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Membrane fatty acid composition and longevity of mammals and birds

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Abstract

The fatty acid composition of membrane lipids varies systematically among species in a manner that is consistent with their metabolic rate and longevity. Because the susceptibility of fatty acids to peroxidation relates directly to their extent of unsaturation, it is possible to calculate a peroxidation index (PI) for membranes through characterization of their specific fatty acid composition. Long-living mammals and birds have membrane lipids with a lower PI than shorter-living species. Bird and mammal species with the same maximum life span also have membrane lipids with essentially the same PI. Exceptionally long-living mammals and birds usually have membrane lipids high in monounsaturates, but low in polyunsaturates, with the consequence that the PI of their membrane lipids is as low as expected for their respective longevity. Longevity variation within species (whether due to calorie-restriction, extended longevity associated with specific strains, queen-worker differences in honey bees or inherited longevity differences among humans) is also associated with differences in membrane composition and PI. Membrane composition is specific for each species and PI appears to generally be resistant to dietary manipulation. It is postulated that membrane fatty acid composition is an important influence on aging and the determination of maximum life span.

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4. Membrane fatty acid composition and longevity of mammals and birds

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Abstract. The fatty acid composition of membrane lipids varies systematically among species in a manner that is consistent with their metabolic rate and longevity. Because the susceptibility of fatty acids to peroxidation relates directly to their extent of unsaturation, it is possible to calculate a peroxidation index (PI) for membranes through characterization of their specific fatty acid composition. Long-living mammals and birds have membrane lipids with a lower PI than shorter-living species. Bird and mammal species with the same maximum life span also have membrane lipids with essentially the same PI. Exceptionally long-living mammals and birds usually have membrane lipids high in monounsaturates, but low in polyunsaturates, with the consequence that the PI of their membrane lipids is as low as expected for their respective longevity. Longevity variation within species (whether due to calorie-restriction, extended longevity associated with specific strains, queen-worker differences in honey bees or inherited longevity differences among humans) is also associated with differences in membrane composition and PI. Membrane composition is specific for each species and PI appears to generally be resistant to dietary manipulation. It is postulated that membrane fatty acid composition is an important influence on aging and the determination of maximum life span.

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4.1. Introduction

Humans have long known that large mammal species, in general, live longer than smaller mammal species. In one of the earliest attempts to understand what determines maximum longevity, Max Rubner [1] formalised this understanding by quantifying the size-related differences in longevities of guinea pigs, cats, dogs, cattle and horses. Twenty-five years earlier he had tested the hypothesis that an animal's metabolic rate was proportional to its surface area and not its body mass. He measured the metabolic rate of different sized dogs and confirmed that small dogs had a faster metabolism than large individuals [2]. Combining these two observations, Rubner [1] calculated that lifetime energy consumption per gram of body mass was approximately the same for each of the five mammal species. Although this wasn't the first time that a link between metabolic activity and life span had been suggested (see Speakman [3] for a very readable account of some earlier proposals), Rubner's calculations attracted much attention. His proposed connection between metabolic rate and longevity was later used by Pearl [4] to explain the observation that low temperature extended the life span of fruit-flies. Pearl's book, "The Rate of Living", resulted in subsequent interpretations of metabolic rate effects on longevity being called the rate-of-living theory of aging. Two things are worth commenting on Rubner's calculations: (1) he didn't include *Homo sapiens* in his calculations, if he had his case would not have been as convincing, as humans, being an exceptionally long-living mammal with a normal mammalian metabolism would have had a huge lifetime energy expenditure compared to his other species, and (2) it was not known at that time that membrane fatty acid composition also varied systematically with body size (indeed, to be fair, very little was known about biology of fats at the time). We will comment on both of these observations later in the manuscript and will propose that longevity and metabolic rate of mammals and birds are both related to the fatty acid composition of their membranes.

