Membrane Fatty Acid Unsaturation, Protection against Oxidative Stress, and Maximum Life Span

A Homeoviscous-longevity Adaptation?

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ABSTRACT: Aging is a progressive and universal process originating endogenously that manifests during postmaturational life. Available comparative evidence supporting the mitochondrial free radical theory of aging consistently indicates that two basic molecular traits are associated with the rate of aging and thus with the maximum life span: the presence of low rates of mitochondrial oxygen radical production and low degrees of fatty acid unsaturation of cellular membranes in postmitotic tissues of long-lived homeothermic vertebrates in relation to those of short-lived ones. Recent research shows that steady-state levels of free radical–derived damage to mitochondrial DNA (mtDNA) and, in some cases, to proteins are lower in long- than in short-lived animals. Thus, nonenzymatic oxidative modification of tissue macromolecules is related to the rate of aging. The low degree of fatty acid unsaturation in biomembranes of long-lived animals may confer advantage by decreasing their sensitivity to lipid peroxidation. Furthermore, this may prevent lipoxidation-derived damage to other macromolecules. Taking into account the fatty acid distribution pattern, the origin of the low degree of membrane unsaturation in long-lived species seems to be the presence of species-specific desaturation pathways that determine membrane composition while an appropriate environment for membrane function is maintained. Mechanisms that prevent or decrease the generation of endogenous damage during the evolution of long-lived animals seem to be more important than trying to intercept those damaging agents or repairing the damage already inflicted. Here, the physiological meaning of these findings and the effects of experimental manipulations such as dietary stress, caloric restriction, and endocrine control in relation to aging and longevity are discussed.

KEYWORDS: advanced maillard products; aging; antioxidants; arachidonic acid; diets; double-bond index; DNA oxidation; docosahexanoic acid; free radicals; 8-hydroxy-deoxyguanosine; linoleic acid; longevity; malondialdehyde; mitochondria; protein oxidation

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INTRODUCTION

The aging process causes a multitude of detrimental changes in the organism at all levels of biological organization, especially limiting maximum functional capacities, decreasing homeostasis, and increasing the probability of death. All those changes are thought to originate from a smaller number of causes continuously operating throughout life. The early proposal that free radicals,[1] especially of mitochondrial origin,[2,3] are among the main causes of aging is increasingly receiving support from scientific studies;[4] but any theory of aging should fit with at least two main characteristics of this process: aging is progressive and endogenous.[5] True aging changes occur throughout life at an approximately similar rate. Thus, the causes of aging cannot be present exclusively in old individuals, they must be present already in young adult individuals, otherwise the young would not become old. They must be present during the whole life span, both in youth and at old age, at roughly the same levels. On the other hand, aging is an endogenous process. This means that exogenous factors like dietary-dependent antioxidants, radiation, or external sources of stress cannot be the causes of aging. It also means that the aging rate of each animal species, and thus maximum longevity (MLSP), is mainly determined by the genes, not by the environment.[6,7] The endogenous character of aging explains why different animal species age at widely different rates under similar environments (varying more than 40-fold among mammalian species) and why the intrinsic aging rate of a given species (unlike its age at death due to external stress) is similar in widely different environments. Survival rate in stressful environments should not be confused with aging. The external environmental conditions strongly modify survival and thus mean life span, whereas genetically determined progressive and endogenous processes mainly control the rate of aging and the longevity of species. Available comparative evidence consistently indicates that there are two basic molecular traits linking aging and oxidative stress: the rate of generation of oxygen radicals (ROS) in mitochondria and the degree of unsaturation of membrane fatty acids. Both are, or lead to, endogenously determined processes that occur continuously throughout life at a characteristic rate in each animal species depending on its longevity.

ANTIOXIDANTS, MITOCHONDRIAL ROS PRODUCTION, AND MAXIMUM LIFE SPAN

Synoptically, the main findings relating antioxidants and free radical production with maximum longevity are the following (FIG. 1):

(1) Endogenous antioxidant enzymes and low-molecular-weight antioxidants are negatively correlated with maximum longevity; that is, long-lived animals, either mammals, birds, or vertebrates in general share a common trait: they have very low levels of endogenous tissue antioxidants. Furthermore, experimental supplementation with antioxidants does not slow the intrinsic aging process. Hence, antioxidants cannot determine longevity (reviewed in Pérez-Campo *et al.*[8]).

