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Commentary

# Life, death and membrane bilayers

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## Summary

**Membrane bilayers are essential elements of life, and the synthesis of the hydrocarbons that make up membrane bilayers may have preceded the appearance of life on Earth. Membrane-associated processes are significant components of metabolism, and the acyl composition of membrane bilayers is associated with metabolic activity in a predictable manner. This has resulted in the 'membrane pacemaker' theory of metabolism, which proposes that the relative balance between monounsaturated and long-chain polyunsaturated acyl chains in membrane bilayers is a fundamental determinant of metabolic rate of a species. The omega-3 polyunsaturated docosahexaenoate is an especially important component of membranes in this regard. Whilst it is suggested that the physical properties of membrane polyunsaturates are important with respect to their influence on metabolic rate, it is their chemistry that is important in aging. Membrane acyl composition is related to maximum lifespan in mammals and birds, probably *via* their role in lipid peroxidation. Calorie restriction modifies acyl composition of membrane bilayers and is associated with decreased membrane lipid peroxidation and lifespan extension. The membrane pacemaker theory of metabolism has given birth to the membrane pacemaker hypothesis of aging, which will require further investigation.**

## Introduction

Life requires membranes. Their universal occurrence in living organisms suggests that the earliest life-forms on the planet also possessed them. Indeed, just as DNA is described as an 'eternal' molecule, membranes might be called 'eternal' structures, since in modern organisms new membranes arise from pre-existing membranes.

Biological membranes generally consist of bilayers of amphipathic molecules held together by non-covalent bonds. In eukaryotic cells, phospholipids are the predominant membrane lipids and consist of a hydrophilic head group to which are attached hydrophobic acyl chains. These acyl chains are either saturated, monounsaturated or polyunsaturated hydrocarbon chains that normally vary from 14 to 22 carbons in length. Fig. 1 presents the structure of two phospholipids, and Table 1 lists acyl chains commonly found in biological membranes. This commentary will concentrate on the emerging role of membrane acyl chains during the life and death of animals.

## Origins: the beginning of life and membrane bilayers

Whilst most speculation about life's origins has centred on biopolymers, especially RNA, there is an alternative 'lipid world' scenario (Segre et al., 2001) where the self-assembly properties of lipids would result in supramolecular structures (such as bilayers) with the ability to enhance energy-dependent synthetic reactions. Such assemblies would become primordial homeostatic systems displaying life-like properties and on which selective processes would act. Fluids collected from hydrothermal vents on the mid-Atlantic ridge indicate abiotic *de novo* synthesis of linear saturated hydrocarbons, including some found in modern membranes (Holm and Charlou, 2001).

The three major domains of life – Archaea, Bacteria and Eucarya – include two prokaryotic domains that differ markedly in the lipids that constitute their membranes (see Itoh et al., 2001). The Archaea inhabit the most extreme environments on Earth and correspondingly have unusual lipids that provide a 'toughness' to their membranes (Hochacka and Somero, 2002). The core of the archaeal membrane consists of isoprenoid chains and not the acyl chains found in Bacteria and Eucarya. Whilst some archaeal membrane lipids are amphipathic, others are described as bipolar lipids with two 40-carbon isoprenoid chains attached at either end to hydrophilic glycerol head groups by ether linkages. These tetraether lipids form 'monolayer' cell membranes that are analogous to bilayer membranes with each side covalently linked to the other. The 40-carbon

isoprenoid chains may also contain isopentane ring structures. Both the tetraether structure of archaeal membrane lipids and the ring structures within the isoprenoid chains provide a protective membrane 'rigidity' in the very high-temperature environments that many of the Archaea inhabit. Some thermophilic Archaea homeostatically increase the number of ring structures within the 40-carbon isoprenoid chains in response to higher growth temperature (Itoh et al., 2001). It is of interest that the sterols, such as cholesterol, that are membrane components in Eucarya are synthesised from isoprenoid chains.

*Lipid structure and function across three domains of life*

The backbone of most membrane lipids in Archaea, Bacteria and Eucarya is the three-carbon glycerophosphate, and the membrane lipids of Bacteria and Eucarya are similar, consisting of fatty acyl chains ester-linked to this three-carbon backbone. In the Bacteria, most is known about lipid metabolism in *Escherichia coli* but although many bacteria follow the *E. coli* paradigm others do not (Rock et al., 1996). Bacteria do not synthesise lipids for energy storage, thus the fundamental function of fatty acid synthesis in bacteria is the production of membranes. The three acyl chains normally synthesised by *E. coli* – palmitate (16:0), palmitoleate (16:1 n-7) and vaccenate (18:1 n-7) – are incorporated into phospholipids by an acyltransferase. Bacterial membrane bilayers contain saturates and monounsaturates but generally lack polyunsaturates.