Rubner's calculations were important in that they suggested aerobic metabolism was responsible, in some way, for an animal's longevity. About fifty years after his contribution, a number of studies suggested that free radicals were natural products of normal aerobic metabolism and were responsible for much of the damage associated with aging. These findings gave rise to the free-radical theory of aging [5], which is now known as the oxidative-stress theory, and is currently the most generally accepted theory of aging (for a recent review see [6]).

4.2. Body size, metabolism, longevity and membrane composition of mammal species

The first observation that the fatty acid composition of membrane lipids varied in a systematic manner among mammals was Gudbjarnason's [7] report of a strong correlation between the docosahexaenoic acid (22:6 n-3) content of cardiac phospholipids and resting heart rate of mammals ranging from mice to whales. Resting heart rate has long been known to correspond with the basal metabolic rate of species and thus Gudbjarnason's report indicated that membrane composition may be related to the body size-metabolic rate relationship. This was of particular interest as it corroborated the findings of a series of studies carried out during the 1980s and 1990s which showed that membrane-associated processes were significant components of metabolic rate and, further, that the speed of these membrane-associated processes varied dramatically among the vertebrate species examined. Couture and Hulbert [8] extended Gudbjarnason's observation and showed that variation in fatty acid composition was not restricted to cardiac phosphospholipids nor was it restricted to the docosahexaenoic acid. It became apparent that membrane lipids from skeletal muscle, cardiac muscle, liver and kidney of mammals with fast metabolic rates contained lower amounts of monounsaturates (MUFA), but had a higher content of polyunsaturated fatty acids (PUFA) compared to mammals with a slow metabolism. Furthermore, species with higher metabolic rates also had a higher degree of polyunsaturation of membrane PUFAs. Insight into the functional basis of membrane fatty acid effects on metabolic rate was provided by a series of species-crossover experiments. These showed that the presence of more polyunsaturated membrane lipids resulted in faster activities of membrane proteins compared to membranes with a higher concentration of MUFAs, and that these effects were related to the physical properties of the membrane lipids and not to the enzymes themselves (for reviews see [9] and [10]).

A long-known difference between PUFA and MUFA is their susceptibility to peroxidative damage. This is because it is the *bis*-allylic hydrogen (H) atoms (i.e. the H attached to single-bonded carbon (C) atoms between double-bonded C) of fatty acids that are most easily removed by free-radicals [11]. Because MUFAs do not have *bis*-allylic $–CH_2-$ groups (i.e. they don't have multiple -CH=CH- groups), they are very resistant to free-radical attack. By contrast, the more polyunsaturated a PUFA molecule is, the more *bis*-allylic $–CH_2-$ groups it possesses, and consequently the more susceptible it is to free radical attack. The removal of a *bis*-allylic H leaves a

C atom with an unpaired electron, i.e. a carbon-centred free radical that, in turn, can interact with a membrane-located oxygen molecule to form a lipid peroxide radical [11]. Holman [12] measured the oxygen consumption during the peroxidation of different fatty acids and showed that the rate of peroxidation of oleic acid (18:1 n-9) was only 2.5% of that measured for linoleic acid (18:2 n-6) while that of the highly polyunsaturated docosahexaenoic acid (22:6 n-3) had a rate 800% faster than linoleic acid. The relative peroxidation rates for different fatty acids allows the calculation of a specific peroxidation index (PI) for membrane lipids based on their particular fatty acid composition (see [6] for details). This ability to encapsulate the susceptibility of a particular mix of fatty acids to peroxidative damage with a single number makes the PI a very useful analytic tool.

In 1985, Cutler [13] reported that tissues from long-living mammals had a lower potential to produce peroxides than tissues from shorter-living mammals. This was extended in the 1990s, by Pamplona and colleagues who showed that the fatty acid composition of phospholipids in several tissues was correlated with maximum life span for a range of mammal species (e.g. 14-16). The relationship between the peroxidation index (PI) of liver mitochondrial phospholipids and maximum life span (MLSP) for 20 species of mammals is shown in **Fig. 1a**, and a similar relationship for PI of skeletal muscle phospholipids and MLSP of 51 mammal species is depicted in **Fig. 1b**.

