Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1757 (2006) 496–508
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Review

Mitochondrial oxidative stress, aging and caloric restriction: The protein and methionine connection

Reinald Pamplona [a], Gustavo Barja [b,*]

[a] Department of Basic Medical Sciences, University of Lleida, Lleida 25008, Spain
[b] Department of Animal Physiology-II, Complutense University, Madrid 28040, Spain

Received 22 November 2005; revised 10 January 2006; accepted 28 January 2006
Available online 24 February 2006

Abstract

Caloric restriction (CR) decreases aging rate and mitochondrial ROS (MitROS) production and oxidative stress in rat postmitotic tissues. Low levels of these parameters are also typical traits of long-lived mammals and birds. However, it is not known what dietary components are responsible for these changes during CR. It was recently observed that 40% protein restriction without strong CR also decreases MitROS generation and oxidative stress. This is interesting because protein restriction also increases maximum longevity (although to a lower extent than CR) and is a much more practicable intervention for humans than CR. Moreover, it was recently found that 80% methionine restriction substituting it for L-glutamate in the diet also decreases MitROS generation in rat liver. Thus, methionine restriction seems to be responsible for the decrease in ROS production observed in caloric restriction. This is interesting because it is known that exactly that procedure of methionine restriction also increases maximum longevity. Moreover, recent data show that methionine levels in tissue proteins negatively correlate with maximum longevity in mammals and birds. All these suggest that lowering of methionine levels is involved in the control of mitochondrial oxidative stress and vertebrate longevity by at least two different mechanisms: decreasing the sensitivity of proteins to oxidative damage, and lowering of the rate of ROS generation at mitochondria.

Keywords: Mitochondria; Methionine restriction; Caloric restriction; Free radical; Aging; DNA damage

1. Introduction

Aging causes a multitude of detrimental changes in the organism at all levels of biological organization, decreases maximum functional capacities and homeostasis, and increases the probability of suffering degenerative diseases and finally death. All these changes likely originate from a relatively small number of main causes that continuously operate throughout the life span. Any theory of aging should be able to explain the progressive and endogenous character of biological aging [1]. Different animal species age at very different rates in similar environments and those rates vary more than 40 fold in mammals. The early proposal by Denham Harman that free radicals [2], especially those of mitochondrial origin [3], are among the main causes of aging, is consistent with the results of many investigations [4–9]. Available evidence supporting the mitochondrial free radical theory of aging is consistent with the widely held notion that the rate of generation of reactive oxygen species (ROS) at mitochondria can be one of the main factors that determine aging rate and thus the maximum life span potential (MLSP). Studies with isolated mitochondria indicate that these organelles are a main source of ROS in healthy normal tissues. Mitochondrial ROS (MitROS) generation

Abbreviations: CEL, [$N^{epsilon}$-(carboxyethyl)lysine in proteins]; CML, [$N^{epsilon}$-(carboxymethyl)lysine in proteins]; CR, caloric restriction; %FRL (% free radical leak), ROS production as % of total electron flow in the mitochondrial respiratory chain; 8-oxodG, 8-oxo-7,8-dihydro-2′deoxyguanosine; MDAL, [$N^{epsilon}$(malondialdehyde)lysine in proteins]; MetR, L-methionine restriction; MitROS, mitochondrial ROS; MLSP, maximum life span potential (maximum longevity); mtDNA, mitochondrial DNA; nDNA, nuclear DNA; PR, protein restriction; ROS, reactive oxygen species

* Corresponding author. Departamento de Fisiología Animal-II, Facultad de Ciencias Biológicas, Universidad Complutense, c/Antonio Novais-2, Madrid 28040, Spain. Fax: +34 91 3944935.
E-mail address: gbarja@bio.ucm.es (G. Barja).

0005-2728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2006.01.009

continuously occurs throughout life at a species-specific rate [7,8,10–14] and is thus endogenously determined. The rate of MitROS production is especially high in highly aerobic tissues like heart, brain, or liver where aging changes are especially marked.

