

Regulation of Membrane Unsaturation as Antioxidant Adaptive Mechanism in Long-lived Animal Species

Alba Naudí,¹ Mariona Jové,¹ Victòria Ayala,¹ Manuel Portero-Otín,¹ Gustavo Barja,² Reinald Pamplona^{1,*}

¹Department of Experimental Medicine, University of Lleida-IRBLleida, Lleida 25008, Spain. ²Department of Animal Physiology II, Complutense University, Madrid 28040, Spain.

ABSTRACT

Oxidative stress resulting from biomolecular oxidative damage due to the imbalance between reactive species production and antioxidant response has become an universal constraint of life-history evolution in animals and a modulator of phenotypic development and trade-offs. Redox balance is an important selective pressure faced by most organisms, and a myriad of mechanisms have evolved to regulate and adjust this balance. This diversity of mechanisms means that organisms have a great deal of flexibility in how they deal with reactive species challenges across time, conditions, and tissue types, as well as that different organisms may evolve different strategies for dealing with similar challenges. In the following paragraphs, we review the adaption of biological membranes as structural antioxidant defense against reactive species evolved by animals. In particular, it is our goal to describe the physiological mechanisms underlying the structural adaption of cellular membranes to oxidative stress, to explain the meaning of this adaptive mechanism, and to review the state of the art about the link between membrane composition and longevity of animal species.

Key words: Fatty acid desaturases, Membrane unsaturation, Oxidative damage, Peroxidizability index, Phylogenomic analysis, Reactive carbonyl species

***Corresponding author:** Prof. Dr. Reinald Pamplona, Department of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, Street Montserrat Roig-2, 25008 Lleida, Spain.

E-mail: reinald.pamplona@mex.udl.cat, Phone: +34-973702442, Fax: +34-973702426

DOI: 10.5530/ax.2011.3.2

INTRODUCTION

The increase in oxygen concentration in the Earth's atmosphere represented an important selective pressure and contributed to set up the pace of evolutionary changes in physiological and metabolic systems.^[1-3] Despite the evolution of the capacity to use oxygen for efficient energy production represented a relevant evolutionary innovation, the redox reactions associated with its use were responsible for the production of free radicals and, more in general, of reactive species (RS). The damaging effects of oxygen and other reactive molecular species on cells are, in essence, due to oxidation of essential cellular components.^[4] Consequently, in addition to evolutionary attempts to avoid or minimize the production of reactive byproducts of oxidative metabolism, another very important feature was the generation and selection of antioxidant defence systems.^[5] The result was a high diversity in molecular and structural antioxidant defences,

as well as in redox signaling pathways perfectly integrated in the cellular metabolic machinery. So, oxidative stress resulting from biomolecular oxidative damage due to the imbalance between RS production and antioxidant response has become a universal constraint of life-history evolution in animals and modulator of phenotypic development and trade-offs.^[6-15]

Redox balance is an important selective pressure faced by most organisms, and a myriad of mechanisms have evolved to regulate and adjust this balance. This diversity of mechanisms means that organisms have a great deal of flexibility in how they manage with RS challenges across time, conditions, and tissue types, as well as that different organisms may evolve different strategies for dealing with similar challenges.^[15] In the following paragraphs, we review the adaption of biological membranes as structural antioxidant defense against RS evolved by animals. In particular, it was our goal to describe the physiological mechanisms underlying the

structural adaption of cellular membranes to oxidative stress and to explain the meaning of this adaptive mechanism, and to review the state of the art about the link between membrane composition and longevity of animal species.

MEMBRANE FATTY ACID COMPOSITION

All living organisms have lipid membranes. Biological membranes are dynamic structures that generally consist of bilayers of amphipathic molecules held together by non-covalent bonds.^[16,17] Phospholipids, the predominant membrane lipids, consist of a hydrophilic head group with attached hydrophobic acyl chains. The wide range of processes in which phospholipids are specifically involved explains the need for diversity in phospholipid structures and fatty acid composition.^[18] This diversity requires complex metabolic and regulatory pathways.^[16,17] In fact, for example, eukaryotic cells invest around 5% of their genes to synthesize all of these lipids.^[19] The membrane phospholipid composition is maintained primarily by feedback regulation of phospholipid biosynthesis. Recent insights have emerged from the study of membrane-bound transcription factors called sterol regulatory element-binding protein (SREBP) that seem to monitor cell membrane composition and to adjust lipid synthesis accordingly.^[20]

The acyl chains are either saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) hydrocarbon chains that normally vary from 14 to 22 carbons in length.^[21] In eukaryotic cells from vertebrate species, the average chain length of a biological membrane is strictly maintained around 18 carbon atoms, and the relative distribution between saturated and unsaturated fatty acids follows a ratio of 40:60.^[22] PUFAs are generally synthesized by the modification of saturated fatty acid precursors that are products of fatty acid synthase. The desaturase enzymes, which are conserved across kingdoms, insert double bonds at specific carbon atoms in the fatty acid chain and the fatty acid elongation system elongates the precursors in two-carbon increments.^[23,24] The fatty acid desaturation pathway and the deacylation-reacylation cycle are the mechanisms responsible for the particular fatty acid composition of cell membranes.