(2) All the comparative studies performed thus far show that the rate of mitochondrial oxygen radical generation is lower in long-lived than in short-lived animals. This occurs in all kinds of species, comparing animals following the "rate of living theory" [9–11] (the inverse relationship between maximum longevity and meta-

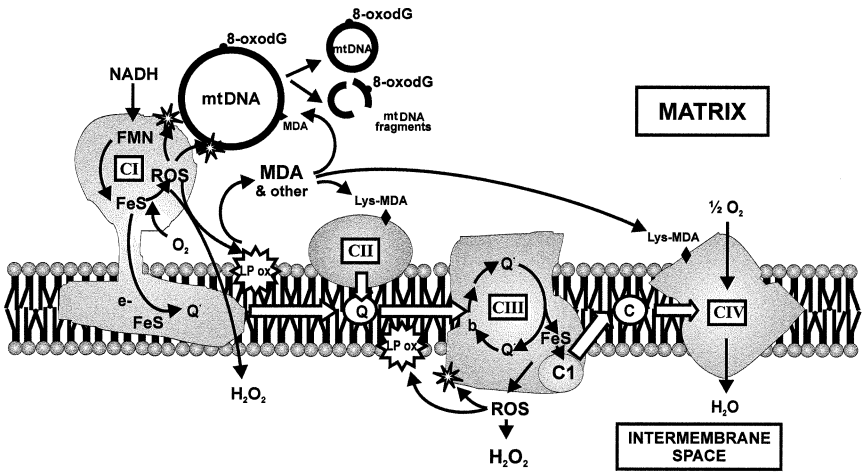

FIGURE 1. Although heart mitochondria can produce ROS at both complexes I and III, the longevity-related difference has been located at complex I in rat versus pigeon.[13] Recent results discard the flavin and suggest that iron–sulphur clusters (FeS) are the ROS generators of complex I.[17] Caloric restriction also decreases mitochondrial ROS generation of rat heart mitochondria specifically at complex I.[18] ROS production causes oxidative damage to mtDNA and to polyunsaturated fatty acids, leading to the formation of carbonyl compounds that, in turn, damage other macromolecules.

bolic rate), as well as in comparisons between animals showing differences in longevity that cannot be explained on the basis of their metabolic rates (e.g., mammals vs. birds).[12–15] Therefore, the rate of mitochondrial ROS generation is a better correlate of maximum longevity and aging rate than the basal rate of oxygen consumption. This is logical since, after all, the potentially damaging agents are the ROS, not ground-state oxygen. Furthermore, it is interesting to note that ROS production does not increase in proportion to the increase in oxygen consumption during state 4 to state 3 energy transition, which can explain the paradox that exercise does not shorten rodent or human longevity.[16] This is so because the degree of reduction of the electron carriers in the respiratory chain, including those containing the ROS generators, increases when electron flow decreases; and the larger the degree of reduction of the generator, the higher will be its rate of ROS production.[16] So, mitochondrial ROS production does not necessarily increase in proportion to mitochondrial oxygen consumption, either across species or in a single species under different physiological conditions, because the free radical leak (the percent of total electron flow leading to univalent O_2 reduction) in the respiratory chain is not a constant. It remains to be tested whether a decrease in the rate of mitochondrial ROS generation in a given species, performed experimentally without deleterious effects, can slow down the aging rate.

(3) Localization studies in heart mitochondria show that complexes I and III can produce H_2O_2 in state 4, but the lower mitochondrial ROS production of pigeons (MLSP, 35 years) in relation to rats (MLSP, 4 years) is localized exclusively at complex I.[13] Recent results discard the flavin and suggest that the iron–sulphur

clusters are the ROS generators of complex I.[17] Interestingly, it has been recently found that the only manipulation that consistently slows down aging, caloric restriction (CR), also decreases mitochondrial ROS generation of rat heart mitochondria specifically at complex I.[18]

(4) It has been recently found that the steady-state levels of oxidative damage in mtDNA (not in nDNA), measured as 8-oxodG in the heart and brain of homeothermic animals (mammals or birds), are also negatively correlated with maximum longevity.[19,20] Mitochondrial ROS damage mtDNA more intensely than nDNA in the heart and brain of homeothermic vertebrates because of the proximity between the source of damage and the target. Thus, the attack rate of mitochondrial ROS against mtDNA can be a major determinant of aging rate.[21–23] This can occur through the repeatedly described accumulation of mtDNA mutations during rodent and human aging.[24,25] The rate of accumulation of those somatic age-related mutations is much quicker in short- than in long-lived animals.[26,27] The hypothesis emerges that the rate of mitochondrial ROS production of each animal determines the rate of ROS attack and flux of oxidative damage through mtDNA[23] and thus the rate of accumulation of mtDNA mutations and the aging rate. Furthermore, it has been found that CR also decreases 8-oxodG in heart mtDNA (not in nDNA) in agreement with a CR-induced decrease in mitochondrial ROS production.[18] Thus, a common mechanism seems to be responsible, at least in part, for decreases in aging rate in a given species (after CR) and between different animal species: a decrease in ROS generation at complex I.