For normal function, membrane bilayers must be 'fluid', allowing lateral movement of membrane components. Phospholipids with two saturated acyl chains will only be able to maintain a fluid state at high temperatures. Unsaturated acyl chains are essential for membrane 'fluidity' in the range of temperatures typical of modern life. The fact that membrane lipids in bacteria and eukaryotes consist of pairs of acyl chains attached to a head group is a means of 'handcuffing' an unsaturate to every saturated acyl chain, ensuring compulsory mixing and thus bilayers that do not laterally separate into 'solid' and 'fluid' patches. This does not mean that all membranes are homogeneous in their fluidity. When grown at low temperature, *E. coli* substitute 18:1 n-7 acyl chains for 16:0 acyl chains, producing phospholipids containing two monounsaturates. This very rapid response, being evident within 30 s of lowering the temperature (Rock et al., 1996), is termed 'homeoviscous adaptation' and results in a relatively constant membrane fluidity.

Compared with bacteria, the eukaryotes have increased the variety of lipids that make up their membrane bilayers.

Table 1. *Some fatty acyl chains found in membrane bilayers of animal cells*

| Common name | Systematic name* | Abbreviation† |
|---|---|---|
| Saturates | | |
| Mystyric | Tetradecanoic | 14:0 |
| Palmitic | Hexadecanoic | 16:0 |
| Stearic | Octadecanoic | 18:0 |
| Arachidic | Eicosadecanoic | 20:0 |
| Unsaturates | | |
| Monounsaturates | | |
| Palmitoleic | *cis*-9-hexadecenoic | 16:1 n-7 |
| Vaccenic | *cis*-11-octadecenoic | 18:1 n-7 |
| Oleic | *cis*-9-octadecenoic | 18:1 n-9 |
| Polyunsaturates | | |
| Omega-6 | | |
| α-Linoleic | *cis*,*cis*–9,12-octadecadienoic | 18:2 n-6 |
| γ-Linolenic | All-*cis*-6,9,12-octadecatrienoic | 18:3 n-6 |
| | All-*cis*-8,11,14-eicosatrienoic | 20:3 n-6 |
| Arachidonic | All-*cis*-5,8,11,14-eicosatetraenoic | 20:4 n-6 |
| | All-*cis*-4,7,10,13,16-eicosapentaenoic | 22:5 n-6 |
| Omega-3 | | |
| α-linolenic | All-*cis*-9,12,15-octadecatrienoic | 18:3 n-3 |
| | All-*cis*-11,14,17-eicosatrienoic | 20:3 n-3 |
| EPA | All-*cis*-5,8,11,14,17-eicosapentaenoic | 20:5 n-3 |
| DHA | All-*cis*-4,7,10,13,16,19-docosahexaenoic | 22:6 n-3 |

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

*Numbers indicate the positions of double bonds numbered from the carboxylic end (see Fig. 1).

†The number preceding the colon indicates the number of carbon atoms; the number following the colon indicates the number of double bonds; the position of the double bond nearest the methyl end is given by the designation 'n (total number of carbon atoms) minus the number of carbon atoms from the end of the chain'.

Notably, they synthesise phosphatidylcholine, a common lipid in eukaryotic membranes (see Fig. 1), as well as non-phospholipid membrane lipids such as sphingolipids and sterols. Compared with prokaryotes, eukaryotes also have additional desaturase enzyme systems capable of introducing extra double bonds into acyl chains (Tocher et al., 1998). There are three types of desaturases, all of which consume molecular oxygen and electrons obtained from an electron transport chain. One type is found in the stroma of plant plastids, a second type are associated with the endoplasmic reticulum and chloroplast membranes of plants and thylakoid membranes of cyanobacteria, whilst the third type are membrane-bound enzymes associated with the endoplasmic reticulum of animal and fungal cells. These enzymes introduce double bonds into acyl chains esterified to coenzyme A. Whilst some invertebrate groups possess the desaturases required for *de novo* production of omega-3 and omega-6 polyunsaturates, these enzymes are absent in vertebrates and many other invertebrates. In such species, polyunsaturates are essential dietary components, although they can elongate and further desaturate short-chain omega-3 and omega-6 polyunsaturates. As animals are heterotrophs and because polyunsaturates both occur in the membranes of eukaryotic cells and are required only in small amounts, polyunsaturates will normally be found in adequate amounts in food.