BIOLOGICAL MEMBRANE AS STRUCTURAL ANTIOXIDANT SYSTEM

The susceptibility of membrane phospholipids to oxidative alterations is related to two inherent traits, the physico-

chemical properties of the membrane bilayer and the chemical reactivity of the fatty acids that compose the membrane.^[22,25] The first property is related with the fact that oxygen and free radicals are more soluble in the fluid lipid bilayer than in the aqueous solution. Thus, membranes contain an interior organic phase, in which oxygen may tend to concentrate. Therefore, these differences in solubility are important when considering the availability of oxygen/free radicals for chemical reactions inside living systems: organic regions may contain more free radicals than aqueous regions^[26,27] and, consequently, membrane lipids become primary targets of oxidative damage. The second and more significant property is related to the fact that PUFA residues of phospholipids are extremely sensitive to oxidation. Every membrane phospholipid contains an unsaturated fatty acid residue esterified to the 2-hydroxyl group of its glycerol moiety. Many of these are polyunsaturated and the presence of a methylene group between two double bonds renders the fatty acid sensitive to ROS-induced damage, their sensitivity to oxidation increasing exponentially as a function of the number of double bonds per fatty acid molecule.^[28,29] Consequently, polyunsaturated fatty acid side chains (two or more double bonds) are much more easily attacked by radicals than are saturated (no double bonds) or monounsaturated (one double bond) side chains. In this scenario, from a given membrane fatty acid profile it is possible to calculate its *peroxidizability index* (PI) by combining this composition with the relative susceptibility of individual fatty acids to peroxidation. So, PI is an approach to the relative susceptibility of a given membrane fatty acid mixture to peroxidative damage. The higher the number the more susceptible, the lower the value of PI, the more resistant to lipid peroxidation is the membrane bilayer.^[25]

Lipid peroxidation generates hydroperoxides as well as endoperoxides, which undergo fragmentation to produce a broad range of reactive intermediates called reactive carbonyl species (RCS) with three to nine carbons in length, the most reactive being α,β -unsaturated aldehydes [4-hydroxy-trans-2-nonenal (HNE) and acrolein], di-aldehydes [malondialdehyde (MDA) and glyoxal], and keto-aldehydes [4-oxo-trans-2-nonenal (ONE) and isoketals].^[30,31] 2-Hydroxyheptanal (2-HH) and 4-hydroxyhexenal (4-HHE) are other significant aldehydic products of lipid peroxidation of PUFAs. These carbonyl compounds, ubiquitously generated in biological systems, have unique properties contrasted with free radicals. For instance, compared with ROS, reactive aldehydes have a much longer half-life (i.e., minutes to hours instead of microseconds to nanoseconds

for most free radicals). Further, the non-charged structure of aldehydes allows them to migrate with relative ease through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the production site. Based on these features alone, these carbonyl compounds can be more destructive than ROS and may have far-reaching damaging effects on target sites within or outside membranes.^[22]

Carbonyl compounds react with nucleophilic groups in macromolecules (lipoxidation reactions) like proteins, DNA, and aminophospholipids, among others, resulting in their chemical, nonenzymatic, and irreversible modification and formation of a variety of adducts and crosslinks collectively named Advanced Lipoxidation Endproducts (ALEs).^[31-33] So, these aldehydes were believed to produce simply “cytotoxic” effects associated with oxidative stress, but evidence is increasing that these compounds can also have specific signaling roles inducing adaptive responses driven to decrease oxidative damage and improve antioxidant defences.^[22,33] Two of these mechanisms are: the regulation of uncoupling protein activity,^[34,35] and the activation of the antioxidant response signaling pathway,^[36-38] both involved in the prevention of oxidative damage effects.

So, the high concentration of PUFAs in cellular membrane phospholipids not only makes them prime targets for reaction with oxidizing agents but also enables them to participate in long free radical chain reactions. With these premises, and maintaining other physiological properties, it is plausible to postulate that a low degree of fatty acid unsaturation in cellular membranes could be advantageous by decreasing their sensitivity to lipid peroxidation. This would also protect other molecules against lipoxidation-derived damage. So, it is proposed that membrane unsaturation acts as a structural antioxidant system and it is adapted to the animal longevity.

MEMBRANE UNSATURATION AND ANIMAL LONGEVITY

The relationship between the degree of membrane unsaturation and longevity has been evaluated in mammals—including humans— and birds, strains of mice that vary in longevity and animal species that are exceptionally long-living, differences in longevity between queen and worker honeybees, strains of *D. melanogaster* that vary in longevity, and from experimental extensions of longevity in rodents, flies and worms. Unfortunately, not data are currently available for exceptional models such as bivalves, bats, and non-human primates.

Thus, it has been found that long-lived animals (birds and mammals, including humans) have a lower degree of total tissue and mitochondrial fatty acid unsaturation and PI than short-lived ones (**Table 1**). In agreement with this, it has been demonstrated that in long-lived animal species a low degree of total tissue and mitochondrial fatty acid unsaturation is accompanied by a low sensitivity to *in vivo* and *in vitro* lipid peroxidation and a low steady-state level of lipoxidation-derived adducts in both tissue and mitochondrial proteins from organs like skeletal muscle, heart, liver, and brain.^[22,61] In this line, lipofuscin, often considered a hallmark of aging also shows an accumulation rate that inversely correlates with longevity.^[62] These findings were consistent with the negative correlation observed between longevity and the sensitivity to lipid autoxidation of mammalian kidney and brain homogenates.^[63]

Interestingly, the findings from a recent work^[57] centered in offspring of long-lived individuals again seem to reinforce the association between membrane unsaturation and longevity. Thus, the fatty acid composition of erythrocyte membranes from 41 nonagenarian offspring were compared with 30 matched controls. Genetic loci were also tested in 280 centenarians and 280 controls to verify a potential genetic predisposition in determining unique lipid profile (allele and genotyping frequencies at endothelial-nitric oxide synthase and delta-5/delta-6 and delta-9 desaturase loci were considered). The results of this study demonstrated significant differences in the lipid composition of erythrocyte membranes derived from nonagenarian offspring versus matched controls. This is indicative of reduced oxidative stress and increased membrane integrity at the cellular level for nonagenarian offspring compared with the general population under investigation. In this context, it is plausible to infer that lipid composition of erythrocyte membranes could represent a useful biomarker of longevity.