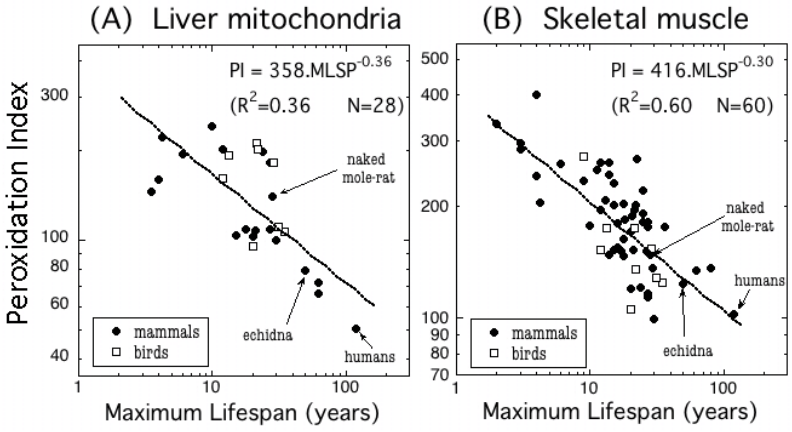

Figure 1. The relationship between maximum life span of mammal and bird species and the peroxidation index of (A) liver mitochondrial phospholipids and (B) skeletal muscle phospholipids. Liver mitochondrial data are those cited by Hulbert [48] combined with those of Pamplona et al [14]. Skeletal muscle data are those cited by Hulbert [48] combined with those from Valecak and Ruf [49]. Data points for naked mole-rat are from [25] and those for echidna are from [24]. The equations in the boxes describe the relationship between the appropriate peroxidation index (PI) and maximum life span (MLSP).

4.3. Body size, metabolism, longevity and membrane composition of bird species

Although less studied than mammals, it has long been known that resting metabolism of birds is related to their body size in a manner similar to mammals. Studies have shown that membrane fatty acid composition also varies with body mass in birds [17,18] in a similar manner to that reported for mammals [19]. However, there is a difference between mammals and birds. In both liver mitochondria and skeletal muscle, there are more n-6 PUFA and less n-3 PUFA in tissue phospholipids from birds compared to similar-sized mammals. Because n-6 PUFA generally have less –CH=CH- units than comparable n-3 PUFA, this means that bird phospholipids generally have both a lower unsaturation index and a lower peroxidation index than equivalent phospholipids from mammals.

Maximal life span is also related to body size in birds but although they have similar metabolic rates, birds on average live considerably longer than similar-sized mammals [6]. This mammal-bird longevity difference has long been known and has been used for many comparative studies. It was also recognized early to be associated with a difference in fatty acid composition of membrane lipids [20-22]. In Figure 1, along with the mammal data, the PI of liver mitochondrial phospholipids and skeletal muscle phospholipids of birds is plotted against their MLSP. What is noticeable from this comparison is that mammals and birds appear to follow the same relationship. In other words, the longer life span of bird species compared to similar-sized mammals is associated with a membrane composition that is less susceptible to peroxidative damage.

4.4. Membrane composition of exceptionally long-living mammals and birds

Within both mammals and birds there are species that are exceptionally long-living with maximum life spans several times higher than predicted from their body size. Some mammalian examples include the naked mole-rat (*Heterocephalus glaber*), the short-beaked echidna (*Tachyglossus aculeatus)* and our own species *Homo sapiens.* These three species have MLSPs that are 4-6 times that predicted from their body mass [6,23,24]. The fatty acid composition of membrane lipids from these species have been measured. For example, the phospholipids of naked mole rats are high in peroxidation-resistant MUFA and low in peroxidation-susceptible PUFA (especially 22:6 n-3) compared to those of similar-sized mice (*Mus musculus*), irrespective of

whether the individuals are young, adult or old [25]. In mice tissues, 27-57% of all phospholipids contain 22:6 n-3, compared to only 2-6% in naked mole rats [26]. Phospholipids from echidnas (~3kg) are similarly high in MUFA and low in PUFA (especially 22:6 n-3) relative to what one would predict for a 3 kg mammal [27]. When the PIs for liver mitochondrial phospholipids and skeletal muscle phospholipids for these three exceptionally long-living mammal species are plotted against their respective MLSPs (see **Figure 1**), they are approximately where one would predict them to be with respect to their longevities. In other words, their membrane fatty acid composition agrees with the PI versus MLSP relationships observed for all mammals and birds.