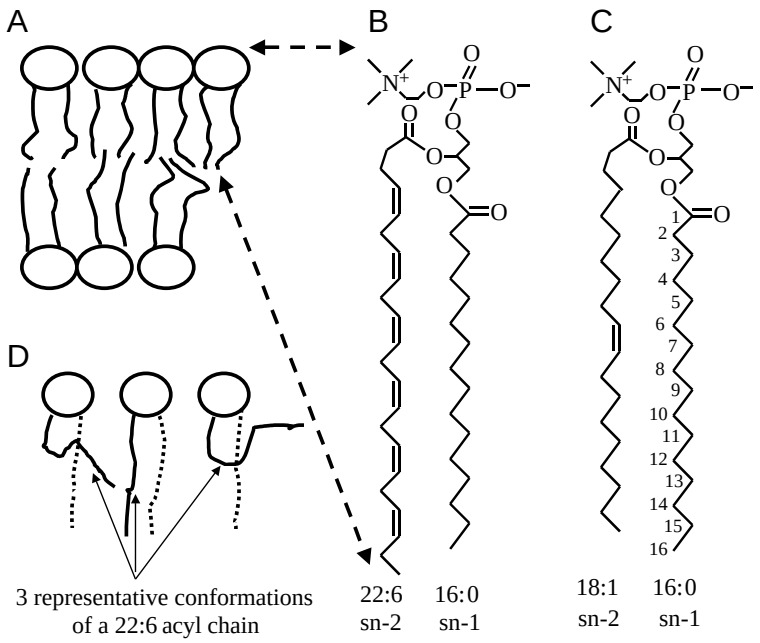


Fig. 1. Diagrammatic representation of a biological membrane bilayer (A), with the molecular structure of two representative phospholipids: a polyunsaturated phosphatidylcholine (B) and a monounsaturated phosphatidylcholine (C). In both structures, neither C nor H are shown, although the carbon atoms are numbered in the right-hand chain in C. (D) Three representative conformations of docosahexaenoic acid (DHA) (22:6) acyl chain (solid line) in a phosphatidylcholine molecule (these conformations are from fig. 12 of Feller et al., 2002). In D, the sn-1 acyl chain is shown as a broken line.

## Metabolism: the middle of life and membrane bilayers

Living costs energy. A substantial part of this cost of living involves membrane-associated activities that are influenced by the acyl composition of the membrane. Eukaryotic cells are distinctive in that they maintain compartments with very different compositions, and thus membrane processes are responsible for the maintenance of associated transmembrane gradients.

### *Composition of metabolic rate*

At a fundamental level, mitochondria maintain a substantial proton gradient across the mitochondrial inner membrane, and this gradient is used by the mitochondrial ATP synthase to manufacture ATP. Most of the oxygen consumed by cells is used by the mitochondrial respiratory chain to maintain this transmembrane proton gradient. However, even when mitochondria are not making ATP, they still consume oxygen and pump protons. Under these conditions, the mitochondrial proton gradient remains constant because there is a balancing proton leak. Mitochondrial proton leak is best characterised in liver mitochondria and is estimated to be responsible for approximately 20% of resting oxygen consumption of mammalian hepatocytes (Porter and Brand, 1995). Mitochondrial proton leak is rarely mentioned in undergraduate texts.

Another significant transmembrane gradient is generated by the $Na^+/K^+$-ATPase, ubiquitous to animal cells. The transplasmalemmal $Na^+$ gradient it maintains is used by a host of other cellular activities, including ion homeostasis and regulation of intracellular volume. It is also the immediate energy source for action potentials in excitable cells and the active uptake of nutrients and transcellular ion transport in some epithelial cells. Its quantitative contribution to energy turnover varies from approximately 10% in liver to approximately 60% in kidney and brain (Clausen et al., 1991).

The resting oxygen consumption of the laboratory rat has been allocated thus: 10% is non-mitochondrial, 20% is related to mitochondrial proton leak and 70% is for mitochondrial ATP production, which is used by the $Na^+/K^+$-ATPase (20–25%), protein synthesis (20–25%), $Ca^{2+}$-ATPase (5%), gluconeogenesis (7%), myosin-ATPase (5%) and ureagenesis (2%), with other activities (including nucleic acid synthesis) constituting 6% (Rolfe and Brown, 1997). A substantial amount of the energy requirements of life is associated with membrane-linked processes. Apart from the obvious mitochondrial proton leak, $Na^+/K^+$-ATPase and $Ca^{2+}$-ATPase, some protein synthesis is a membrane-associated activity and some non-mitochondrial oxygen consumption is associated with membranes. For example, the desaturases are oxygen-consuming membrane-associated enzyme complexes. Their quantitative contribution to this non-mitochondrial oxygen consumption is currently unknown.