While longevity can differ dramatically between mammal and bird species, there can also be significant longevity differences within a species. For example, populations of two wild-derived strains of mice display extended longevity (both mean and maximum longevity) compared to genetically heterogenous laboratory mice when kept under identical conditions.^[64] The PI of both skeletal muscle and liver phospholipids of the two wild-type mice strains with the extended longevity was significantly smaller than that of the laboratory mice.^[51] This is interesting because, since the different mice strains were fed the same diet, it shows that the differences in membrane composition between species are not determined by dietary differences but is

Table 1. Comparative studies between membrane unsaturation (peroxidizability index, PI) and longevity in animal species (by chronological order).

Species compared	Organ	PI long-lived species	References
Rat-Pigeon-Human	Liver mitochondria	Lower	[39]
SAM-R/1 vs SAM-P/1 mice	Liver	Lower	[40]
8 mammalian species	Liver mitochondria	Lower	[41]
Rat vs pigeon	Heart mitochondria	Lower	[42]
Mouse vs canary	Heart	Lower	[43]
Mouse vs parakeet	Heart	Lower	[43]
Rat vs pigeon	Liver mitochondria	Lower	[44]
Rat vs pigeon	Heart mitochondria and microsomes	Lower	[44]
8 mammalian species	Heart	Lower	[45]
7 mammalian species	Liver	Lower	[46]
8 mammalian species	Liver mitochondria	Lower	[47]
Rat vs pigeon	Skeletal muscle	Lower	[48]
Mouse, parakeet, canary	Brain	Lower	[49]
8 mammalian species	Heart	Lower	[50]
Strains of mice (Idaho, Majuro and WT)	Skeletal muscle and liver	Lower	[51]
Naked-mole rat vs mouse	Skeletal muscle mitochondria and Liver mitochondria	Lower	[52,53]
12 mammalian species and 9 bird species	Skeletal muscle	Lower	[25]
10 mammalian species and 8 bird species	Liver mitochondria	Lower	[25]
Queen honey bees vs workers	Head, thorax, abdomen	Lower	[54]
13 bird species	Heart	Lower	[55]
Echidna vs mammals	Liver, liver mitochondria, and ç Skeletal muscle	Lower	[56]
Humans (long-lived vs control group)	Erythrocytes	Lower	[57]
<i>D. melanogaster</i> (long-lived mutant strains)	Whole organism and mitochondria	Lower	[58, #]
<i>C. elegans</i> (long-lived mutant strains)	Whole organism	Lower	[59]
Rat vs pigeon	Erythrocytes, heart, kidney, liver, skeletal muscle (whole tissue and mitochondria)	Lower	[60]
<i>D. melanogaster</i> (wild-type strains)	Whole organism and mitochondria	Lower	#

#R. Pamplona, unpublished results

genetically controlled. In this line, it is also interesting that in the senescence-accelerated mouse (SAM) strain, the SAM-prone mice have greater levels of the highly polyunsaturated peroxidation-prone fatty acids 22:6 n-3 and 20:4 n-6 and lower levels of the less peroxidation-prone 18:2 n-6 PUFA in their membranes, and consequently

have a greater PI than the SAM-resistant mice.^[40] SAM-prone mice also show greater degrees of lipid peroxides in their tissues than do SAM-resistant mice.

Two exceptionally long-living mammalian species (naked mole-rats and echidnas) also have membrane fatty acid profiles that are resistant to lipid peroxidation

as one would predict from their longevities. Thus, when membrane fatty acid composition was measured in tissues from naked mole-rats, the longest-living rodents known with a recorded longevity exceeding 28 years,^[65] it was found that they have very low levels of 22:6n-3 in their tissue phospholipids compared to mice. Although both mice and naked mole-rats have similar levels of total unsaturated fatty acids in their tissue phospholipids, the low 22:6n-3 levels of the naked mole-rats result in lower PI and more peroxidation-resistant membranes in skeletal muscle and liver mitochondria.^[52,53] In a similar way, the echidna *Tachyglossus aculeatus*, a monotreme mammal from Australia that is exceptionally long-living and with a documented longevity of 50 years, also have a membrane composition resistant to lipid peroxidation.^[56] Accordingly, membrane lipids of echidna tissues (skeletal muscle, liver and liver mitochondria) were found to have a lower content of polyunsaturates and a higher content of monounsaturates, resulting in a low PI and, consequently, indicating that the cellular membranes of echidnas is peroxidation-resistant. Interestingly, when the calculated peroxidation index was plotted against maximum longevity, the echidna values conformed to the relationship for mammals in general.^[56]