One very interesting aspect of the two graphs presented in **Figure 1** is that they have similar slopes (i.e. PI is proportional to the -0.36 power of MLSP for liver mitochondrial phospholipids, and to the -0.30 power of MLSP for skeletal muscle phospholipids). From these values, one can calculate the effect a percent change in one parameter will have relative to percent change in the other. Thus for every 22% decrease in PI of liver mitochondrial phospholipids and 19% decrease in PI of skeletal muscle phospholipids, there is a doubling of MLSP. The quantitative similarity of these two relationships may signal a fundamental functional relationship between membrane composition and maximum longevity that only further research will reveal.

One interesting aspect arises from the data for these three exceptionally long-living mammal species. From work described earlier in this contribution, we would expect from their membrane composition that these three species might have metabolic rates lower than expected for their body size. This is the case for both naked mole-rats and echidnas which have metabolic rates that are ~40% lower than predicted from their body mass [27,28]. These reduced metabolic rates are not sufficiently large to fully explain these species increased longevity from a rate-of-living perspective (although it can explain part of their exceptional longevity). *Homo sapiens*, however, has a metabolic rate commensurate with that predicted for a typical mammal of its body mass, thus differing from both naked mole-rats and echidnas. For both naked mole-rats and echidnas, the fatty acid composition of liver total phospholipids and liver mitochondrial phospholipids are essentially the same [24,25]. This is a common pattern among the mammals and birds for which we have characterized both mitochondrial and total tissue phospholipids over the years [e.g. 18,19,29]. However, this may not be the case for *Homo sapiens*. There are limited data for the membrane fatty acid composition of human tissues and we know of only one data set for the fatty acid composition of mitochondrial phospholipids from humans. This is for human liver mitochondria [30] and appears to differ dramatically from three

reports of fatty acid composition of total phospholipids from livers of normal healthy humans [31-33]. Liver total phospholipids are reported to contain n-3 PUFA but they are absent in liver mitochondrial phospholipids (**Figure 2**). This is of interest as the health effects of n-3 PUFA essentially relate to plasma-membrane events rather than mitochondrial membrane. Such a dichotomy, suggests that mitochondrial membranes may be especially important for aging and that the exceptional longevity of *Homo sapiens* might be associated with the exclusion of peroxidation-prone n-3 PUFA from their mitochondrial membrane lipids. However, unfortunately all the humans values reported in **Figure 2** come from different laboratories and there is considerable variation in the data from these different laboratories. There is a need for at least one laboratory to determine whether the exceptionally-longevous *Homo sapiens* excludes n-3 PUFA from mitochondrial membranes but not other cellular membranes in the same tissue.

Within birds also, there are species and groups that are exceptionally long-living compared to other birds. Two avian groups that have a number of long-living species are the petrels and albatrosses (Procellariformes) and the parrots (Psittaciformes). A recent comparison between Procellariformes (petrels) and Galliformes (fowl) showed that the 4.6-fold difference in MLSP was associated with differences in the fatty acid composition of heart phospholipids [34]. The petrels had heart phospholipids high in MUFA but low in PUFA (mainly n-6 PUFA) compared to the fowl. The PI of heart