2. Variations in oxidative damage and DNA mutations during aging

Tissue antioxidants help to control oxidative stress in vivo, but antioxidant interception and scavenging of ROS is not 100% efficient. There is always a certain level of oxidative damage to macromolecules even in healthy unstressed animals. Such oxidative damage affects cellular lipids, proteins and DNA in tissues. The results of many investigations suggest that the steady-state levels of markers of oxidative damage to those macromolecules can increase with age in organs like liver, heart and brain. This was observed for proteins [9,15–17] and lipids [18–20], although such increases were not always detected [21]. ROS can also attack DNA directly at the sugar-phosphate backbone or at the bases. This produces many different oxidatively modified purines and pyrimidines, including the most commonly measured 8-oxo-7,8-dihydro-2′deoxyguanosine (8-oxodG), as well as single and double strand breaks in both rats and humans [22–24]. Various studies have found increases in steady-state levels of 8-oxodG during aging in brain, heart or liver nuclear (nDNA) or mitochondrial DNA (mtDNA) in rodents and humans [25–31], though again such increases were not always observed [29,32]. In any case, the increases in oxidative damage to macromolecules are not strong enough to be the result of a life-long accumulation. Such hypothetical accumulation would not be consistent with the widespread presence of many forms of repair of oxidative damage in tissues. Instead, those increases with age likely represent a resetting of the flux of oxidative damage and repair of cellular macromolecules at a higher steady-state level in old individuals [33] that contributes to age-related deterioration. That flux also occurs more rapidly in short-lived and in ad libitum-fed (AL) animals compared to long-lived and caloric restricted (CR) ones, respectively. One of the most important life-long consequences of oxidative damage to tissues during aging can be the constant generation of somatic DNA mutations in postmitotic cells, like those in mtDNA [8,34,35], because they can accumulate during aging. It is well known that mtDNA mutations accumulate with age in heart, brain and other tissues [34–36]. Although individual mtDNA mutations reach only low levels in old individuals, the mean aggregate mutational load of all kinds of mutations can be much higher. It is also possible that a relatively small number of mutations cause important deleterious and permanent effects during aging in the cells harboring them, in nearby cells of the same tissue or perhaps even in cells situated far away in other organs. For instance, it has been proposed that anaerobic mutated cells induce plasma LDL oxidation leading to amplification of damage in many other organs of the body [37]. Also, continuous generation of mtDNA fragments could induce genomic instability that would not be detected due to the heteroplasmic nature of mtDNA in most cells.

In agreement with a key role of MitROS and mtDNA in aging, it has been found that 8-oxodG is normally present in mtDNA at levels around 10 fold higher than in nuclear DNA (nDNA) [30,38,39]. Most interestingly, the level of somatic mutations in mtDNA is also around 10 fold higher than in nDNA [40], suggesting a cause–effect relationship between the rate of MitROS generation and the production and rate of accumulation of mtDNA mutations with age. Although the mitochondrion seems to lack some particular forms of DNA repair such as that of pyrimydine dimers (induced by UV radiation), it is now known that its capacity to repair 8-oxodG is similar or even higher than that in the nucleus [41]. A most probable reason for the higher level of oxidative damage in mtDNA than in nDNA is its closeness to or even its contact with the main ROS generator of healthy cells, the inner mitochondrial membrane [7,13,14]. Other differences, such as the lack of histones and polyamines, could also contribute to this difference. Furthermore, more direct evidence that increases in mtDNA mutations can increase aging rate has been recently obtained in mutant mice [42] although it is not definitively clear if this model corresponds to accelerated aging or accelerated pathology.

Oxidative damage is not limited to direct attack by ROS, since cross-reactions of side and final products of peroxidation of different kinds of macromolecules also occur. This is the case for glycoxidation and lipid peroxidation-derived reactions, which can finally lead to protein and DNA modification. Thus, non-enzymatic oxidative reactions of carbohydrates and lipid substrates (Maillard reaction or carbonyl-amine reactions) [43], and lipid peroxidation products, generate protein adducts like $N^{epsilon}$-(Malondialdehyde)lysine (MDAL), $N^{epsilon}$-(Carboxymethyl)lysine (CML), or $N^{epsilon}$-(Carboxyethyl)lysine (CEL) [17,43]. The study of the possible aging-related variations in such damage markers or in highly specific (measured by GC/MS) protein carbonyls like glutamic or aminoadipic semialdehydes in the intracellular proteins of postmitotic tissues is only beginning.