Honeybees (*Apis mellifera*) and flies (*D. melanogaster*) provide another example of variation in longevity within a species. In the honey bee, depending on what they are fed, female eggs become either workers or queens.^[66] So, queens and workers share a common genome. However, the longevity of queens is an order-of-magnitude longer than workers. In order to test if differences in membrane composition could be involved it was compared the fatty acid composition of phospholipids of queen and worker honey bees.^[54] The cell membranes of both young and old honey bee queens were highly monounsaturated with very low content of PUFAs. Newly emerged workers shown a similar membrane fatty acid composition to queens but within the first week of hive life, they increase the polyunsaturate content and decrease the monounsaturate content of their membranes, probably as a result of pollen consumption. This means their membranes likely become more susceptible to lipid peroxidation in this first week of hive life. So, the results again support the suggestion that membrane composition might be an important factor in the determination of longevity. Assuming the same slope of the relationship between membrane PI and longevity as previously observed for mammal and bird species, it was proposed^[54] that the 3-fold difference in PI of phospholipids of queens and workers is large enough to account for the

order-of-magnitude difference in their longevity. In another approach, these predictions have also been tested in a comparison among three wild type strains of *D. melanogaster* differing in their longevities (a long-lived strain: Oregon R, and two short-lived strains: Canton S and Dahomey). The results also confirm the present of an inverse correlation between membrane unsaturation and lipoxidation-derived molecular damage and longevity. So, the longer the longevity of the *Drosophila* strain, the lower is the membrane unsaturation (R. Pamplona, unpublished results).

Are experimental extensions in longevity accompanied by attenuations of membrane unsaturation and lipoxidation-derived molecular damage? This question is a key issue that goes beyond correlation to establish a causative role for membranes and lipoxidative stress in the determination of longevity. In order to clarify whether the low membrane unsaturation of long-lived animals protects their cellular components from lipid oxidation and lipoxidation-derived molecular damage, studies of experimental dietary modification of *in vivo* membrane fatty acid unsaturation have been performed.^[67-69] These studies were specially designed to partially circumvent the homeostatic system of compensation of dietary-induced changes in membrane unsaturation which operates at tissue level. The obtained findings demonstrate that lowering the membrane unsaturation of cellular membranes protects post-mitotic tissues against lipid peroxidation and lipoxidation-derived macromolecular damage.

The relevance of membrane unsaturation in determining longevity has also been recently reinforced by using *Drosophila* as experimental model.^[58] In this study, it was created transgenic strains of *Drosophila* that express yeast NDI1 (in yeast, the single-subunit NADH dehydrogenase Ndi1 serves as a non-proton-translocating alternative enzyme that replaces complex I, bringing about the reoxidation of intramitochondrial NADH) ubiquitously. NDI1 expression mitigated the aging associated decline in respiratory capacity and the accompanying increase in mitochondrial free radical production, and resulted in decreased accumulation of markers derived from a lipoxidative stress in aged flies, resulting in an increased longevity. This lower lipoxidation-derived damage in the long-lived strains is also linked to an adaptive response with a low degree in membrane unsaturation (R. Pamplona, unpublished results).

In another approach with *C. elegans* as experimental model,^[59,70] it was analyzed the fatty acid profile of lipids extracted from strains of *C. elegans* that vary in longevity by ~10-fold, and report several significant log-linear

correlations between longevity and fatty acid composition. The results are strongly influenced by two mutant strains (*daf-2* and *age-1*, both long-lived mutant strains linked to a disruption of the insulinlike-signaling pathway) that show the greatest longevity. Their findings can be summarized thus; comparing the shortest-living to longest-living strains total monounsaturates increased from 34% to 48%, total polyunsaturates decreased from 37% to 26%, and PI decreased from 141 to 81. Interestingly, these changes were remarkably similar to correlations between PI and longevity reported previously for mammals and birds. Furthermore, it was also found a reduction in *C. elegans* longevity with the addition of PUFAs to their diet. All together, these findings clearly support a role for membrane unsaturation in the determination of longevity.

Finally, available evidences in favour for a relationship between membrane unsaturation and longevity proceed from nutritional interventions that extend longevity in experimental models. Thus, caloric (CR), as well as protein (PR) and methionine (MetR) restriction –*nutritional interventions that increase longevity*– attenuates age-related changes in the degree of membrane unsaturation and the level of lipoxidation-derived protein damage in a variety of tissues and animal species.^[71-73] Thus, a decrease in membrane unsaturation, lipid peroxidation and lipoxidation-derived damage has been reported in tissues (liver, heart, and brain) from these dietary restrictions in rats and mice.^[74-84] CR has also been shown to reduce levels of lipofuscin in tissues of rodents and *C. elegans*.^[62,85-88] From these studies it can be inferred that the magnitude of the change is modest for membrane unsaturation (between 2.5-10%) than that for the lipoxidation-derived molecular damage (between 20-40%) likely due to the added effect of the lower mitochondrial free radical generation also induced by these nutritional interventions. In addition to the moderate but significant effect on membrane unsaturation, these nutritional interventions show an effect that is directly related to the percent of the dietary restriction applied, being both protein and methionine restriction (80% MetR) even more intense and effective than caloric restriction. It is suggested from available data that the effects of CR on membrane unsaturation could be divided in three stages depending of CR duration in rats. During short-term CR periods, decreases in the rate of mitochondrial free radical production and lipoxidation-derived protein damage are observed in some tissues together with minor changes in membrane fatty acid composition. If CR is applied for several weeks-months, changes in particular

fatty acids with moderate or no changes in PI occur, although the magnitude of the changes depends on the organ and the intensity of the restriction. Finally, in long-term CR, the beneficial effects on free radical production, PI, and lipoxidation-derived damage are evident. In fact, CR diminishes the slope of the relationship between age and age-related lipid peroxidation. Thus, the CR manipulation seems to trigger an adaptive response protecting the most basic requirements of membrane integrity.

