be primarily considered as the basic unit of biological membranes and that the evolution of polyunsaturated fats (i.e. those which contain more than one double bond between carbon atoms in the hydrocarbon chain) is best understood to serve membrane function. Furthermore, the evolutionary 'loss' (in early animals) of the enzymes responsible for the synthesis of polyunsaturated fats from saturated and monounsaturated fats [which contain zero and one carbon–carbon double bond(s), respectively], as well as the enzymes responsible for making omega-3 fats from omega-6 fats resulted in these two types of fatty acids becoming separate essential components of animal diets (Box 1). Their nutritional importance is under-appreciated, and their effects on the biology of animals are best explained via the effect of these essential fats on membrane composition. In this Commentary, I begin by describing the structure of membrane lipids, the early synthesis of saturated fats by lipogenesis and the later evolution of polyunsaturated fatty acids, which are important for movement in biological membranes. I go on to discuss the separate dietary essentiality of omega-3 and omega-6 and the effect of their balance in the diet on membrane composition. Throughout the Commentary, I will refer to fatty acids by a numbering system rather than their chemical name (e.g. arachidonic acid is 20:4ω-6). The first number refers to number of carbon atoms in the chain, the second is the number of double bonds, and the suffixes ω-6 and ω-3 identify whether it is an omega-6 or an omega-3 fatty acid, respectively.

Lipid membranes and transmembrane gradients are essential for life

Cell membranes are essential features of life as we know it, and Archaea, Bacteria and Eukarya all have cells surrounded by a lipid-based membrane as their basic unit. The Archaea (which will not concern me here) have lipid membranes based on isoprenoid chains giving them a special 'toughness' to cope with the extreme

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Box. 1. Sources of omega-6 and omega-3 in the diet: seeds, leaves and meat

Plants can synthesise all four fatty acid classes (saturated, monounsaturated, omega-6 polyunsaturated and omega-3 polyunsaturated); however, most animals are unable to synthesise either omega-6 or omega-3 fats, instead obtaining these from plants. The polyunsaturated fats synthesised by plants are 18-carbon fatty acids, with the omega-6 being 18:2ω-6 and the omega-3 being 18:3ω-3. Both of these 18-carbon polyunsaturated fats are found in animals, along with 20- and 22-carbon polyunsaturated fats: animals are able to both elongate and further desaturate the plant-sourced omega-6 (to make 20:4ω-6) and omega-3 (to make 20:5ω-3 and 22:6ω-3).

There are consistent patterns regarding the balance between omega-6 and omega-3 in different types of plant-derived foods. 'Seeds' (e.g. grains, nuts) and food derived from seeds (e.g. vegetable oils) overwhelmingly contain omega-6 fats; many contain virtually no omega-3 fat (e.g. seeds and oil from corn, sunflower, safflower, peanut, cottonseed, coconut and palm are essentially devoid of omega-3). Few seeds (e.g. linseed, chia) are known to have more omega-3 than omega-6. In contrast, the polyunsaturated fats in 'leaves' are predominantly omega-3 fats; for example, the polyunsaturated fats in spinach, cabbage and lettuce are 70–90% omega-3 and only 10–30% omega-6 (USDA food database: https://fdc.nal.usda.gov/index.html). Thus, leaf-eaters or grass-eaters will contain significant amounts of omega-3 in their tissues, whereas seed-eaters or grain-eaters will contain mainly omega-6 fats.

Meat or products from animals (e.g. dairy) generally contain a reasonable balance between omega-6 and omega-3 fats. However, if a mammal or bird eats seeds (e.g. grain-fed), both its meat and products will be higher in omega-6 and lower in omega-3 than if it eats leaves (e.g. grass-fed). Fish, and marine food in general, is high in omega-3 because the plants at the base of the marine food chain are essentially 'leaves' (predominantly algae, which unlike terrestrial plants also contain 20- and 22-carbon omega-3). Like ourselves, fish are unable to synthesise omega-3, and have an essential dietary requirement for omega-3 fats. In the 1960s, corn and corn oil were tried as a substitute for the normal food of fish in aquaculture but this resulted in a lethal condition called 'transport shock', caused by omega-3 deficiency (Castell et al., 1972).