(5) Proteins are also directly modified by ROS, leading to the formation of oxidatively modified amino acids. Furthermore, proteins are indirectly modified by reactive carbonyl compounds resulting from oxidation of carbohydrates and polyunsaturated fatty acids, with the final formation of advanced maillard products (AMPs).[28] Several fundamental findings relate this protein damage and aging rate: (a) the accumulation of protein damage during aging in extracellular matrix and tissues;[28,29] (b) the concentration[30,31] and the rate of accumulation[32] of protein damage is higher in short- than in long-lived animal species; and (c) the concentration[33] and rate of accumulation[32] of protein damage is lower in food-restricted than in *ad libitum*–fed animals.

MEMBRANE FATTY ACID UNSATURATION AND MAXIMUM LIFE SPAN

The available comparative studies indicate that maximum longevity is inversely related to mitochondrial free radical production and mtDNA and tissue protein oxidative damage. Although these very important characteristics are consistent with the free radical theory of aging, additional factors related to other macromolecules can also lead to a low level of oxidative damage in long- versus short-lived animal species.

Among cellular macromolecules, polyunsaturated fatty acids (PUFA) exhibit the highest sensitivity to ROS-induced damage, their sensitivity to oxidation exponentially increasing as a function of the number of double bonds per fatty acid molecule.[34] A low degree of fatty acid unsaturation in cellular membranes, and particularly in the inner mitochondrial membrane, may be advantageous by decreas-

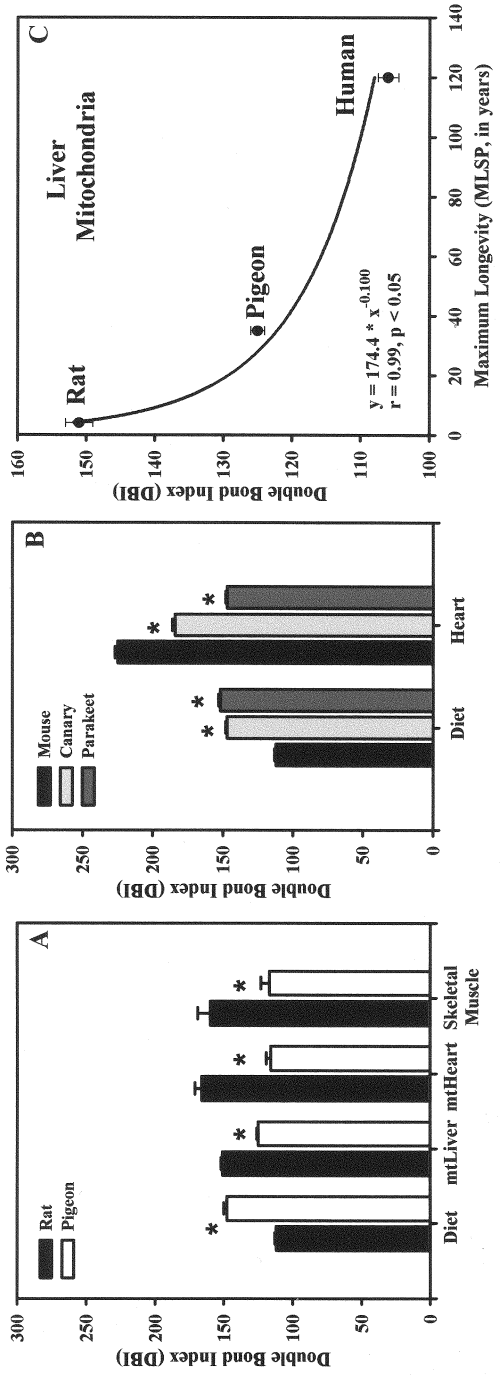

FIGURE 2. (A) Double-bond index (DBI) of fatty acid present in the diet and tissue lipids (liver[35] and heart[30] mitochondria and skeletal muscle) of rats (MLSP, 4 years) and pigeons (MLSP, 35 years). (B) DBI of fatty acids present in the diet and heart lipids of mice (MLSP, 3.5 years), canaries (MLSP, 24 years), and parakeets (MLSP, 21 years).[37] **(C)** Relationship between liver mitochondria DBI and maximum longevity (MLSP, in years) in vertebrate species (DBI was calculated from data in Ref. 35). DBI = [(Smol% monoenoic × 1) + (Smol% dienoic × 2) + (Smol% trienoic × 3) + (Smol% tetraenoic × 4) + (Smol% pentaenoic × 5) + (Smol% hexaenoic × 6)].