Globally, all these comparisons a) support an important role for membrane fatty acid composition in the determination of longevity, b) reinforce the idea that the connection between membrane unsaturation and longevity is not restricted to vertebrates, and c) suggest that membrane composition is regulated in a species-specific way.

MECHANISM RESPONSIBLE FOR THE LONGEVITY-RELATED DIFFERENCES IN MEMBRANE UNSATURATION

The membrane acyl composition of the animal species studied indicates that their biological membranes maintain a similar a) fatty acid average chain length (18 carbon atoms), b) ratio of saturated versus unsaturated fatty acids (ratio 40:60), and c) phospholipid distribution irrespective of animal longevity.^[22] The low PI observed in long-lived species are due to changes in the type of unsaturated fatty acid that participates in membrane composition. So, there is a systematic redistribution between the types of PUFAs present from highly unsaturated fatty acids such as 22:6n-3, 20:5n-3, and 20:4n-6 in short-lived animals to the less unsaturated 18:3n-3, 18:2n-6, and 18:1 in the long-lived ones, at mitochondrial and tissue level.^[25,61] Furthermore, the PI of the respective diets did not correlate with longevity. This indicates again that the contribution of the variations in the degree of unsaturation of dietary fats to the inter-species differences is, if any, very modest.^[25,61]

The mechanisms responsible for the longevity-related differences in fatty acid profile can be related, in principle, to the fatty acid desaturation pathway, and the deacylation-reacylation cycle. The available estimates of delta-5 and delta-6 desaturase activities indicate that they are several folds lower in long-lived species than in short-lived ones.^[25,59,61,89] This can explain why e.g. 22:6n-3 and 20:4n-6 decreases and 18:2n-6 and 18:3n-3 increases, from short- to long-lived animals, since desaturases are the rate-limiting enzymes of the n-3 and n-6 pathways synthesizing

the highly unsaturated PUFAs 20:4n-6 and 22:6n-3 from their dietary precursors, 18:2n-6 and 18:3n-3, respectively. 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. The fact that acyltransferase/n-6 desaturase activity ratio is about 10:1 in tissues^[90] reinforces the idea that regulation of desaturases can be the main limiting factor responsible for the observed membrane unsaturation-longevity relationship. However, a role for a phospholipid-specific deacylation-reacylation system can not be discarded since it has been observed that the longevity-related redistribution particularly affects the phosphatidylcholine and phosphatidylethanolamine fractions in liver mitochondria, and does not modify cardiolipin.^[47,53]

In accordance with this interpretation, a recent study^[91] with a phylogenomic approach to identify the genetic targets of natural selection for elongated longevity in mammals has been published. In this work, comparing the nonsynonymous and synonymous evolution of 5.7 million codon sites across 25 species, shows that genes involved in lipid composition (and particularly desaturation system) have collectively undergone increased selective pressure in long-lived species. So, cellular membrane has apparently been the optimized feature.

SIGNIFICANCE

Animals with a high longevity have a low degree of membrane fatty acid desaturation based in the redistribution between types of PUFAs without any alteration in the total (%) PUFA content, average chain length, and phospholipid distribution. This may be viewed as an elegant evolutionary strategy, because it decreases the sensitivity to lipid peroxidation and lipoxidation-derived damage to cellular macromolecules without strongly altering fluidity/microviscosity, a fundamental property of cellular membranes for the proper function of receptors, ion pumps, and transport of metabolites. This would occur because membrane fluidity increases acutely with the introduction of the first and less with the second double bond (due to their introduction of “kinks” in the fatty acid molecule), whereas additional (the third and following) double bonds cause few further variations in fluidity.^[92] This is so because the kink has a larger impact on fluidity when the double bond is situated near the centre 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 the sensitivity to lipid peroxidation, however, double bonds increase it irrespective of their location at the centre or laterally on the fatty acids.^[28] Thus, by substituting fatty acids with four or six double bonds by 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 would be essentially maintained. This hypothesis, reminiscent of membrane acclimation to different environments at PUFA level in poikilotherms and bacteria, has been denominated *homeoviscous longevity adaptation*.^[61]

ACKNOWLEDGEMENTS

Investigations of the author of this review have been supported in part by I + D grants from the Spanish Ministry of Science and Innovation (BFU2008-00335/BFI and BFU2011-23888), and BSCH-UCM (2009-2010) to G.B; and grants from the Spanish Ministry of Education and Science (BFU2009-11879/BFI), the Spanish Ministry of Health (RD06/0013/0012), and the Generalitat of Catalunya (2009SGR735) to R.P.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med.* 2000; 108:652-59.
2. Lane N. Oxygen. The molecule that made the world. Oxford University Press, UK; 2002.
3. Embley TM, Martin W. Eukaryotic evolution, changes and challenges. *Nature.* 2006; 440:623-30.
4. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, UK; 2007.
5. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radic Res.* 1999; 31:261-72.
6. von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. Good genes, oxidative stress and condition-dependent sexual signals. *Proc Royal Soc London B.* 1999; 266:1-12.
7. Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Chastel O, et al. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution.* 2006; 60:1913-24.
8. Costantini D, Dell’Omo G. Environmental and genetic components of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J Comp Physiol B.* 2006; 176:575-79.
9. Bize P, Devevey G, Monaghan P, Doligez B, Christie P. Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology.* 2008; 89:2584-93.