Finally, it is worth noting that cats (and likely felids in general) have 'lost' the enzymes to make the 20- and 22-carbon from 18-carbon polyunsaturated fats (Rivers et al., 1975). This means they must obtain their long-chain omega-3 and omega-6 already pre-formed in their diet. Since these 20- and 22-carbon fats are only found in meat, these mammals have become 'obligate' meat-eaters.

environments they inhabit (Hochachka and Somero, 2002). Both bacterial and eukaryotic cell membranes consist of predominantly phospholipid molecules with a 3-carbon backbone, a 'water-loving' ionized head group attached to one of the carbons and 'water-fearing' fatty acid chains attached to each of the other two carbons. Thus, each membrane lipid molecule has a fatty acid pair: hence their description as diglycerides. This structure means membrane lipids are 'amphipathic' (the end of the molecule with the head group is hydrophilic whereas the other end with two fatty acid chains is lipophilic); consequently, membrane lipid molecules self-organise to form a bilayer structure. The internal part of the membrane is a hydrocarbon environment, relatively impermeable to water, ions and other charged molecules. The charged outer parts of the membrane lipid molecules interact with water and water-soluble molecules.

The existence of the lipid bilayer allows the environment inside the cell to be different to the outside environment. Whether a cell is alive or dead can be determined by measuring this inside–outside difference. For example, in a bacterial cell, the existence of a transmembrane proton gradient is a sign that it is alive (Nelson and Cox, 2005). In mitochondria and chloroplasts, the presence of a transmembrane proton gradient is a sign that they are functional and can manufacture ATP (Nelson and Cox, 2005). In animal cells, this ATP is used to create another gradient at the plasma membrane surrounding the animal cell – the trans-plasmalemma Na^+ gradient. This gradient, in turn, is used as a primary energy source for other cellular activities: the trans-plasmalemma gradients of other ions appear to be linked to the Na^+ gradient in a self-regulatory near-equilibrium system (Masuda et al., 1990); consequently, maintenance of this transmembrane Na^+ gradient is used to maintain cell volume, pH and ion homeostasis, as well as being the energy source for active uptake or expulsion of other important molecules and also for action potentials in nerve and muscle cells (Hulbert and Else, 2000). The importance of transmembrane ion gradients in the metabolic rate of animals is discussed in more detail elsewhere (Hulbert and Else, 1999, 2000).

Lipogenesis: the creation of membrane fats

For most of us, our introduction to fat is when it is pointed out as that yellow/white part of the meat on our dinner plate. Our treatment and teaching of fat is dominated by this perception of it as an energy store. Yet, when considered from an evolutionary perspective, fat as an energy store is a relatively late arrival. For much of evolutionary history, the primary importance of fat (more precisely, fatty acid chains) was as fundamental parts of cell membranes. Most biochemistry evolved in bacteria, but bacteria such as *Escherichia coli* synthesise fatty acids to make biological membranes, not to store energy. The fatty acid synthase of *E. coli* consists of seven separate polypeptide enzymes, whereas in the fatty acid synthase of vertebrates, these same seven enzymes have become parts of a single large polypeptide (Nelson and Cox, 2005, p. 794). Seven genes have merged to become a single gene for a single enzyme (with seven catalytic sites). This evolutionary change emphasises the importance of the process for making fatty acids. The product of both bacterial and animal fatty acid synthase is palmitic acid, a sixteen-carbon saturated fatty acid (often written as 16:0) that is the main fatty acid found in the membranes of *E. coli* (Ishinaga et al., 1979). Although the product of the fatty acid synthase enzyme is 16:0, this 16-carbon saturated fatty acid is also the source of the other fatty acids found in membranes. For example, it is elongated to form stearic acid (18:0) by an elongation enzyme system (Nelson and Cox, 2005).

When I first started teaching biochemistry, I followed standard practice and introduced fatty acid synthesis as the way of converting the energy of carbohydrates to energy storage as fat, specifically triglycerides. I did not appreciate that an intermediate in the process of converting carbohydrates to triglycerides was actually a membrane lipid. This intermediate is phosphatidate (an amphipathic diglyceride and possibly the simplest phospholipid); the process of producing a triglyceride from this diglyceride involves removal of the phosphate group and its replacement by another fatty acyl chain (Nelson and Cox, 2005, p. 805). In this way, an amphipathic diglyceride (a membrane fat) is converted to a completely lipophilic triglyceride (a storage fat).

Movement in membrane bilayers: the evolution of polyunsaturated fatty acids

The physical properties of fatty acid chains that make up the membrane lipid molecules are the predominant influence on movement in membrane bilayers. In phospholipid bilayer membranes made of phosphatidylcholine molecules in which both fatty acid chains are 18:0, lateral movement in the membrane bilayer will only be possible at temperatures above 55°C, as this is the melting temperature of this

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phospholipid; the bilayer will be 'solid' at lower temperatures. If all fatty acid chains are 16:0, then it is only at temperatures above 41°C that the membrane bilayer will be 'fluid' (i.e. exist in a physical state whereby lateral movement is possible) (Silvius, 1982). For bacteria that live in hot environments (e.g. thermal vents) membranes in which all fatty acyl chains are saturated would be adequate for maintaining the living state. However, they could not maintain a living state at lower temperatures.