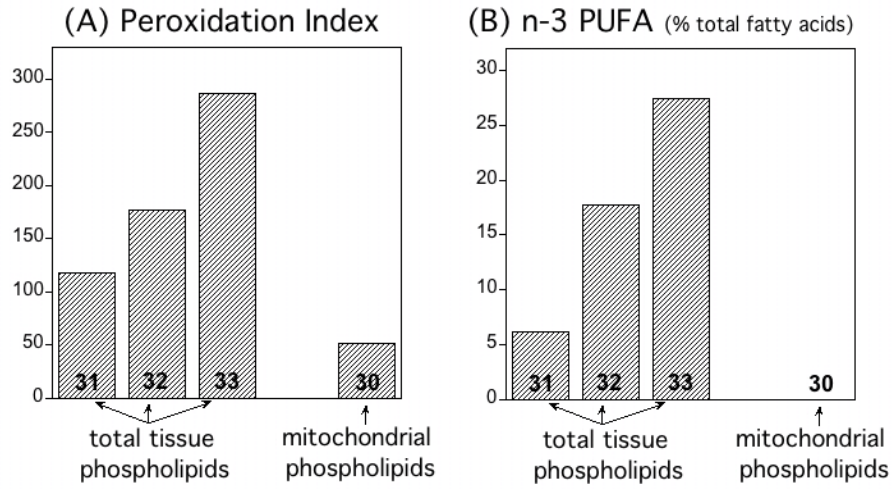

Figure 2. A comparison of (A) Peroxidation Index and (B) n-3 PUFA content of total phospholipids and mitochondrial phospholipids of human liver. The data for total liver phospholipids are from references [31-33], while the data for liver mitochondrial phospholipids are from reference [30].

phospholipids from the long-living petrels was, on average, 36% lower that for the shorter-living fowl. Interestingly, this difference was of the magnitude expected from the relationships for skeletal muscle and liver mitochondrial phospholipids (see **Figure 1**) in relation to the petrel-fowl difference in MLSP. It would be of interest to know if the exceptional longevity of parrots compared to other birds is also associated with a distinctive membrane fatty acid composition.

4.5. Membrane composition and longevity within species

Longevity has also been shown to vary within species. We will consider four such examples here and examine whether this within-species variation is also associated with variation in the fatty acid composition of membrane lipids. The four examples are: (1) life span extension of rodents experimentally induced by calorie-restriction, (2) longer life spans observed in some wild-derived strains of mice, (3) queen-worker longevity differences in female honey bees, and (4) inherited differences in longevities among humans.

Calorie-restriction is the most investigated experimental treatment that alters aging and was first shown by Laganiere and Yu in 1987 to decrease the relative abundance of peroxidation-prone fatty acids in membrane lipids [35]. A number of other studies have since verified this original observation (for review see [6]). A recent study of mice showed that (i) these changes are very rapid compared to other biochemical changes induced by calorie-restriction, and (ii) are relative to the degree of calorie-restriction [36]. In **Figure 3A** the PI for liver mitochondrial phospholipids following one-month of different levels of calorie-restriction for these mice are plotted against the life span determined on different mice but fed the same energy-deficient diets [37]. It can be seen from this figure that the relationship between longevity-extension and changes in membrane composition induced by calorie-restriction is similar to that observed comparing different mammal and bird species.

In mice, two wild-derived strains have been shown to have an extended life span compared to a genetically heterogenous laboratory mice strain kept under identical conditions and fed identical food in the laboratory [38]. The fatty acid composition of both liver and skeletal muscle phospholipids differs among the three mice strains [39], with the decrease in PI being related to the degree of longevity-extension. The data for skeletal muscle phospholipids are plotted in **Figure 3B** from which it can be seen that the relationship between MLSP and PI within mice is not dissimilar to the relationship observed among mammals and birds in general.