ing their sensitivity to lipid peroxidation. This would also protect other molecules against lipoxidation-derived damage. In agreement with this, it has been found that long-lived animals have a lower degree of total tissue and mitochondrial fatty acid unsaturation (low double-bond index [DBI]) than short-lived ones. Thus, an early study found that the DBI of liver mitochondrial phospholipids was lower in pigeons (MLSP, 35 years) than in rats (MLSP, 4 years) and was also lower in humans (MLSP, 122 years) than in pigeons (FIG. 2C).[35] These results were later confirmed by another independent laboratory.[36] Further, also comparing rat versus pigeon, the same result was obtained in heart mitochondria[33] and skeletal muscle (unpublished results) (FIG. 2A). Nevertheless, the possibility remained that the low DBI found in pigeon (order *Columbiformes*) has a physiological significance unrelated to MLSP. To discern whether a low DBI is a general characteristic of these highly long-lived animals, additional bird species were studied. It was then observed that the DBI in heart of both canary (MLSP, 24 years; order *Passeriformes*) and parakeet (MLSP, 21 years; order *Psittaciformes*) was lower than that of mice (MLSP, 3.5 years) (FIG. 2B).[37] A negative correlation between DBI and MLSP was also obtained in mitochondrial phospholipids[38,39] and heart[40] (FIG. 3) and liver[32] total phospholipids of mammals with different longevities.

Concerning the physiological meaning of the decrease in the degree of unsaturation in long-lived animals, various possibilities have been presented. Some authors[41] have proposed that mammals of large body size have a low DBI in order to decrease their metabolic rates, because the lower the DBI of a membrane, the lower is its permeability to ions (and ion pumping is one of the main determinants of metabolic rate). The permeability to Na^+ and K^+ in liver hepatocytes[42] and to H^+ in the inner mitochondrial membranes[43] also correlates negatively with body size. Whereas this possibility could be true for mammals of different sizes, it cannot explain the low DBI of birds because they have a metabolic rate similar or even higher than that of mammals of similar size. But the studied birds and the mammals of large body size share a common trait: their maximum longevity is very high (they age slowly). Thus, it can be hypothesized that the low DBI of long-lived homeotherms (either mammals or birds) could have been selected during evolution to decrease membrane lipid peroxidation and its lipoxidative consequences to other cellular macromolecules including proteins[44] and DNA.[45] Thus, the low fatty acid unsaturation of long-lived mammals of large body size would protect their tissues against oxidative damage, and, simultaneously, it could contribute to lowering their metabolic rate. But the more general relationship in all homeotherms (either mammals or birds) is the negative association between DBI and MLSP, not between DBI and metabolic rate. The low DBI of birds does not fit with their very high metabolic rates, whereas it does

FIGURE 3. Relationship between maximum longevity (MLSP) and double-bond index (DBI, *upper left*), *in vivo* lipid peroxidation (*upper right*), *in vitro* lipid peroxidation (*lower left*), and the lipoxidation-derived protein damage marker malondialdehyde-lysine (*lower right*) in heart phospholipids of eight mammalian species. The MLSP of the selected species are the following: mouse (*Mus musculus*), 3.5 years; rat (*Rattus norvegicus*), 4 years; guinea pig (*Cavia porcellus*), 8 years; rabbit (*Oryctolagus caniculus*), 13 years; sheep (*Ovis aries*), 20 years; pig (*Sus scrofa*), 27 years; cow (*Bos taurus*), 30 years; and horse (*Equus caballus*), 46 years. Values are means ± SEM. Data for DBI, and *in vivo* and *in vitro* lipid peroxidation have been obtained from Ref. 40.