10. Costantini D. Oxidative stress in ecology and evolution: lessons from avian studies. *Ecol Lett.* 2008; 11:1238-51.
11. Dowling DK, Simmons LW. Reactive oxygen species as universal constraints in life-history evolution. *Proc Royal Soc B.* 2009; 276:1737-45.
12. Monaghan P, Metcalfe NB, Torres R. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurement and interpretation. *Ecol Lett.* 2009; 12:75-92.
13. Costantini D. Complex trade-offs in the pigeon (*Columba livia*): egg antioxidant capacity and female serum oxidative status in relation to diet quality. *J Comp Physiol B.* 2010; 180:731-39.
14. Metcalfe NB, Alonso-Alvarez C. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct Ecol.* 2010; 24:984-96.
15. Pamplona R, Costantini D. Molecular and structural antioxidant defences against oxidative stress in animals. *Am J Physiol Regul Integr Comp Physiol.* doi: 10.1152/ajpregu.00034.2011.
16. Yeagle P. The membranes of cells. Academic Press, San Diego; 1993.
17. Vance DE, Vance JE. Biochemistry of lipids, lipoproteins and membranes. Elsevier Science BV, Amsterdam, The Netherlands; 1996.
18. Dowhan W. Molecular basis for membrane phospholipid diversity: why are there so many lipids? *Annu Rev Biochem.* 1997; 66:199-232.
19. Van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nature Rev Mol Cell Biol.* 2008; 9:112-24.
20. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB. Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*. *Science.* 2002; 296:879-83.
21. Wallis JG, Watts JL, Browse J. Polyunsaturated fatty acid synthesis: what will they think of next?. *Trends Biochem Sci.* 2002; 27:467-73.
22. Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim Biophys Acta Bioenergetics.* 2008; 1777:1249-62.
23. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu Rev Nutr.* 2004; 24:345-76.
24. Guillou H, Zadavec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog Lipid Res.* 2010; 49:186-99.
25. Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol Rev.* 2007; 87:1175-1213.
26. Moller M, Botti H, Batthyany C, Rubbo H, Radi R, Denicola A. Direct measurement of nitric oxide and oxygen partitioning into liposomes and low density lipoprotein. *J Biol Chem.* 2005; 280:8850-54.
27. Gamliel A, Afri M, Frimer AA. Determining radical penetration of lipid bilayers with new lipophilic spin traps. *Free Radic Biol Med.* 2008; 44:1394-1405.
28. Holman RT. Autoxidation of fats and related substances. In: Holman RT, Lundberg WO, Malkin T (eds) *Progress in Chemistry of Fats and Other Lipids*, Pergamon Press, London, 51-98; 1954.
29. Bielski BH, Arudi RL, Sutherland MW. A study of the reactivity of HO₂/O₂⁻ with unsaturated fatty acids. *J Biol Chem.* 1983; 258:4759-61.
30. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med.* 1991; 11:81-128.
31. Thorpe SR, Baynes JW. Maillard reaction products in tissue proteins: new products and new perspectives. *Amino Acids.* 2003; 25:275-81.
32. West JD, Marnett LJ. Endogenous reactive intermediates as modulators of cell signaling and cell death. *Chem Res Toxicol.* 2006; 19:173-94.
33. Pamplona R. Advanced lipoxidation end-products. *Chem Biol Interact.* 2011; 192:14-20.
34. Ehtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otín M, et al. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J.* 2003; 22:4103-10.
35. Brand MD, Affourtit C, Esteves TC, Green K, Lambert AJ, Miwa S, et al. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med.* 2004; 37:755-67.
36. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, et al. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci USA.* 2004; 101:2040-45.
37. Copple IM, Goldring CE, Kitteringham NR, Park BK. The Nrf2-Keap1 defence pathway: role in protection against drug-induced toxicity. *Toxicology.* 2008; 246:24-33.
38. Giles GI. Redox-controlled transcription factors and gene expression. In: Jacob C, Winyard PG, eds. *Redox signaling and regulation in biology and medicine*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 245-270; 2009.
39. Pamplona R, Prat J, Cadenas S, Rojas C, Pérez-Campo R, López Torres M, et al. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. *Mech Ageing Dev.* 1996; 86:53-66.
40. Park JW, Choi CH, Kim MS, Chung MH. Oxidative status in senescence accelerated mice. *J Gerontol A Biol Sci Med Sci.* 1996; 51:B337-45.
41. Pamplona R, Portero-Otín M, Riba D, Ruiz C, Prat J, Bellmunt MJ, et al. Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J Lipid Res.* 1998; 39:1989-94.
42. Pamplona R, Portero-Otín M, Requena JR, Thorpe SR, Herrero A, Barja G. A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. *Mech Ageing Dev.* 1999; 106:283-96.
43. Pamplona R, Portero-Otín M, Riba D, Ledo F, Gredilla R, Herrero A, et al. Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse. *Aging (Milano).* 1999; 11:44-49.
44. Gutiérrez AM, Reboredo GR, Arcemis CJ, Catalá A. Non-enzymatic lipid peroxidation of microsomes and mitochondria isolated from liver and heart of pigeon and rat. *Int J Biochem Cell Biol.* 2000; 32:73-79.
45. Pamplona R, Portero-Otín M, Ruiz C, Gredilla R, Herrero A, Barja G. Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. *Mech Ageing Dev.* 2000; 112:169-83.
46. Pamplona R, Portero-Otín M, Riba D, Requena JR, Thorpe SR, López-Torres M, et al. Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in