3. Rate and sites of mitochondrial oxygen radical generation and longevity

Mitochondria are responsible for the majority of cellular O_2 consumption and are a major source of ROS in healthy cells [44]. Oxidative stress could be related to aging, in principle, through variations in ROS generation, ROS elimination, or both. Antioxidants, however, although possibly involved in protection against various age-related diseases, do not seem to control the rate of aging. This is inferred from four different kinds of studies. First, contrary to early hypotheses, it is known that the levels of endogenous tissue antioxidants do not decrease during aging [7,45]. Second, at first glance, the slow rate of aging of long-lived animals could be due to a constitutively higher endogenous antioxidant defence system. However, the reverse situation was consistently found in the investigations from five independent laboratories that reported such kind of comparative data [12,13], reviewed in [46]. The tissue levels of

antioxidant enzymes and low molecular weight endogenous antioxidants negatively correlate with the MLSP of mammals and other vertebrates. Third, a large number of studies have shown that experimentally increasing tissue antioxidants through dietary supplementation, pharmacological induction, or transgenic techniques sometimes moderately increases mean longevity but does not change MLSP in mammals (see [7,47] for review). In line with this, a recent report found up to 17–21% increases in mean life span and only around a 10% increase in MLSP in mice after a 50 fold increase in mitochondrial catalase [48]. A fourth line of evidence comes from studies in which genes for particular antioxidants are knocked out. These animals can show different pathologies but their rates of aging do not seem to be affected [47,49].

Although antioxidants do not determine the rate of aging, their negative correlation with maximum longevity strongly indicates that the endogenous rate of free radical production in tissues in vivo must be lower in long-lived than in short-lived animals. If long-lived animals had high rates of ROS production together with their very low levels of endogenous antioxidants, their tissue cells would not be able to maintain oxidative stress balance, they would lose homeostasis and would die young instead of being long-lived. For instance, humans have 10 fold less tissue GSH-Px activity than short-lived rodents [50], and this is the main enzyme responsible for decomposing the low micromolar levels of H_2O_2 continuously produced in aerobic tissue cells, because its K_M for H_2O_2 is various orders of magnitude smaller than that of catalase. Decreasing MitROS production instead of increasing antioxidants or repair systems makes sense when considered from the point of view of evolution of longevity among species. It would be very inefficient to generate large amounts of ROS and, afterwards, try to intercept them before they reach macromolecules like DNA, or even worse, try to repair DNA after heavily damaging it. This is even more true taking into account: (a) the high energetic cost of continuously maintaining high levels of antioxidant and repair molecules in the tissues of long-lived species, (b) the capacity of all kinds of animals (short- or long-lived) to transitorily induce these protective molecules when needed in higher amounts, and (c) the close proximity between sources of ROS and macromolecular targets of special importance for aging like mtDNA (mitochondrial DNA). Instead, lowering the rate of ROS generation near its main aging-related target decreases its damage much more efficiently and at much lower cost. Antioxidants will still be important to maintain oxidative stress homeostasis and cell survival at many other cellular sites, but they could not change the rate of aging because they could not strongly decrease final forms of damage to mtDNA (somatic mutations) when it is situated very close to or even in contact with the MitROS generation sites at the inner mitochondrial membrane [12–14].