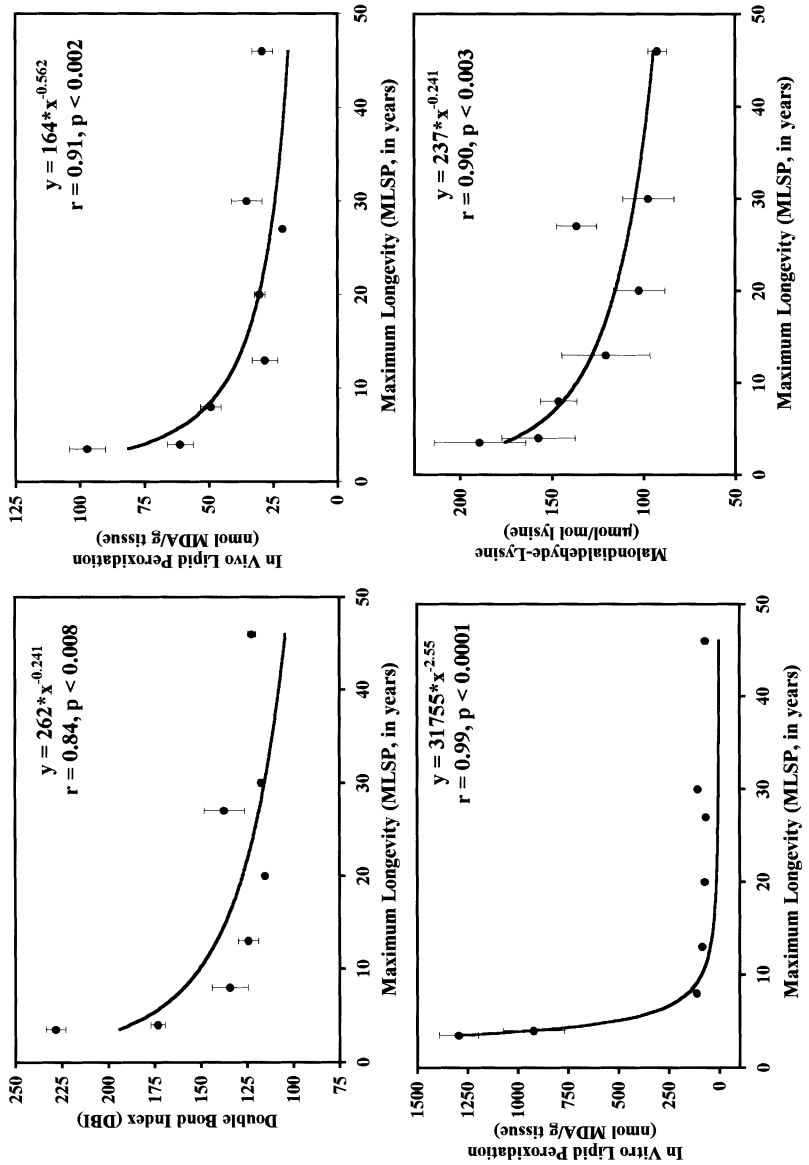

FIGURE 3. *See preceding page for legend.*

fit with their high longevity. Undoubtedly, factors other than DBI must be responsible for the high metabolic activity of birds.

Oxidation of PUFA leads to the formation of hydroperoxides and endoperoxides, which undergo fragmentation to yield a broad range of reactive intermediates, including alkanals, alkenals, hydroxyalkenals, glyoxal, and malondialdehyde (MDA).[46] These carbonyl compounds, and possibly their peroxide precursors, react with nucleophilic groups in proteins, resulting in chemical modification of the protein. The modification of amino acids in proteins by products of lipid peroxidation results in the chemical, nonenzymatic formation of a variety of adducts, including malondialdehyde-lysine (MDA-lys) and N^{e}-carboxymethyllysine (CML) among others,[28] which may be useful as indicators of protein oxidative stress *in vivo*. In this context, it has been demonstrated that in long-lived animal species a low degree of total tissue and mitochondrial fatty acid unsaturation (low DBI) is accompanied by a low sensitivity to *in vivo* and *in vitro* lipid peroxidation[32,33,35,37,40] and a low concentration of the lipoxidation-derived adducts MDA-lys and CML in several tissues and mitochondrial proteins[32,33] (see FIG. 3 for heart phospholipids). Independent experiments have also demonstrated a negative correlation between sensitivity to lipid autoxidation and MLSP in brain and kidney homogenates from different mammalian species.[47]

Because correlation does not imply causation, in order to ascertain whether the low DBI protects mitochondria of long-lived animals by decreasing lipid oxidation and protein lipoxidation, an experimental dietary study of *in vivo* modification of the DBI of rat heart mitochondria was performed. The diets used were specially designed to partially circumvent the homeostatic system of compensation of dietary-induced changes in DBI that operates at tissue level. For this purpose, Wistar rats were fed for 7 weeks with semipurified AIN-93G diets containing 10% menhaden oil (rich in n-3; UFA group), or 9.5% hydrogenated coconut plus 0.5% corn oil (SAT group) as the sole source of fat. The addition of 0.5% corn oil in the SAT group was included to avoid homeostatic (DBI) reactive increases in mead acid (20:3n-9) in the SAT group. The analysis of heart mitochondria showed that the dietary manipulation was successful, since the DBI was lower in the SAT than in UFA group (FIG. 4, left panel). The decrease in the DBI significantly lowered *in vivo* levels of lipid peroxidation, protein carbonyls, MDA-lysine, and CML in heart mitochondria[48] (FIG. 4). These observations demonstrate that lowering the DBI of tissue cellular membranes protects against lipid and lipoxidation-derived protein peroxidation. This strengthens the notion that the relatively low DBI of the membranes of long-lived animals could have evolved to protect them from oxidative damage.

The fatty acid profiles of the mammals and birds studied indicate that their biological membranes maintain a similar fatty acid average chain length (around 18 carbon atoms), and a similar ratio of saturated versus unsaturated fatty acids irrespective of animal longevity. The low DBI observed in long-lived species is obtained by modulating the type of unsaturated fatty acid that participates in membrane composition. So, there is a systematic redistribution between the types of PUFAs present from the highly unsaturated arachidonic (20:4n-6, AA) and docosahexaenoic (22:6n-3, DHA) acids in short-lived animals to the less unsaturated linoleic acid (18:2n-6, LA), and, in some cases, to linolenic acid (18:3n-3, LNA) in the long-lived ones at the mitochondrial and tissue levels[32,33,35,37-40] (see FIG. 5 for LA and DHA).