- mammalian species with long life spans. *J Gerontol A Biol Sci Med Sci.* 2000; 55:B286-91.
47. Portero-Otín M, Bellmunt MJ, Ruiz MC, Barja G, Pamplona R. Correlation of fatty acid unsaturation of the major liver mitochondrial phospholipid classes in mammals to their maximum life span potential. *Lipids.* 2001; 36:491-98.
 48. Portero-Otín M, Requena JR, Bellmunt MJ, Ayala V, Pamplona R. Protein nonenzymatic modifications and proteasome activity in skeletal muscle from the short-lived rat and long-lived pigeon. *Exp Gerontol.* 2004; 39:1527-35.
 49. Pamplona R, Portero-Otín M, Sanz A, Ayala V, Vasileva E, Barja G. Protein and lipid oxidative damage and complex I content are lower in the brain of budgerigards and canaries than in mice. Relation to aging rate. *AGE.* 2005; 27:267-80.
 50. Ruiz MC, Ayala V, Portero-Otín M, Requena JR, Barja G, Pamplona R. Protein methionine content and MDA-lysine protein adducts are inversely related to maximum life span in the heart of mammals. *Mech Ageing Dev.* 2005; 126: 1106-14.
 51. Hulbert AJ, Faulks SC, Harper JM, Miller RA, Buffenstein R. Extended longevity of wild-derived mice is associated with peroxidation-resistant membranes. *Mech Ageing Dev.* 2006; 127:653-57.
 52. Hulbert AJ, Faulks SC, Buffenstein R. Oxidation-resistant membrane phospholipids can explain longevity differences among the longest-living rodents and similarly-sized mice. *J Gerontol A Biol Sci Med Sci.* 2006; 61:1009-18.
 53. Mitchell TW, Buffenstein R, Hulbert AJ. Membrane phospholipid composition may contribute to exceptional longevity of the naked mole-rat (*Heterocephalus glaber*): a comparative study using shotgun lipidomics. *Exp Gerontol.* 2007; 42: 1053-62.
 54. Haddad LS, Kelbert L, Hulbert AJ. Extended longevity of queen honey bees compared to workers is associated with peroxidation-resistant membranes. *Exp Gerontol.* 2007; 42:601-9.
 55. Buttemer WA, Battam H, Hulbert AJ. Fowl play and the price of petrol: long-living Procellariiformes have peroxidation-resistant membrane composition compared with short-living Galliformes. *Biol Lett.* 2008; 4:351-4.
 56. Hulbert AJ, Beard LA, Grigg GC. The exceptional longevity of an egg-laying mammal, the short-beaked echidna (*Tachyglossus aculeatus*) is associated with peroxidation-resistant membrane composition. *Exp Gerontol.* 2008; 43:729-33.
 57. Puca AA, Andrew P, Novelli V, Anselmi CV, Somalvico F, Cirillo NA, et al. Fatty acid profile of erythrocyte membranes as possible biomarker of longevity. *Rej Res.* 2008; 11:63-72.
 58. Sanz A, Soikkeli M, Portero-Otín M, Wilson A, Kempainen E, McIlroy G, et al. Expression of the yeast NADH dehydrogenase Ndi1 in *Drosophila* confers increased lifespan independently of dietary restriction. *Proc Natl Acad Sci USA.* 2010; 107: 9105-10.
 59. Shmookler Reis RJ, Xu L, Lee H, Chae M, Thaden JJ, Bharill P, et al. Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging.* 2011; 3:125-47.
 60. Montgomery MK, Hulbert AJ, Buttemer WA. The long life of birds: the rat-pigeon comparison revisited. *Plos One.* 2011; 6:e24138.
 61. Pamplona R, Barja G, Portero-Otín M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation?. *Ann NY Acad Sci.* 2002; 959:475-90.
 62. Terman A, Brunk UT. Lipofuscin. *Int J Biochem Cell Biol.* 2004; 36:1400-04.
 63. Cutler RG. Peroxide-producing potential of tissues: inverse correlation with longevity of mammalian species. *Proc Natl Acad Sci USA.* 1985; 82:4798-4802.
 64. Miller RA, Harper JM, Dysko RC, Durkee SJ, Austad SN. Longer life spans and delayed maturation in wild-derived mice. *Exp Biol Med.* 2002; 227:500-8.
 65. Buffenstein R. The naked mole-rat: a new long-living model for human aging research. *J Gerontol A Biol Sci Med Sci.* 2005; 60:1369-77.
 66. Winston ML. The biology of the honey bee. Cambridge MA: Harvard University Press; 1987.
 67. Herrero A, Portero-Otín M, Bellmunt MJ, Pamplona R, Barja G. Effect of the degree of fatty acid unsaturation of rat heart mitochondria on their rates of H₂O₂ production and lipid and protein oxidative damage. *Mech Ageing Dev.* 2001; 122:427-43.
 68. Portero-Otín M, Bellmunt MJ, Requena JR, Pamplona R. Protein modification by advanced Maillard adducts can be modulated by dietary polyunsaturated fatty acids. *Biochem Soc Trans.* 2003; 31:1403-5.
 69. Pamplona R, Portero-Otín M, Sanz A, Requena J, Barja G. Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain. *Exp Gerontol.* 2004; 39:725-33.
 70. Hulbert AJ. Longevity, lipids and *C. elegans*. *Aging.* 2011; 3:81-2.
 71. Yu BP. Membrane alteration as a basis of aging and the protective effects of calorie restriction. *Mech Ageing Dev.* 2005; 126:1003-10.
 72. Pamplona R, Barja G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. *Biochim Biophys Acta Bioenergetics.* 2006; 1757:496-508.
 73. Pamplona R, Barja G. An evolutionary comparative scan for longevity-related oxidative stress resistance mechanisms in homeotherms. *Biogerontology.* doi: 10.1007/s10522-011-9348-1.
 74. Lambert AJ, Portero-Otín M, Pamplona R, Merry BJ. Effect of aging and caloric restriction on specific markers of protein oxidative damage and membrane peroxidizability in rat liver mitochondria. *Mech Ageing Dev.* 2004; 125:529-38.
 75. Laganieri S, Yu BP. Anti-lipoperoxidation action of food restriction. *Biochem Biophys Res Comm.* 1987; 145:1185-91.
 76. Pamplona R, Portero-Otín M, Bellmunt MJ, Gredilla R, Barja G. Aging increases Nepsilon-(carboxymethyl)lysine and caloric restriction decreases Nepsilon-(carboxyethyl)lysine and Nepsilon-(malondialdehyde)lysine in rat heart mitochondrial proteins. *Free Radic Res.* 2002; 36:47-54.
 77. Pamplona R, Portero-Otín M, Requena JR, Gredilla R, Barja G. Oxidative, glycoxidative and lipoxidative damage to rat heart mitochondrial proteins is lower after 4 months of caloric restriction than in age-matched controls. *Mech Ageing Dev.* 2002; 123:1437-46.
 78. Sanz A, Gredilla R, Pamplona R, Portero-Otín M, Vara E, Tresguerres JA, et al. Effect of insulin and growth hormone on rat heart and liver oxidative stress in control and caloric restricted animals. *Biogerontology.* 2005; 6:15-26.
 79. Sanz A, Caro P, Ayala V, Portero-Otín M, Pamplona R, Barja G. Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. *FASEB J.* 2006; 20:1064-73.
 80. Ayala V, Naudi A, Sanz A, Caro P, Portero-Otín M, Barja G, et al. Dietary protein restriction decreases oxidative protein damage, peroxidizability index, and mitochondrial complex I content in rat liver. *J Gerontol A Biol Sci Med Sci.* 2007; 62:352-60.