The dominant influences on the resting metabolic rate of different animal species are body size, body temperature and whether the species is ectothermic or endothermic. With respect to body size, most studies have involved mammals, but the findings seem generally applicable to other groups. Resting metabolic rate of mammals varies allometrically with body mass with the exponent approximating 0.73–0.75. An interesting finding has been that, although metabolic variation is large (mass-specific metabolic rate of a mouse is about 20 times that of a cow), the relative composition of energy metabolism appears constant with body size. For example, many processes (e.g. protein turnover, RNA turnover and ethane exhalation) in mammals vary with the same allometric exponent as resting metabolic rate (e.g. Topp et al., 1995), which means they constitute a constant proportion of total metabolic rate irrespective of the metabolic intensity and body size of the mammal. Part of the allometric variation in metabolic rate is due to variation in relative tissue size and part is due to variation in mass-specific tissue metabolism. Tissue metabolism varies allometrically in mammals, as does the *in vitro* mass-specific sodium pump activity (Couture and Hulbert, 1995). The fact that the allometric exponents for these activities are similar indicates that sodium pump activity is a constant proportion of total cellular metabolic activity. Similarly, the oxygen consumption of mammalian hepatocytes varies allometrically and the proportion of total hepatocyte oxygen consumption devoted to ATP production, mitochondrial proton leak and non-mitochondrial processes is relatively constant irrespective of body size (Porter and Brand, 1995).

The difference in the resting metabolism of ectotherms and endotherms has been analysed by the study of reptiles and mammals matched for body mass and body temperature. The reptile species chosen are desert lizards *Amphibolurus nuchalis* and *A. vitticeps*, with preferred body temperatures of 37°C, thus allowing comparison of the rate of cellular activities not complicated by the effects of temperature. Resting metabolism is approximately 7-fold greater in the endothermic mammals (mice and rats) compared with the ectothermic reptiles and this is associated with larger tissues and greater mass-specific tissue metabolism in the mammals (Brand et al., 1991). The contribution of ATP production, mitochondrial proton leak and non-mitochondrial processes to total oxygen consumption of hepatocytes is similar in the reptile and mammal (Brand et al., 1991), whilst the proportion of tissue metabolism devoted to sodium pump activity is also similar (Else and Hulbert, 1987).

### *‘Membrane pacemaker’ theory of metabolism*

From both the allometric comparison of metabolism in mammals and the ectotherm–endotherm comparison, the same conclusions are reached. These are: (1) the large differences in the whole-organism metabolic rate are partly a cellular phenomenon and (2) the relative composition of metabolism in different species is similar. Resting metabolic rate appears to consist of linked processes such that when one varies, all vary in unison. This suggests that there is a single factor (or a small number of factors) that influences all (or many) of these processes: i.e. a pacemaker for metabolism. The importance of membrane-associated processes in determining overall metabolic rate suggests that membranes may be the site of such a pacemaker. It has been proposed that both the amount of membranes and their acyl composition, notably the relative balance between monounsaturated and polyunsaturated acyl chains, especially docosahexaenoic acid (DHA), are a pacemaker for metabolic activity (Hulbert and Else, 1999, 2000). Space limitations restrict our discussion here to the compositional aspects of membranes.

The evidence supporting the ‘membrane pacemaker’ theory is of two types: (1) the acyl composition of membrane bilayers varies in a manner similar to variations in metabolic rate and (2) variations in acyl composition of membrane bilayers influence membrane-associated processes. These influences are such that high DHA content is normally associated with increased activity of the membrane-associated processes.

Although they have the same body temperature, the acyl composition of tissue phospholipids in the endothermic mammal had a much greater unsaturation index (number of double bonds per 100 acyl chains) than that of the reptilian phospholipids. The mammalian membrane bilayers had a greater content of the omega-3 polyunsaturated DHA than did the reptilian membrane bilayer but a lower content of the monounsaturated 18:1 n-9 (Hulbert and Else, 1989). In a similar manner, the tissue phospholipids of small mammals have a higher unsaturation index than those of large mammals, although the total percentage of unsaturates does not vary with body size in mammals. The membrane bilayers of small mammals are generally high in DHA but low in 18:1 n-9 compared with those of large mammals. These trends in acyl composition are manifest in phospholipids from all tissues examined except brain, which are highly polyunsaturated in all mammals irrespective of body size (Hulbert et al., 2002c). The acyl chain that shows the greatest allometric variation is DHA, which has exponents of –0.19, –0.21, –0.34 and –0.40 in liver, kidney, heart and skeletal muscle, respectively (Hulbert et al., 2002c). Recently, the same trends have also been observed in the pectoral muscle phospholipids of birds, with DHA content having an exponent of –0.28 (Hulbert et al., 2002a). These exponents are similar to the –0.27 for mass-specific metabolic rate. DHA content represents the greatest variation in body composition yet recorded for different-sized mammals or birds. Fig. 2 shows some of these allometric relationships for skeletal muscle in both mammals and birds.