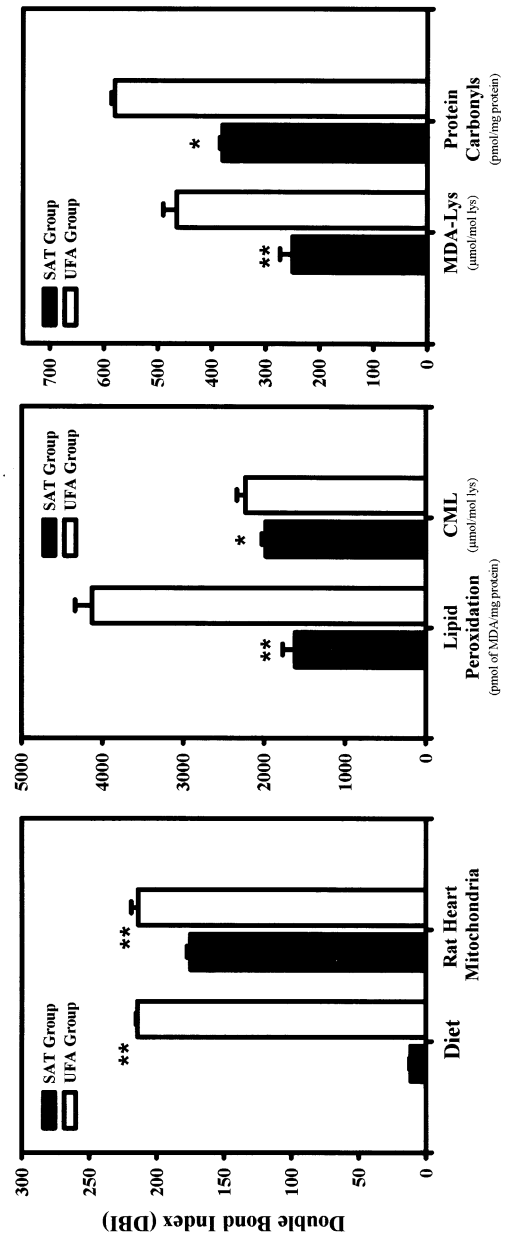

FIGURE 4. ***Left*: double-bond index (DBI) of the dietary fats and total phospholipids in heart mitochondria of rats fed saturated or unsaturated diets.**[48] *Right*: lipid and protein peroxidation markers in heart mitochondria of rats fed saturated or unsaturated diets.[48] Values are means ± SEM.

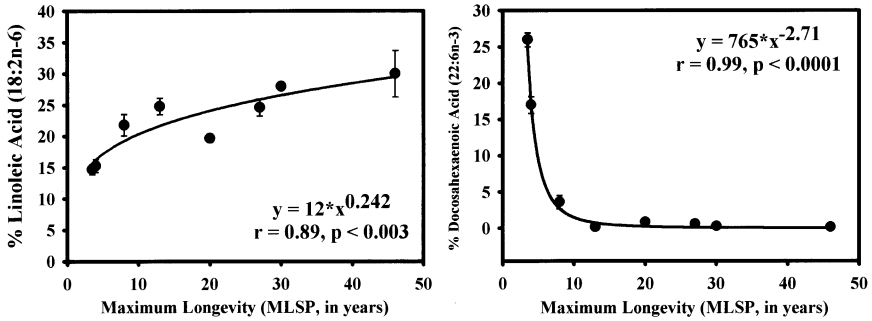

FIGURE 5. Relationship between maximum longevity (MLSP) and linoleic acid (18:2n-6) and docosahexaenoic acid (22:6n-3) contents in heart phospholipids of eight mammalian species.[40] The MLSP of the selected species are indicated in the figure legend of FIGURE 3. Values are means ± SEM.

Furthermore, we have shown that the interspecies differences were not due in any case to dietary PUFAs, since diet DBI did not correlate with MLSP.

Although those findings had never been described as a function of MLSP, two previous comparative reports existed in mammals relating fatty acid unsaturation to body size. In the first one, it was observed that DHA acutely decreased as body size increased in the order mouse–rat–rabbit–man–whale,[49] which is also an order of increasing MLSP, although this was not commented on by the authors. In the second report,[41] it was found that the DBI was negatively correlated with body size in the heart, skeletal muscle, and kidney cortex of five species (mouse–rat–rabbit–sheep–cattle); whereas in the liver the negative trend did not reach statistical significance, and in the brain no correlation was observed. The fatty acids mainly responsible for these differences were again DHA and AA, which decreased as body size increased, and LA, which showed progressively larger levels in animals of larger size.