81. Gómez J, Caro P, Naudí A, Portero-Otín M, Pamplona R, Barja G. Effect of 8.5% and 25% caloric restriction on mitochondrial free radical production and oxidative stress in rat liver. *Biogerontology*. 2007; 8:555-66.
82. Naudí A, Caro P, Jové M, Gómez J, Boada J, Ayala V, et al. Methionine restriction decreases endogenous oxidative molecular damage and increases mitochondrial biogenesis and uncoupling protein 4 in rat brain. *Rej Res*. 2007; 10:473-84.
83. Caro P, Gómez J, López-Torres M, Sánchez I, Naudí A, Jové M, et al. Forty percent and eighty percent methionine restriction decrease mitochondrial ROS generation and oxidative stress in rat liver. *Biogerontology*. 2008; 9:183-96.
84. Caro P, Gomez J, Sanchez I, Naudi A, Ayala V, López-Torres M, et al. Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria. *Rej Res*. 2009; 12:421-34.
85. Enesco HE, Kruk P. Dietary restriction reduces fluorescent age pigment accumulation in mice. *Exp Gerontol*. 1981; 16:357-61.
86. De AK, Chipalkatti S, Aiyar AS. Some biochemical parameters of ageing in relation to dietary protein. *Mech Ageing Dev*. 1983; 21:37-48.
87. Rao G, Xia E, Nadakavukaren MJ, Richardson A. Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. *J Nutr*. 1990; 120:602-9.
88. Gerstbrein B, Stamatias G, Kollias N, Driscoll M. In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. *Aging Cell*. 2005; 4:127-37.
89. Pamplona R, Barja G. Aging rate, free radical production, and constitutive sensitivity to lipid peroxidation: insights from comparative studies. In: Van Zglinicki T (ed) *Biology of aging and its modulation series*. Vol. 1. Aging at the molecular level. Kluwer Academic Publisher, New York, pp 47-64; 2003.
90. Ivanetich KM, Bradshaw JJ, Ziman MR. Delta 6-desaturase: improved methodology and analysis of the kinetics in a multi-enzyme system. *Biochim Biophys Acta*. 1996; 1292: 120-32.
91. Jobson RW, Nabholz B, Galtier N. An evolutionary genome scan for longevity-related natural selection in mammals. *Mol Biol Evol*. 2010; 27:840-47.
92. Brenner RR. Effect of unsaturated fatty acids on membrane structure and enzyme kinetics. *Progr Lipid Res*. 1984; 23:69-96.