In comparison to a single -C-C- bond, which allows free rotation around the single covalent bond, when a double bond connects two carbon atoms, -C=C-, such rotation is not possible and the connection is rigid (Nelson and Cox, 2005, p. 13). Introducing such a double bond into a fatty acyl chain dramatically affects the movement of the whole hydrocarbon chain and lowers the temperature at which a fluid membrane can be maintained. For example, a membrane bilayer of phosphatidylcholine molecules with 18:0–18:1 as the pair of fatty acyl chains will not solidify until 6°C, whereas one with a 16:0–18:1 pair will remain fluid down to −2°C (Silvius, 1982). The introduction of the desaturase enzyme system, responsible for producing monounsaturated fatty acids from saturated fatty acids, opened up a huge number of additional environments for early bacteria. It is likely that this is the selective advantage that favoured the evolution of these enzyme systems. I know of no other reason why unsaturated fatty acids would have been necessary for early prokaryotes.

The membrane lipids of *E. coli* contain mixed pairs of 16:0, 18:0, 14:0, 16:1, 18:1 and 14:1. This bacterium does not have any polyunsaturated fats in its membranes (Ishinaga et al., 1979). When *E. coli* are grown at 37°C, most of their membrane fats have pairs of saturated–monounsaturated fatty acids, followed in abundance by saturated–saturated pairs and monounsaturated–monounsaturated pairs (Ishinaga et al., 1979). When *E. coli* are subjected to low temperatures, they actively replace the 16:0 in their membrane fats with 18:1 in order to maintain fluid membranes. This change is very rapid, being evident within 30 s of the temperature change (Rock et al., 1996).

However, the simplistic concept of 'fluidity' does not do justice to understanding movement in biological membranes, where there appears to be a 'Goldilocks' level of molecular movement, as discussed above. Such a requirement possibly explains the evolution of additional 'desaturase' enzyme systems that insert extra double bonds into monounsaturated fatty acyl chains to produce polyunsaturated fatty acids. Each desaturase enzyme system is specific for where in the fatty acyl chain it will convert a single -C-C- bond to a double -C=C- bond (Nelson and Cox, 2005). This means that the specific desaturase determines where in the profile of a bilayer membrane the double bonds are located. Monounsaturated acyl chains have double bonds approximately half-way between the alpha carbon (located on the outside edge of the bilayer) and the omega carbon (located in the very middle of the bilayer). This means that a monounsaturated bilayer membrane will have a layer of double bonds approximately a quarter of the way into the bilayer from either edge but no double bonds in the middle half of the bilayer (Fig. 1).

Polyunsaturated fatty acid chains have multiple double bonds spread throughout the acyl chain and, unlike monounsaturated chains, will contribute double-bonded carbons to the middle of a membrane bilayer. The two classes of polyunsaturated fatty acids, omega-6 and omega-3, differ in the position of their most terminal -C=C- unit. Omega-6 fatty acids have no double bonds in the last six carbons of the chain before the terminal omega carbon. Omega-3 fatty acids have no -C=C- units in the last three carbons before the omega-carbon. This means that in a bilayer membrane of lipids with 18-carbon acyl chains (i.e. the bilayer is 36 carbons wide), that omega-6 acyl chains contribute double-bonded carbons to the outer-third on each side of the bilayer but no -C=C- units to the middle third of the bilayer, where only the omega-3 fatty acyl chains will contribute such double-bonded carbons (Fig. 1). This distinction between these two types of polyunsaturated fats means that they have different effects on the profile of molecular movement in biological membranes.

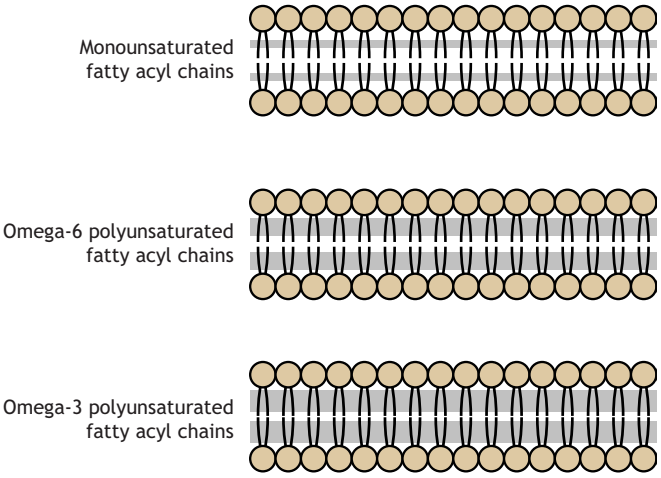

Fig. 1. Cross-section of biological membrane bilayers. Location of double-bonded carbons contributed by monounsaturated, omega-6 polyunsaturated and omega-3 polyunsaturated fatty acyl chains (shown by grey highlighted regions).