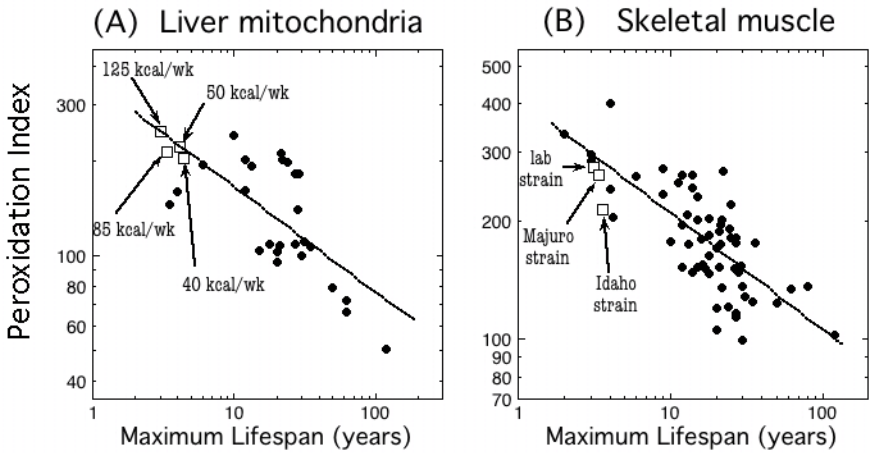

Figure 3. A comparison of within-species longevity-variation with between-species longevity-variation. In both the left hand and right hand figures the circles represent the data points for both mammal and bird species previously shown in figure 1. (A) The relationship between maximum life span (MLSP) and peroxidation index (PI) of liver mitochondrial phospholipids. The open square symbols show the data points for mice subjected to four different levels of energy intake. MLSP data from [37] while PI data are from [36]. (B) The relationship between maximum life span (MLSP) and peroxidation index (PI) of skeletal muscle phospholipids. The open square symbols show the data points for three strains of mice. MLSP data from [38] while PI data are from [39].

Another example of within species variation in longevity relates to social insects such as honey bees (*Apis mellifera*). Female honey bees can become either 'queens' or 'workers', depending on the type of food they receive in the larval stage. Although genetically identical, 'queens' can live for years while 'workers' only live weeks (or months if they are prevented from moving to the foraging phase). During the adult stage there is another key difference between 'queens' and 'workers'. Within the first week of hive-life workers commence eating pollen while queens are fed mouth-to-mouth by secretions from workers throughout their adult life and consequently, 'queens' are not allowed to consume pollen during their adult life in the hive [40]. This is of interest because pollen has a very high PUFA content (see **Figure 4**) and worker larvae, newly emerged workers, young queens and very old queens all have phospholipids with a low PUFA content but very high MUFA content. However, although phospholipids from newly emerged workers are low in PUFA, at the end of the first week of hive life (when they commence consumption of pollen) there is a dramatic increase in PUFA content of their phospholipids which remains high in both older hive bees (~3 weeks post-eclosion) and bees returning to the hive after a foraging trip (~ 5 weeks post-eclosion). These findings [41] suggest that membrane fatty acid

composition may explain the queen-worker longevity differences observed within female honey bees. We have plotted the PI of phospholipids from honey bees and pollen in **Figure 4** to illustrate these changes. Interestingly, if one assumes a similar slope between MLSP and PI of phospholipids in bees as observed in figure 1 for mammals and birds, then the ~3-fold difference in PI of phospholipids between queens and workers is large enough to explain the order-of-magnitude difference in their longevity.

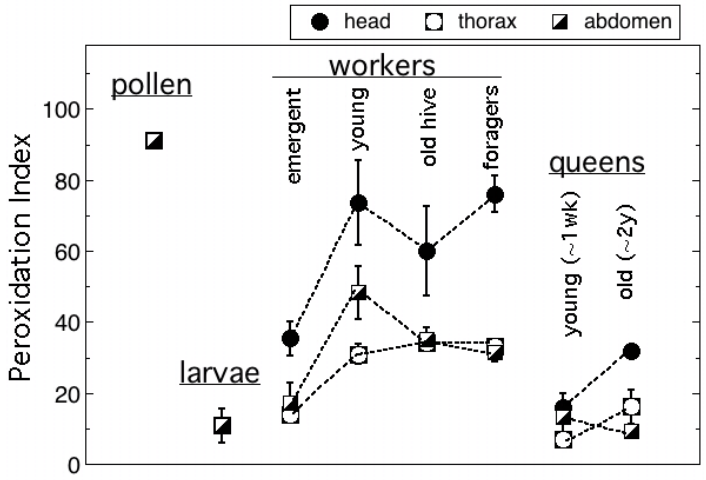

Figure 4. A comparison of the peroxidation index (PI) of phospholipids from pollen and different life stages of the female honey bee (*Apis mellifera*). Data are from ref [41]. Pollen values are for pollen collected from the legs of returning forager bees. Larvae values are for whole larvae, while those for workers and queens are presented separately for head, thorax and abdomen. Error bars represent $\pm$ 1 SEM.