These results suggest that cellular and/or subcellular mechanisms exist to bring about the observed distinctive distribution of acyl groups in the cellular and mitochondrial membrane phospholipids among different vertebrates. Two mechanisms may be implied in determining the fatty acid profile observed in tissue and mitochondrial total lipids or phospholipid classes: the fatty acid desaturation pathway and the deacylation–reacylation cycle. The estimation of delta-5 and delta-6 desaturase activities indicated that they were various magnitudes lower in long-lived species compared with short-lived ones.[32,33,40] This can explain why DHA and AA decrease and LA and/or LNA increase from short- to long-lived animals. The same was also postulated in the studies referred to above, since desaturases are the limiting enzymes in the pathways of n-3 and n-6 synthesis of the highly unsaturated PUFAs AA (20:4n-6) and DHA (22:6n-3) from their dietary precursors, LA (18:2n-6) and LNA (18:3n-3), respectively. The authors concluded that the main factor responsible for the different DBIs was the possession of low delta-5/-6 desaturase activities in animals of large body size.[41] Thus, desaturation pathways would make available *in situ* the n-6 and n-3 fatty acids to phospholipid acyltransferases in order to remodel the phospholipid acyl groups, postulating the presence of constitutively low spe-

cies-specific desaturase activities in long-lived animals.[32,33,40] The finding that the acyltransferase to n-6 desaturase activity ratio is about 10:1 in tissues[50] also suggests that the desaturases are the main limiting factor responsible for the observed DBI–longevity relationship.[32,33,40]

The presence of constitutively low desaturase activities in long-lived animals can explain why feeding corn oil (rich in LA) to primates increases mainly LA (to 30% of total fatty acids) instead of AA (only to 10% of total) in their tissues,[51] whereas in short-lived rodents dietary LA leads to strong increases in AA. Similarly to those primates, human monastic communities that chronically consume only corn oil as the main dietary fat source (67% rich in LA) have lipid profiles with around 30% LA but only 9% AA in their lipoproteins.[52] Also, standard diets of mammals (i.e., rats, mice, cows, and horses) and even of birds contain the precursors in the n-6 and n-3 PUFA families LA and LNA at similar levels (35–41% and 1–2%, respectively, in the four species) and do not contain DHA (this extremely unsaturated fatty acid is not added to commercial diets because it oxidizes too easily to be stable at room temperature). However, tissue DHA reached 10% in mice and 3.6% in rats, whereas it was below 0.6–0.3% in cows and horses, which also showed low AA/LA ratios.

The low DBI of long-lived animals is based in a redistribution between types of PUFAS without any alteration in the total (%) PUFA content or in the average chain length. This is an elegant evolutionary strategy, since it allows the reduction of sensitivity to lipid peroxidation and lipoxidation-derived damage to cellular macromolecules without altering fluidity/microviscosity, a fundamental property of cellular membranes for the proper function of receptors, ion pumps, and transport of metabolites, among other functions. This occurs because membrane fluidity is known to increase strongly with the formation of the first and (less) with the second double bonds by means of their introduction of "kinks" in the fatty acid molecule, whereas additional (the third and subsequent) double bonds cause few further variations in fluidity.[53] This is so because the kink has a larger impact on fluidity when the double bond is situated near the center of the fatty acid chain (first double bond) than when it is situated progressively nearer to its extremes (next double bond additions). In the case of sensitivity to lipid peroxidation, however, double bonds increase it irrespective of being situated at the center or laterally on the fatty acids. Thus, by substituting fatty acids with four or six double bonds with those having only two (or sometimes three) double bonds, the sensitivity to lipid peroxidation is strongly decreased in long-lived animals; whereas the fluidity of the membrane is essentially maintained. We call this phenomenon, reminiscent of membrane acclimation to temperature at PUFA level in poikilotherms, the *homeoviscous longevity adaptation in homeotherms*.

The adjustment of the DBI of each organ and species independently of the diet indicates that it is an endogenous trait under genome control. This occurs through PUFA-induced repression of the expression of genes controlling PUFA synthesis, whereas PUFA-deficient diets increase the expression of those genes.[54] These genome-based mechanisms are responsible for the decreases in PUFAn-6 induced by diets rich in PUFAn-3 as well as for the reverse, which occur mainly trough variations in delta-5/-6 activities.[55] If both n-3 and n-6 PUFA are absent from the diet, the unusual fatty acid mead acid (20:3n-9) can still be synthesized from oleic acid (18:1n-9, OA), a process also controlled by delta-5/-6 desaturases.[56] In that situation 20:3n-9 can reach a level as high as 15% of total fatty acids to maintain the tissue DBI. These compensations are overlooked in dietary-based studies in which only the

fatty acid composition of the diets, but not that of the tissues, is measured. Their unnoticed occurrence can be a reason why some studies on the effects of dietary oils differing in their PUFA content have resulted in the lack of or small changes in tissue oxidative damage[57] or survival,[58] and they stress the need for genetic manipulations to actually alter the DBI of the tissues to a large extent.