In 1974, it was shown that although a lowered growth temperature for *E. coli* resulted in increased content of unsaturated fats in the bacterial membranes, the measured viscosity of the membranes (which is the inverse of membrane fluidity) was approximately the same at the different growth temperatures (Sinensky, 1974). Subsequently, the temperature-induced change in membrane fatty acyl composition was called 'homeoviscous adaptation'. It is both rapid and widespread, being also present in plants (Thompson and Nozawa, 1984) and animals (Cossins et al., 1981). It has been especially studied in fish, in which both monounsaturated and polyunsaturated fatty acid composition of membranes change (Hazel and Williams, 1990). Hazel (1995) proposed that the changes in monounsaturated fats were enough to ensure that membranes were fluid at low temperatures, and suggested that the increase in polyunsaturated fat content of membranes at low temperature was more than just maintaining a fluid membrane but was also important for the 'dynamics' of membrane lipid–protein interactions

Influence of polyunsaturated fats on membrane function

Membrane fatty acyl chains affect the activity of proteins located in the membrane bilayer. One example, related to homeoviscous adaptation, was the finding that membrane lipids extracted from cold-acclimated fish cause greater reactivation of a delipidated membrane enzyme than do those from warm-acclimated fish (Hazel, 1972). Similarly, cold-acclimated trout increase the activity of the sodium pumps in red blood cell membranes, which is associated with an increased polyunsaturated content of membrane lipids but not associated with an increased number of pumps (Raynard and Cossins, 1991).

In a comparison of tissues from endothermic and ectothermic vertebrates, considerable variation in Na^+,K^+-ATPase enzymatic activity was observed both between tissues and between species. Although the concentration of sodium pumps (i.e. Na^+,K^+-ATPase) varied dramatically between tissues in a species, pump concentration was similar for each tissue among species. When the individual molecular activity of sodium pumps was calculated for each tissue and species, a simpler pattern emerged. The molecular activity (at 37°C) of the sodium pumps in mammalian tissues averaged ∼8000 ATP min^{-1} (irrespective of tissue source) compared with ∼2500 ATP min^{-1} for the ectothermic vertebrate tissues (Else et al., 1996). That this difference was due to the membrane lipids surrounding the sodium pump, and not due to the sodium pump itself, was experimentally demonstrated by a series of membrane-crossover experiments where the molecular activity of rat sodium pumps was measured surrounded by toad membrane lipids, and that of toad sodium pumps was measured surrounded by rat membrane lipids (Else and Wu, 1999). This was confirmed by a second series of membrane-crossover experiments comparing sodium pumps from cattle and crocodiles (Wu et al., 2004). That these effects are likely to be related to movement within the membrane bilayer is supported by the observation that molecular activity of the sodium pump in rats and toads is strongly correlated with the lateral pressure exerted by the membrane lipids surrounding the pump (Wu et al., 2001), with greater lateral pressure being due to greater movement of membrane lipids.

Protein-mediated processes located on membranes sometimes involve multiple protein–protein interactions. Many of these processes are classified as ‘G-protein cascades’, and because such processes involve lateral movement of membrane proteins, membrane fatty acid composition can have significant influence, as discussed below. Such cascades connect a receptor to an effector via intermediate G-proteins and have two important features: (i) they allow amplification of a signal and (ii) they can include a wide variety of types of both receptor and effectors. The receptors as a group are called ‘G-protein-coupled receptors’ (GPCRs), and include receptors for hormones, neurotransmitters and inflammatory mediators, as well as for senses such as sight, taste, smell and touch. The effectors include different types of enzymes (Nelson and Cox, 2005). An indication of the importance of these systems in biology is that, since 1947, nine Nobel Prizes have been awarded for various aspects of GPCR and G-protein signalling (https://en.wikipedia.org/wiki/G_protein).

The densely packed membranes of retinal cells contain a G-protein cascade. The first membrane protein in the sequence is rhodopsin (which carries the visual pigment). Once activated by a photon, rhodopsin collides with and activates a membrane-bound G-protein. A subunit of the activated G-protein, in turn, interacts with and activates a membrane-associated phosphodiesterase enzyme, which lowers the concentration of c-GMP in the cytoplasm. This influences membrane-bound ion channels, eventually resulting in neuronal impulses in the optic nerve. Although described above as a ‘one-on-one’ sequence, one activated receptor (rhodopsin) can activate several hundred G-proteins, and once the effector (phosphodiesterase enzyme) is activated, it can degrade several hundred c-GMP molecules. Consequently, it has been calculated that one photon can result in the conversion of thousands of c-GMP molecules (Arshavsky and Burns, 2014).