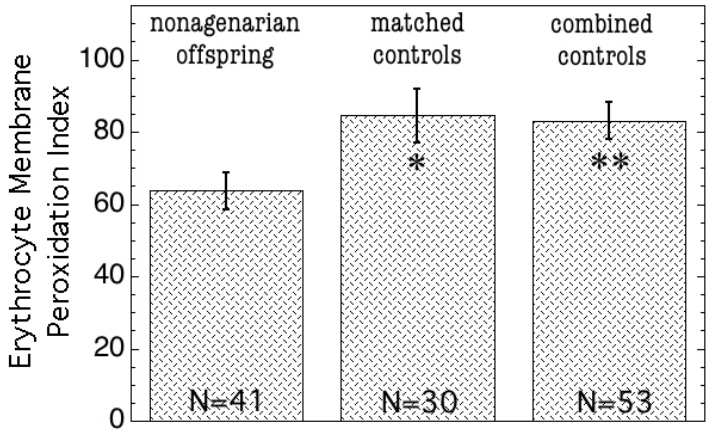

Figure 5. Comparison of the peroxidation index (PI) of human erythrocyte membrane lipids. Data are from ref [43]. Error bars represent $\pm$ 1 SEM and sample size is shown in each bar. Both controls are significantly different (* P=0.02 and ** P=0.009) from nonagenerian offspring.

Longevity is partly inherited and a study of 2872 Danish twin-pairs [42] suggest the heritability of longevity in humans is ~0.25. This means that children of long-living parents are likely to differ from children of short-living parents in biochemical characteristics associated with longevity. In this light, it is interesting that the fatty acid composition of phospholipids of erythrocytes from children of nonagenarians (90-year-olds) differ significantly from those of both matched and unmatched control samples [43]. In **Figure 5** we present the PI for erythrocyte phospholipids reported for these three groups of adult humans.

4.6. Dietary fats and membrane composition

It is obvious from some of the studies cited above that membrane fatty acid composition is regulated. For example, the long-lived wild-derived mice had phospholipids that differed in their fatty acid composition from the laboratory mice even though they were kept in the same environment and fed the same food [38,39]. Similarly, although the diet PUFA of the petrels would be overwhelmingly n-3 PUFA (because of their fish and cephalopod diet) these were largely excluded from their membrane lipids which contained much higher levels of n-6 PUFA than n-3 PUFA [34]. Thus, although we know that membrane fatty acid composition is not just a reflection of dietary abundance, we know very little of how membrane fatty acid composition is regulated. Studies in rat hepatocytes show that among hundreds of different phospholipid molecular species present in membranes, only four molecular species of phosphatidylcholine and phosphatidylethanolamine are synthesised *de novo* [44]. These *de novo* phospholipids are rapidly remodelled by deacylation-reacylation cycles and it appears likely that a series of membrane-bound acyl transferases, which are responsible for the reacylation process, are also responsible for determining the specific membrane fatty acid composition. This remodelling process is so rapid that when hepatocytes are subjected to oxidative stress, there is no observable change in the fatty acid composition of phospholipids. This is because the peroxidised PUFA are being removed and replaced at equal rates to peroxidation damage by newly synthesised PUFA from the triglycerides present in the hepatocytes [45].