Although these relationships are for total tissue phospholipids (i.e. pooled membrane bilayers), the same trends exist when subcellular membranes are independently analysed. Liver mitochondrial phospholipids are more polyunsaturated and less monounsaturated in mammals compared with similar-sized lizards with the same body temperature (Brand et al., 1991; Brookes et al., 1998). The same difference is observed in small mammals compared with large mammals (Porter et al., 1996) and in small birds compared with large birds (M. D. Brand, N. Turner, P. L. Else and A. J. Hulbert, unpublished).

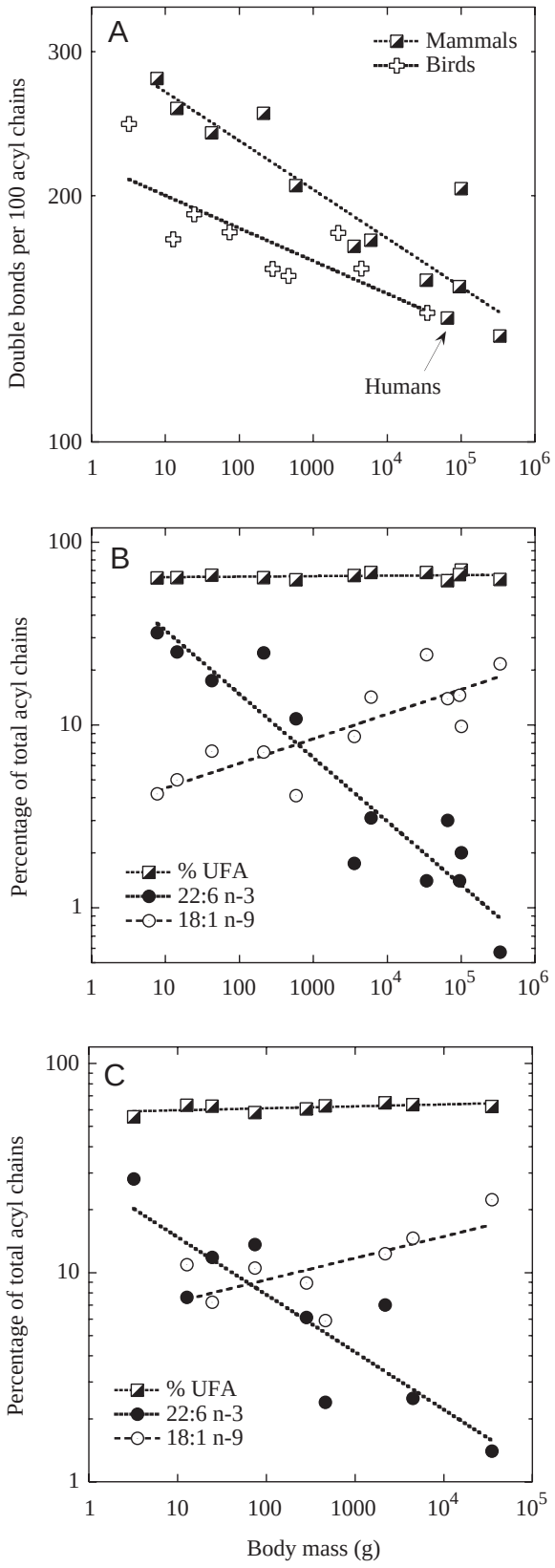


Fig. 2. The relationship between body mass of mammal and bird species and acyl composition of their skeletal muscle phospholipids. (A) The unsaturation index for birds and mammals. (B,C) Percentage of total unsaturates (UFA) and percentage composition of two individual acyl chains, the monounsaturated oleic acid (18:1 n-9) and the polyunsaturated docosahexaenoic acid (22:6 n-3), for mammals (B) and birds (C). The mammal data are from Hulbert et al. (2002c) and the bird data are from Hulbert et al. (2002a).

### *Membrane polyunsaturation and membrane-associated function*

Liver mitochondrial proton leak varies allometrically with body mass in mammals and is related to differences in membrane acyl composition, especially DHA content (Porter et al., 1996). Similarly, in birds, liver mitochondrial proton leak is allometrically related to body mass and correlated with membrane acyl composition (M. D. Brand, N. Turner, P. L. Else and A. J. Hulbert, unpublished). Although liver mitochondrial proton leak from other ectotherms is not as low as that from the desert lizards, it is correlated with differences in acyl composition (Brookes et al., 1998; Hulbert et al., 2002b). When the data for mammals and ectothermic vertebrates are combined, liver mitochondrial proton leak is positively correlated with DHA content of mitochondrial phospholipids ($r$=0.66, $P$<0.01, $N$=26) and negatively correlated with the content of the monounsaturated 18:1 n-9 ($r$=–0.38, $P$<0.05, $N$=26). The difference in proton leakiness of mitochondria from different tissues in the rat can be similarly associated with differences in the acyl composition of their mitochondrial phospholipids (Rolfe et al., 1994). Other evidence is that proton leak of liver mitochondria from mice increased both when membrane DHA content was increased *in vivo*, by feeding menhaden oil, and *in vitro*, by lipid fusion (Stillwell et al., 1997).