The amplification of the retinal G-protein cascade has been measured in artificial membrane vesicles containing rhodopsin, G-proteins and the phosphodiesterase effector enzyme by measuring enzyme activity following stimulation with light (at a level within the normal range experienced by retinal cells). Control vesicles were made from membrane lipids that contained only 18:0–22:6ω-3 fatty acid pairs (which is the dominant membrane lipid in retinal membranes). When vesicles were made from membrane lipids with 18:0–22:5ω-3, there was no difference in effector enzyme activity. However, when vesicles were made from membrane lipids with 18:0–22:5ω-6 fatty acid pairs, the effector enzyme activity was halved (Mitchell et al., 2003). This experiment is relatively little known but, in my opinion, is very significant; it shows that the amplification of this particular G-protein cascade in a membrane containing omega-3 fats is double that in a membrane containing an equivalent omega-6 fat. In this experiment, the position of the double-bonded carbons is more significant than the number of double-bonded carbons. Furthermore, the G-proteins are primarily located on the cytoplasmic surface of the membrane and attached to the membrane bilayer by 14- and 15-carbon acyl chains embedded in the membrane. Thus, this experiment demonstrates that interactions that take place on the surface of the membrane can be strongly influenced by conditions in the very middle of the bilayer.

Amplification varies between different signalling systems and is associated with the number of G-proteins (relative to the receptor), how long the various proteins remain ‘activated’ and also their lateral movement in the bilayer. This lateral movement will affect how many collisions there are between activated receptors and G-proteins and also between activated G-proteins and effectors. It is the lateral movement that membrane fats can influence. Whether this difference between omega-3 and omega-6 content applies to other G-protein cascades is, to my knowledge, unknown, but in my opinion is probable; this is a question that would benefit from future investigation. I posit that the effect is unlikely to be restricted to the visual system. For example, when rats are maintained on an omega-3 deficient (but omega-6 adequate) diet they display not only visual deficiencies, but also loss of cognitive skills, as well as deficits in odour and spatial discrimination (Niu et al., 2004).

Omega-6 and omega-3 are separate dietary essential fatty acids

The capacity to synthesise saturated and monounsaturated fats from non-lipid sources is present in bacteria, plants and animals. Omega-6 fats are made by modifying monounsaturated fats, whereas omega-3 are synthesised by modifying omega-6 fats, with the enzymes responsible for both of these processes being present in plants but not in most animals. Consequently, most animals must obtain both of these polyunsaturated fats pre-formed in their diet. Saturated and monounsaturated fats are not essential dietary components for animals. (For a metabolic chart describing the synthesis of different fatty acids from 16:0, see Hulbert and Abbott, 2011.)

A key story relating to animal nutrition is that once the enzymes required to synthesise a particular molecule are ‘lost’ (either because of mutation, or because they are no longer transcribed/translated etc.) then, as long as the particular molecule is part of the animal’s food, there is no selective disadvantage. It is at this evolutionary moment that the particular molecule moves from being optional to an essential part of the animal’s diet. This is the case for vitamins and for some amino acids, and so it is for omega-6 and omega-3 fats. The nematode *Caenorhabditis elegans*, unlike ‘higher’ animals, still possesses all the enzymes required to synthesise omega-6 and omega-3 fats (Shmookler Reis et al., 2011); thus, for this simple animal, neither type of polyunsaturated fat is dietary essential. Presumably because its diet consists of bacteria (such as *E. coli*, at least in the laboratory) that contain only saturated and

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monounsaturated fats, there remains strong selective pressure for these simple animals to retain the ability to synthesise both types of polyunsaturated fats. As long as cellular membranes are part of the food of an animal (which is an almost universal occurrence), the animal will obtain the essential ingredients required to make its own membranes.

Fat was unexpectedly shown to be needed in the diet of rats during research investigating vitamin E (Burr and Burr, 1929). It was quickly determined that what was actually 'essential' was a specific omega-6 fat (18:2ω-6, with the suggestion that other unsaturated fats may also be essential, Burr and Burr, 1930). Later, it was shown that this omega-6 fat was essential in the diet of many other animals, including humans. Then, in 1978, following a gun accident, a 6-year-old girl had to be fed intravenously for the rest of her life; after about a year she developed severe symptoms that no-one could explain. It was discovered that her intravenous food contained only omega-6 but no omega-3 fats. When her intravenous food was replaced with one containing both omega-6 and omega-3 fats the symptoms went away. This was the first evidence that humans have a separate 'essential' requirement for omega-3, as well as for omega-6 fat, in their diet (Holman et al., 1982).