Membrane fatty acid composition is strongly regulated as long as an adequate supply of PUFAs are present in the diet. The acyl transferases are highly selective for polyunsaturates but do not discriminate well between n-6 and n-3 PUFA [46]. A recent study investigating the relationship between diet fatty acid profile and the fatty acid composition of membrane lipids in rats showed that saturated fatty acid content and MUFA and total PUFA

content of muscle membrane lipids was unresponsive to diet. However, the study did show that the balance between n-3 PUFA and n-6 PUFA in membrane lipids was responsive to the balance between these two PUFA classes in the diet [47]. The relationship between diet PI and muscle phospholipid PI in rats from this study is shown in **Figure 6**. Although balance between dietary n-3 and n-6 PUFAs influenced muscle phospholipid PUFA balance [47], it can be seen from Figure 6 that the PI of muscle phospholipids was unresponsive to large variation in the PI of the diet. This is presumably because both n-3 and n-6 PUFA are capable of peroxidative damage and their substitution for each other had no influence on overall peroxidative susceptibility of the skeletal muscle membrane lipids.

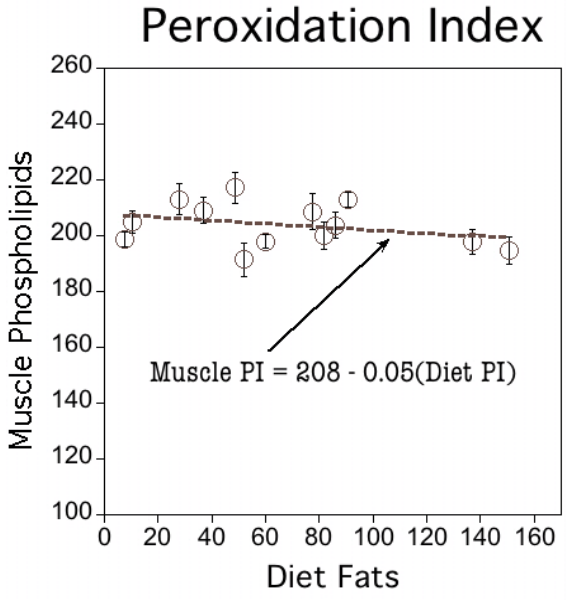

Figure 6. The relationship between the peroxidation index (PI) of the diet and that of skeletal muscle phospholipids in the rat. PI data points have been calculated from the data in ref [47]. Error bars represent $\pm$ 1 SEM. Rats were fed the diets for 8 weeks and diets had 25% energy as fat. They were identical in every respect except for their fatty acid profile.

4.7. Conclusions

The finding that the fatty acid composition of membrane lipids varies between species was initially unexpected as most other aspects of cell composition vary little between species. Variation in membrane composition of mammals and birds is related to both variation in their resting metabolic rates and to variation in their maximum longevities. Because both of these

parameters are related to body size, Max Rubner's studies connecting metabolic rate and life span, was actually comparing species with different membrane fatty acid compositions without him being aware of this fact. We propose that it is the physical properties that different membrane fatty acid compositions impart to membranes that are responsible for the different levels of cellular metabolic rate, while it is the chemical properties of these membranes (namely their propensity to peroxidative damage) that are related to differences in longevity. The fact that saturated and monounsaturated fats are very resistant to peroxidation while the more polyunsaturated a PUFA molecule is, the more susceptible it is to peroxidation, allows a peroxidation index to be derived for a specific membrane fatty acid composition. In mammals and birds, the peroxidation index of membrane lipids is inversely related to maximum life span, and exceptionally long-living mammal and bird species share the same relationship. Longevity-variation within species (either due to calorie-restriction, genetic strain, worker-queen status in honey bees, or inherited longevity variation in humans) also shows similar relationships. Membrane composition is a regulated variable which appears to be relatively unresponsive to dietary fat profile. This consistent finding of longevity-associated variation in membrane fatty acid composition has been proposed to be not just a response to different maximum longevities, but has been suggested to have a more causal role in aging and the determination of life span. More detailed discussion of this interpretation is beyond the current contribution and is presented elsewhere [6,48].

4.8. Acknowledgements

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