In endothermic vertebrates, the molecular activity of individual $Na^+/K^+$-ATPase units is several times that in ectothermic vertebrates (Else et al., 1996), and these differences in molecular activity are related to the different membrane lipids. When delipidated, kidney and brain microsomes from the endothermic rat (*Rattus norvegicus*) and ectothermic cane toad (*Bufo marinus*) both show a decrease in $Na^+/K^+$-ATPase molecular activity. Relipidation with the original microsomal lipids restores molecular activity to normal levels; however, when relipidated with microsomal lipids from the other species, the molecular activity is more like that of the other species (Else and Wu, 1999). These ‘species-crossover’ results show that membrane lipid environment is a significant factor determining $Na^+/K^+$-ATPase molecular activity. The mechanism by which membrane lipids affect enzyme activity is unknown; however, physical characteristics of the membrane lipids appear important. A strong relationship between the packing of membrane lipids and $Na^+/K^+$-ATPase molecular activity in tissues from rats and toads has been observed (Wu et al., 2001). The statistical correlations of molecular activity with the physical properties were stronger than the correlations with acyl compositional parameters, emphasising that membrane acyl composition probably influences the molecular activity of

this membrane protein *via* its effects on the physical environment of the membrane bilayer.

Polyunsaturated acyl chains are also associated with many other rapid membrane-associated activities. For example, DHA content is elevated in high-frequency skeletal muscle such as the flight muscle of hummingbirds (*Archilocus colubris*) and the rattlesnake (*Crotalus atrox*) tail-shaker muscle (Infante et al., 2001). Retinal membranes have a high DHA content and this is associated with high activity of visual system G-proteins (Litman and Mitchell, 1996). Several other examples are described by Hulbert and Else (1999, 2000).

In what at first appears to be a contradiction of the membrane pacemaker theory, many aquatic organisms, especially fish, have highly polyunsaturated membranes. This is associated with adaptation to cold environments and the effect of low temperatures slowing physiological processes. It is also associated with increased metabolic activity. Fish mitochondrial membranes have a higher DHA content than those of mammals and are leakier to protons (Brookes et al., 1998). In fish, cold acclimation generally involves an increase in both monounsaturate and polyunsaturate content (especially DHA) of membrane bilayers, and these changes result in altered activity of membrane-associated proteins (Hazel, 1995). For example, $Na^+/K^+$-ATPase molecular activity is increased by cold acclimation in trout (*Oncorhynchus mykiss*; Raynard and Cossins, 1991), and mitochondrial lipids from cold-acclimated goldfish (*Carassius auratus*) exhibit a greater reactivation of delipidated mitochondrial enzyme than do those from warm-acclimated fish (Hazel, 1972). Lee (1991) has proposed that the effects of membrane lipids on membrane proteins are not related to membrane fluidity. Similarly, Hazel (1995) has suggested that the increase in DHA is not directly related to homeoviscous adaptation, as the increase in monounsaturates is adequate for maintaining membrane fluidity. He suggested that the elevated DHA may have some other function.

### *Polyunsaturated DHA: synthesis, structure and function in eukaryotic membranes*

Recent studies have highlighted the physical properties that DHA imparts to membranes. Molecular dynamic simulations suggest that there are hundreds of conformations likely for DHA in membrane bilayers (Feller et al., 2002). Several of these conformations have the methyl end of the molecule located at the outer edge of the bilayer rather than in the middle of the membrane bilayer, as is normally shown in static diagrams (see Fig. 1). These simulations present an image of DHA thrashing about in the hydrocarbon core of the membrane bilayer. Such molecular movement of DHA in a membrane bilayer suggests that it will likely speed up, in a relatively non-specific manner, many processes catalysed by membrane proteins.