Regulation of membrane composition: importance of diet omega-6 and omega-3 balance

The relationship between dietary fatty acid composition and tissue membrane composition has been studied in the rat (Abbott et al., 2010, 2012). This study involved 12 moderate-fat diets that varied widely in their content of saturated, monounsaturated, omega-6 and omega-3 fatty acids (with both the omega-6 and omega-3 being only 18-carbon fats). The fatty acid composition of storage fats reflected the diet composition, but this was not the case for membrane fats, in which the saturated, monounsaturated and (total) polyunsaturated fatty acid composition was relatively constant irrespective of diet composition (Fig. 2). However, membrane composition was strongly affected by the balance between omega-3 and omega-6 in the diet.

The fatty acid which showed the most variation in membrane composition was arachidonic acid (20:4ω-6). Because there was no 20:4ω-6 in the diet, this important omega-6 fat had been synthesised from 18:2ω-6 in the diet before it was incorporated into membrane lipids. What was also unexpected was that, in all tissues, the balance between omega-3 and omega-6 in the diet was a much better predictor of membrane 20:4ω-6 content than was diet 18:2ω-6

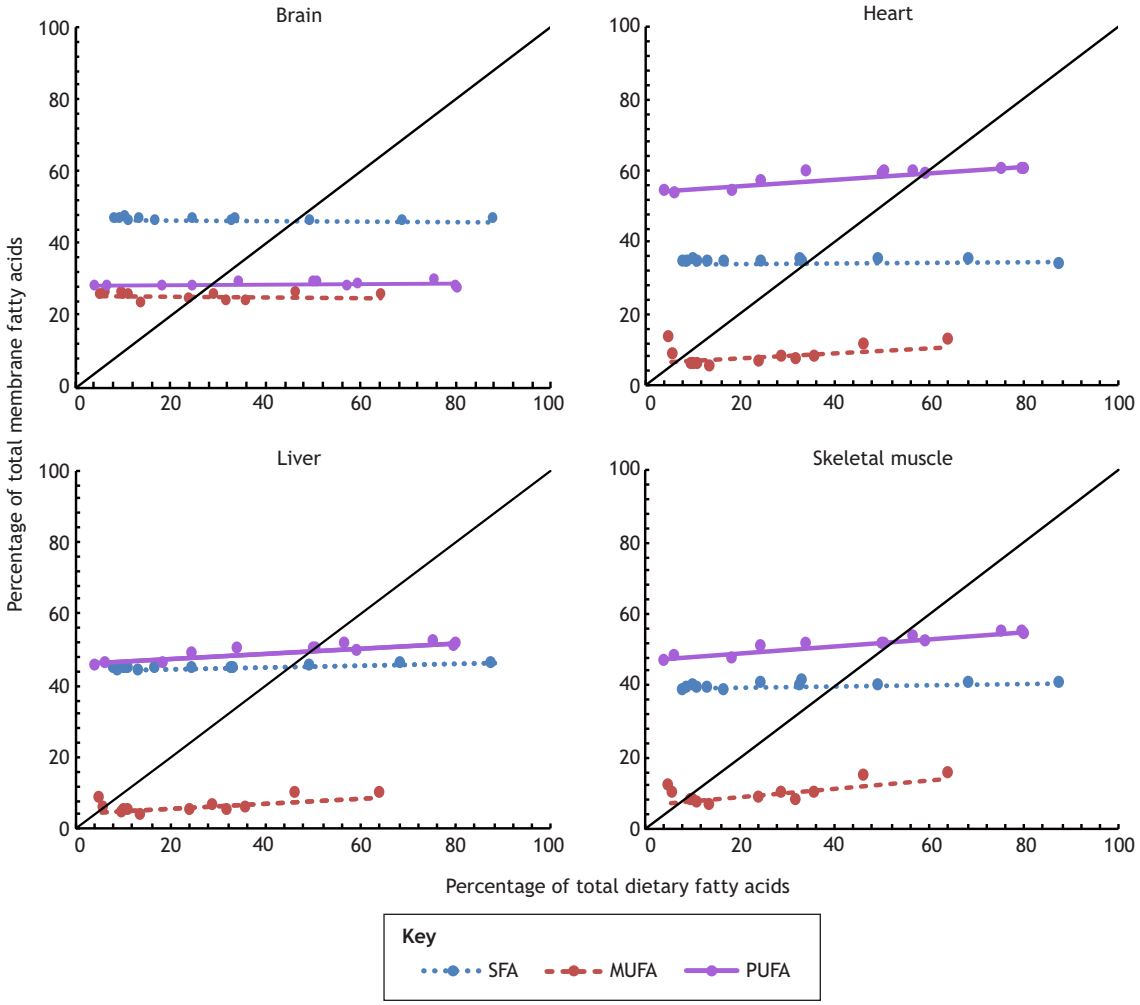

Fig. 2. The homeostatic regulation of membrane fatty acid composition of tissues from the rat in relation to dietary fatty acid composition. For each tissue, SFA is the relationship for saturated fatty acids, MUFA is the relationship for monounsaturated fatty acids, and PUFA is the relationship for total polyunsaturated fatty acids. In each graph, the diagonal line is the line of conformity, where membrane composition equals diet composition. As can be seen for all four tissues, membrane composition is relatively constant despite wide variation in diet composition. Data are taken from Abbott et al. (2012).

content (Fig. 3). There are two pathways for a fatty acid to appear in a membrane phospholipid; the first is via '*de novo*' synthesis of a phospholipid, and the second is via 'remodelling' of membrane lipids. The remodelling pathway is catalysed by acyltransferase enzymes, and previously it has been shown for rat liver cells that 20:4ω-6 appears in membrane lipids only via this pathway (Schmid et al., 1995). The acyltransferase-mediated remodelling pathway is predominantly responsible for maintenance and regulation of membrane fatty acid composition and is very rapid. For example, it is so rapid that it can replace polyunsaturated fatty acids damaged by oxidative stress as fast as they are damaged, thus maintaining a relatively constant membrane fatty acid composition (GironCalle et al., 1997). Early studies found that although acyltransferases are highly selective for polyunsaturated fatty acids they do not discriminate well between omega-6 and omega-3 (Lands et al., 1982). This means that whether an omega-6 or omega-3 is added to the membrane lipid during membrane remodelling will depend on the relative abundance (or balance) of omega-3 and omega-6 fatty acids in the vicinity of the enzyme (Fig. 3).