Many previous studies have shown that increased levels of very unsaturated fatty acids like AA and DHA can have detrimental effects in various tissues. Examples of this include decreases in respiratory control and increases in proton leak in mitochondria, increased mitochondrial breakage and dysfunction, peroxisome proliferation, fatal ventricular fibrillation in rats, neurological damage, increased lipid peroxidation in association with various diseases, increased incidence of death from apoplexy, or sudden cardiac death in humans. Increases of more than one order of magnitude in AA (to 500 mM) occur in the brain during ischemia and even concentrations of AA and eicosapentaenoic acid (20:5n-3, EPA) in the much lower 20–40 mM range uncouple mitochondria and cause tissue edema.[59] Hypermetabolic uncoupling effects of thyroid hormones on rat liver mitochondria are due, to a great extent, to increased AA/LA ratios caused by increases in desaturase activities induced by the hormone, whereas LA is considered a "proton plug" or coupler (see Ref. 60 for review). Furthermore, the largest amounts of unsaturated fats in the healthy human diet must be present as OA or LA, fatty acids with low degrees of unsaturation; whereas beneficial levels of dietary n-3 PUFAs (the n-3 "paradox") occur only at low 1% optimum dietary levels. These beneficial effects, which seem mainly related to avoidance of blood coagulation and perhaps to promotion of apoptosis of heavily damaged cells,[61] are observed in humans, whose low delta-5/-6 desaturase activities limit the conversion of dietary LNA to the highly unsaturated fatty acids like DHA.

Finally, there are other observations that suggest a role for fatty acid desaturation in the determination of aging rate: (1) Decreases in the less unsaturated LA and LNA and increases in the highly unsaturated AA, 22:4n-3, 22:5n-3, and DHA membrane fatty acids have been described during aging in rat liver[62,63] and heart[49]; whereas fasting decreases delta-5/-6 desaturases, and caloric restriction avoided the age-related increases in DBI by increasing OA and LA and decreasing 22:4n-3, 22:5n-3, DHA, and the peroxidizability index in rat liver microsomal and mitochondrial phospholipids.[62] (2) The senescent accelerated prone mouse (SAM-P) has higher levels of the very unsaturated AA and DHA and peroxidizability index and lower levels of LA than SAM-resistant controls.[64] (3) Most interestingly, Eskimos are human populations showing unusually low incidence of coronary heart disease, psoriasis, rheumatoid arthritis, and asthma and have very low levels of AA in plasma phospholipids due to a genetic lack of delta-5 desaturase activity that persists even after changing them to an LA-rich diet.[65] All these facts raise the possibility that variations in desaturase activities can explain part of the changes in aging rate occurring in those models.

In summary, up to now, only two oxidative stress–related traits correlate with the maximum longevity of animals in the appropriate sense: the rate of mitochondrial oxygen radical generation and the degree of unsaturation of membrane fatty acids (FIG. 6). These two molecular traits are significantly lower in all the long-lived homeothermic vertebrates so far studied when compared to short-lived ones, and their values can be main causes of the low aging rate of long-lived animals. The two

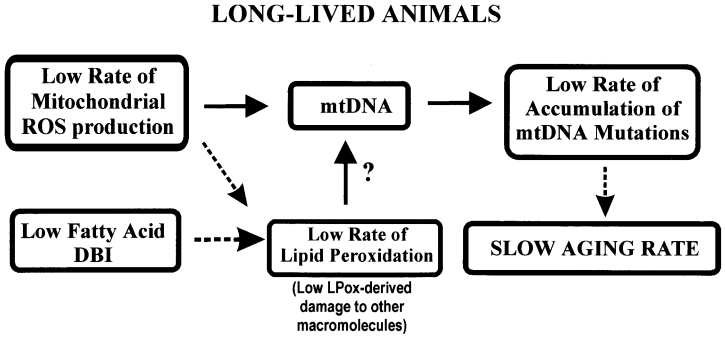

FIGURE 6. Long-lived animals show low rates of mitochondrial ROS production,[15] low steady-state levels of oxidative attack to mtDNA,[19] and low rates of accumulation of mtDNA mutations.[26,27] The low double-bond content (DBI) of cellular membranes in long-lived animals[35–40] can also contribute to a decrease in aging rate by lowering lipoxidation-derived damage to proteins and mtDNA.

MLSP-related traits are species specific, and they are thus genetically determined characteristics that would continuously cause a slow rate of accumulation of irreversible damage in long-lived animals. Thus, they fit with the concept that causes of aging must be endogenous and must operate progressively. Both work through a common mechanism: they decrease the rate generation of endogenous damage. This makes sense evolutionarily, since those kinds of mechanisms are less energetically expensive and much more efficient than increasing antioxidants or repair in order to keep a high MLSP.[23] Furthermore, both determine rate processes (rates of ROS production and of lipid- or lipoxidation-derived peroxidation), which is consistent with their hypothesized role of also controling a rate process: aging.

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