The synthesis of DHA from short-chain omega-3 polyunsaturates involves peroxisomes and is thus different to the synthesis of other membrane acyl chains (Sprecher, 2000). Regulation of the acyl composition of membrane bilayers is both *via* the elongase and desaturase enzyme systems as well as *via* the constant deacylation–reacylation processes of membrane remodelling. In rat hepatocytes, only four molecular species (16:0/18:1, 16:0/18:2, 16:0/22:6 and 18:1/18:2) of phospholipids are synthesised *de novo* and all other molecular species are made by the deacylation–reacylation of these four molecular species (Schmid et al., 1995). Whilst it is not completely clear what particular membrane property is being regulated, the observation that an acyltransferase enzyme is influenced by its surrounding membrane lipid environment (Fyrst et al., 1996) suggests that membrane acyl composition may be regulated at this level. Changes in acyl composition of membrane bilayers can be very rapid, occurring within minutes in cultured cells (Chakravarthy et al., 1986).

The unsaturation of membrane bilayers varies predictably between different tissues but is not very responsive to diet. Omega-6 and omega-3 polyunsaturates are essential components of the diet for higher animals. If polyunsaturates are not present in the diet, the systems involved in regulation of membrane acyl composition will result in greater amounts of both 18:1 n-9 and 20:3 n-9 (an unusual polyunsaturate that higher animals can synthesise *de novo*) in phospholipids. The latter acyl chain is indicative of dietary polyunsaturated fatty acid (PUFA) deficiency, as it is normally not observed in tissue phospholipids. In some animals, gut organisms may also be a significant source of PUFA synthesis. The homeostatic regulation of membrane bilayer composition relative to diet, as well as the tissue specificity, is illustrated in Fig. 3.

The increase in metabolic rate associated with the evolution of endothermy is associated with elevated DHA in membranes and this may have resulted from co-opting the same enzymatic processes used by the ectothermic vertebrates during

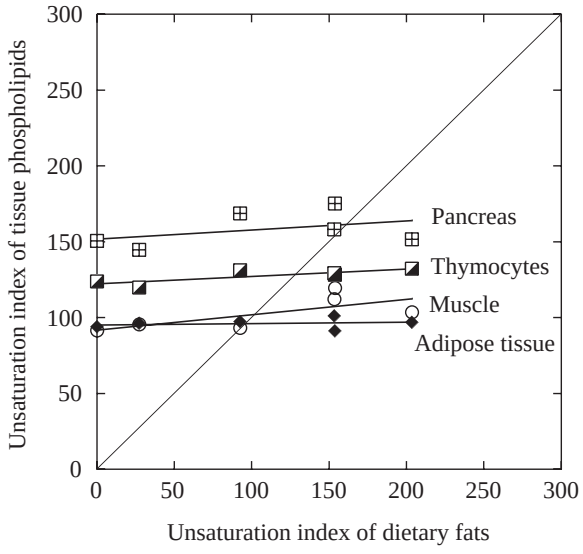


Fig. 3. The relationship between the unsaturation index of dietary fats and the unsaturation index of phospholipids from four tissues in the rat. The diagonal line represents the line of equality where membrane composition is the same as dietary composition. The data are all taken from Soriguer et al. (2000).

homeoviscous adaptation to cold (Hulbert and Else, 1999). It is intriguing to speculate that the differences in metabolic rates of mammals associated with food habits may be due to a distinctive acyl composition of particular foods. For example, are the low metabolic rates of naked mole rats (*Heterocephalus glaber*) and termite eaters due to a diet severely deficient in polyunsaturates? Future investigations into such questions will need to take into account the role of gut microrganisms, but the membrane pacemaker theory of metabolism provides a framework for investigating such mechanistic explanations. For example, Pan and Storlien (1993) have shown that the acyl composition of the diet significantly alters the metabolic rate of rats.

## Death: the end of life and membrane bilayers

### *Membranes and maximum lifespan*

Most multicellular animals eventually die and, although death is not always from old age, the maximum possible length of life is a species characteristic. Membrane bilayer composition may also have a role in determining this maximum lifespan (MLSP). Whilst the physical properties imparted to membranes by their acyl chains influence metabolic rate, it is their chemical properties that are responsible for their role in aging. An extensive discussion of aging mechanisms is not possible here because of space limitations but some general comments follow.

A link between body size, metabolic rate and lifespan was first suggested about a century ago and was elaborated later into the 'rate of living theory' of aging. In the 1950s, it was given a molecular basis with the 'free radical theory' of aging (Harman, 1956). Taking antioxidant defences into account, it has evolved over the past 50 years into an 'oxidative stress theory'. This theory has as its basis the rate of aerobic metabolism and is currently the most popular theory of aging. However, it has a number of problems yet to be resolved. For example, among mammals, humans and bats are long-lived for their size yet have a typically mammalian level of metabolism (Austad and Fischer, 1991). Similarly, birds have metabolic rates slightly higher than mammals but, on average, have lifespans more than twice as long as similar-sized mammals (Holmes and Austad, 1995).