The finding that dietary omega-3/omega-6 balance determines membrane 20:4ω-6 content is significant because, although I have stressed the importance of polyunsaturated fats for the physical properties of membrane bilayers, they are also important in a wide variety of cell-signalling systems, and this is especially true for 20:4ω-6. Membrane lipids that contain a 20:4ω-6 acyl chain are the main sources of eicosanoid signalling molecules (e.g. prostaglandins, prostacyclins, leukotrienes, thromboxanes), which are involved in myriad body functions (including stimulation of adipogenesis, inflammation, fever, pain, blood clotting, allergic reactions) (Nelson and Cox, 2005). They are also the source of another important group of signalling molecules, the endocannabinoids, which are involved in regulation of appetite, memory, energy balance, stress response and the immune system (Watkins, 2019). The relative abundance of 20:4ω-6 in membrane lipids will affect the production of a large number of these signalling molecules and will thus influence body function. For example, in one study, a diet-induced two-fold change in 20:4ω-6 content of white blood cell membranes resulted in a five-fold increase in prostaglandin production (Peterson et al., 1998).

Conclusions and final comments

Biological membranes composed of lipid bilayers are found in all life forms, and fatty acids are essential parts of such membrane lipids. Lipogenesis, the synthesis of a saturated fatty acid, is best understood as the process evolved early by prokaryotes to make membrane components. The synthesis of fat as an energy store is proposed to be a later evolutionary modification of the biochemistry originally used for the manufacture of biological membranes. The evolution of enzyme systems to make monounsaturated and, in turn, omega-6 and omega-3 polyunsaturated fats from these saturated fats allowed the maintenance of fluid membrane bilayers. The later evolutionary loss of the enzyme systems responsible for manufacture of omega-6 and omega-3 fats resulted in them becoming separate essential components of the diet of most animals. The separate nutritional essentiality of omega-3 and omega-6 fats and their sometimes opposing effects is relatively underappreciated outside biomedical research, where they have been (and continue to be) the subject of significant investigation (e.g. Blasbalg et al., 2011; Saini and Keum, 2018; Jang and Park, 2020). The influence of omega-6 and omega-3 fats on (i) the physical properties of membrane bilayers, and (ii) the signalling processes sourced from membrane fatty acids provide a number of mechanistic pathways to explain their different effects. Investigators in at least two areas of comparative physiology have shown that the balance between omega-3 and omega-

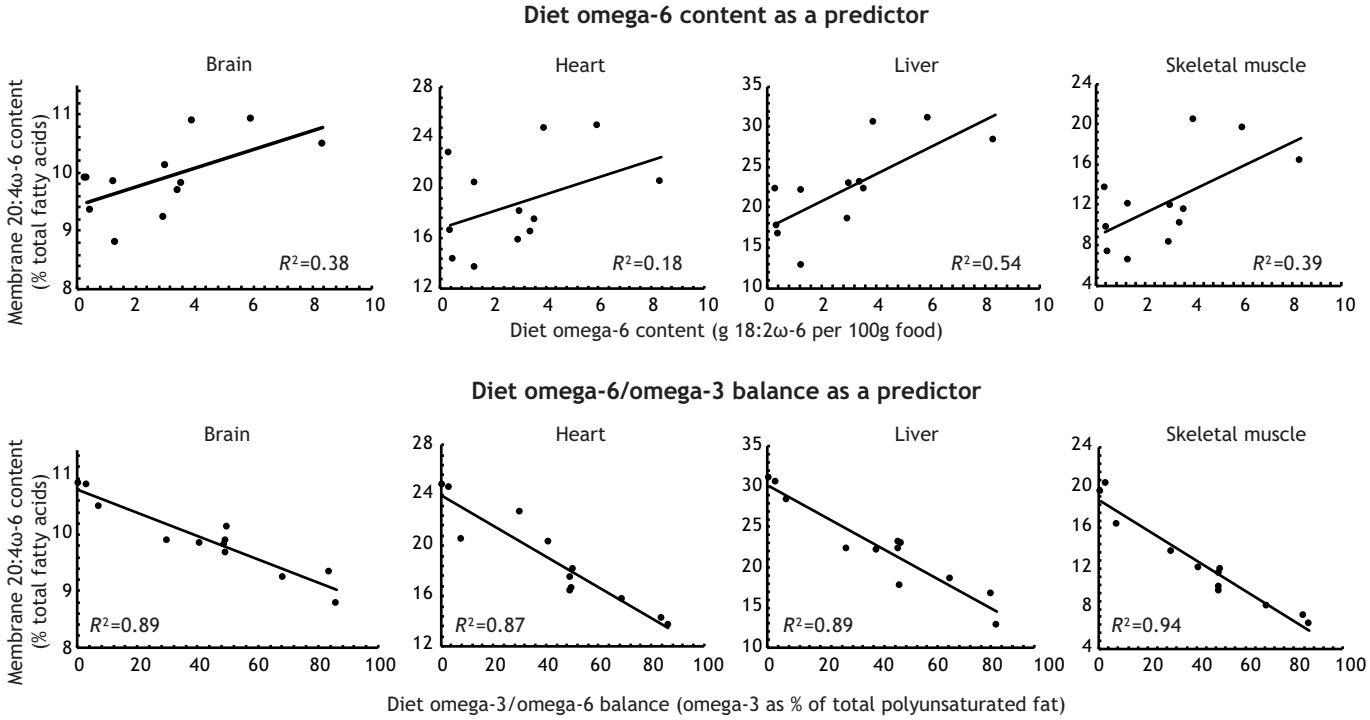

Fig. 3. A comparison of the relationship of membrane 20:4ω-6 content from the rat in relation to dietary 18:2ω-6 content and omega-3/omega-6 balance. Data are shown for diet 18:2ω-6 content (top) or total polyunsaturated fat in the diet that is omega-3 (bottom). All diets had a total fat content of 25% dietary energy. As can be seen from the respective R^2 values, dietary omega-3/omega-6 balance is a much better predictor of membrane 20:4ω-6 content than is diet 18:2ω-6 content (Data are taken from Abbott et al., 2010, 2012).

6 in the diet is important. For example, in hibernators ranging from marmots to dormice it has been shown that dietary omega-6 is beneficial for normal hibernation but that dietary omega-3 is not (Hill and Florant, 2000; Ruf and Arnold, 2008; Girourd et al., 2018). Dietary omega-6 and omega-3 have different effects on the treadmill endurance of rats (Ayre and Hulbert, 1997) and have been proposed to be important in long-distance bird migration (Weber, 2009; McWilliams et al., 2020). Awareness that different foods differ in their omega-6 and omega-3 contents will enable a more sophisticated analysis of the links between diet choice and the natural biology of animals.

As a final comment, I note that we are only beginning to understand the implications of the balance between omega-3 and omega-6 fats in the human diet. Although most animals have a relatively constant diet, we humans are especially diverse (both between individuals and over time) in the types of food we consume. Over the last half-century, the modern human food chain has emphasised omega-6 and diminished omega-3 intake, largely because of: (i) a shift from animal fats to vegetable oils, (ii) an increase in grain-fed meat and dairy, and (iii) a decline in full-fat dairy products from grass-fed livestock (an important source of omega-3). In the opinion of the current author and others, these diet trends are likely to be responsible for the increased incidence of obesity and other modern epidemics of chronic disease, but that is a story for another time.

Competing interests
The author declares no competing or financial interests.

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