The most favoured current view of death from old age is that it is the result of accumulated damage from reactive oxygen species (ROS) that are an inevitable byproduct of mitochondrial oxygen consumption. Proteins, carbohydrates, nucleic acids and lipids are all targets of such oxidative damage. In lipids, it is the carbon atoms between the –C=C– units found in polyunsaturated acyl chains that are most susceptible to oxidative attack (Halliwell and Gutteridge, 1999). Saturated and monounsaturated acyl chains lack such carbon atoms. The long-chain polyunsaturates in membrane bilayers of mitochondria, however, are very susceptible to damage, both chemically and also because of their location close to the site of ROS production. Membrane lipid peroxidation is an autocatalytic chain reaction, and many of its products, including hydroxynonenal (from omega-6 PUFA) and hydroxyhexanal (from omega-3 PUFA), are themselves very potent damagers of proteins (Halliwell and Gutteridge, 1999).

The low level of phospholipid unsaturation in large mammal species has been related to decreased lipid peroxidation and lipoperoxidative tissue damage and has been suggested to be an adaptation to their long MLSPs (Pamplona et al., 1998, 2000). Although both mammals and birds show allometric variation in membrane acyl composition, there are differences between these two groups of endotherms. Birds have a lower unsaturation index in their muscle phospholipids than do mammals (Fig. 2A), which is related to a higher ratio of n-6 PUFA to n-3 PUFA (Hulbert et al., 2002). It has been proposed that this lower unsaturation in birds is related to their longer lifespan compared with similar-sized mammals (Pamplona et al., 1996). The proposal that the MLSP difference between birds and mammals is related to a lower rate of mitochondrial ROS production in birds (Ku and Sohal, 1993) has recently been questioned (St Pierre et al., 2002). If the acyl composition of membrane bilayers is involved in explaining the association of MLSP with body size, as well as the difference between birds and mammals, then we might expect the same relationship for both birds and mammals. Unsaturation index of muscle phospholipids differs between birds and mammals (Fig. 2A), but when the peroxidizability index of muscle phospholipids is plotted against MLSP (Fig. 4), birds and mammals (including humans) all appear to follow the one relationship. Similar relationships exist for other tissues.

Physiological treatments that extend lifespan can also give insight into the mechanisms underlying aging. Calorie restriction is the only physiological treatment known to extend lifespan in a wide range of animals (Sohal and Weindruch, 1996). During calorie restriction, metabolic rate is not reduced

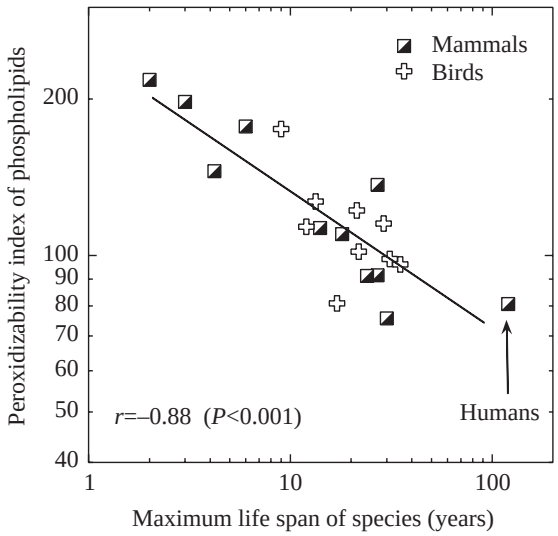


Fig. 4. The relationship between maximum lifespan of mammal and bird species and the peroxidizability index of their skeletal muscle phospholipids. The mammal data have been recalculated from Hulbert et al. (2002c) and the bird data from Hulbert et al. (2002a).

but there is a substantial decrease in lipid peroxidation in rats. This is not attributable to changes in membrane vitamin E content but is associated with changes in membrane acyl composition of both mitochondria and microsomes, resulting in a decreased susceptibility of these membrane bilayers to lipid peroxidation (Laganiere and Yu, 1987). Calorie restriction also modifies acyl composition of muscle membranes (Cefalu et al., 2000), as well as both phosphatidylcholine and phosphatidylethanolamine in liver (Jeon et al., 2001), such as to decrease their ability to undergo lipid peroxidation.

## Conclusions

For a long time, membrane bilayers have been presented as relatively passive components of eukaryotic cells. The patterns regarding membrane acyl composition of animal tissues that have recently become known and the importance of membrane bilayers in the determination of metabolic activity of animals have been synthesised into the membrane pacemaker theory of metabolism. This theory emphasises the physical properties that acyl chains give to membrane bilayers. The potential role of membrane acyl composition in aging processes and the determination of the maximum lifespan of a species represents an exciting area of future investigation that emphasises the chemical properties of these important components of living systems.

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