In agreement with a main role for ROS generation in aging, almost every investigation performed to date has shown that the rate of MitROS production in liver, heart, brain and other tissues is lower in long-lived than in short-lived species [11–14,51–54]; reviewed in [7,8]. This occurs in all kinds of long-lived homeothermic vertebrates independently of their mass-adjusted rates of O_2 consumption, which is low in animals of large body size and high in animals of small size. That characteristic can thus explain why endogenous tissue antioxidants correlate negatively with MLSP across species: long-lived animals have constitutively low levels of antioxidants because they produce ROS at a low rate. The studies performed in birds (very long-lived homeotherms) are especially illustrative since these animals live much longer than mammals of similar body size, even though their metabolic rates are similar to or even higher than those of the similar-sized mammals. In spite of their high rates of O_2 consumption, the three bird species of different families studied to date (parakeets, canaries and pigeons: MLSPs of 21, 24 and 35 years, respectively) have low rates of MitROS generation compared to mice and rats (MLSPs of 3.5 and 4 years) [14,51,53–55]. In many (not all) cases this is possible because the percent of total electron flow in the respiratory chain directed to ROS production (% free radical leak, %FRL) is lower in long- than in short-lived animals (see [7] for review). A role for MitROS production in the control of aging is also supported by current studies in invertebrates [56], fungi [57] and cultivated cells [58].

The site in the respiratory chain where ROS production is lower in long-lived than in short-lived animals has also been studied. Oxygen radical generation at the respiratory chain has been classically attributed to a complex III generator (mainly to complex III ubisemiquinone). However, in agreement with early information obtained in submitochondrial particles [59,60], it was found that complex I also contains an important ROS generator in intact functional heart and brain mitochondria [51,52]. This was soon confirmed by various different laboratories [61–66]. Nowadays the concept that both complex I and complex III produce ROS is widely accepted in the scientific literature and is already part of mainstream basic knowledge [67]. Furthermore, available studies indicate that the respiratory complex responsible for the lower MitROS generation of long-lived species is complex I [51–53]. Concerning the identity of the ROS generator inside complex I there is current debate. Some studies have suggested a role for flavin mononucleotide [63,68], while others favour complex I linked ubisemiquinones [64,66]. Investigations of the laboratory of one of us (GB) [69], soon confirmed by other investigations [61,62], localized the complex I ROS generator between the ferricyanide reduction site and the rotenone binding site. This finding eliminates the flavin from consideration and suggests that the source of ROS may be the complex I FeS clusters situated in that region, although a role for complex I-linked ubisemiquinones cannot be ruled out at present. All these FeS clusters are situated in the hydrophilic complex I domain facing the matrix compartment where mtDNA is located.

4. Oxidative damage to mitochondrial DNA, lipids and proteins and longevity

ROS can damage many different kinds of cellular macromolecules including lipids, proteins and DNA. Damage to DNA may be the most important for aging because it can lead to irreversible loss or alteration of genetic information in

postmitotic cells. mtDNA is situated very close to or even in contact with the site of mitochondrial ROS production. Since long-lived vertebrates have low rates of MitROS generation, this should affect the level of oxidative damage and somatic mutations in their mtDNA. In agreement with this notion it has been found that brain and heart 8-oxodG in mtDNA correlates negatively with maximum longevity in mammals and birds [39]. This agrees with the lower urinary excretion of 8-oxo-7,8-dihydroguanine of long-lived animals [70]. In addition, the levels of 8-oxodG were around 10 fold higher in mtDNA than in nDNA in the brain and heart of all the 11 studied mammalian and bird species studied [39,71], a difference similar to that observed for spontaneous mutations comparing both DNAs. That suggests that the flux rates of both ROS attack on and repair of DNA are much higher in the mtDNA of short-lived than long-lived animals, and are also much higher in the mtDNA than in the nDNA of all species irrespective of their longevity [33]. The higher rate of MitROS production of short-lived animals may be an important cause of their much faster rate of accumulation of mtDNA mutations during aging. A similar degree of accumulation of mtDNA mutations occurs after 70–100 years in humans, after 35–50 years in chimpanzees [72] and after only 2–3 years in mice [73].

Unsaturated fatty acids of cellular membranes are the macromolecules most susceptible to oxidative damage in cells, and this sensitivity increases as a function of their number of double bonds. We have found that the cellular membranes of long-lived mammals and birds have low degrees of fatty acid unsaturation in mitochondria and tissues including liver, heart, skeletal muscle, and kidney, and this constitutively protects their cellular membranes, proteins and DNA against lipid peroxidation-derived damage [74–77]; reviewed in [78,79]. This is shown in Fig. 1A for heart phospholipid fatty acids in the heart of 8 animal species with different MLSP [76]. The total number of double bonds (DBI=double bond index) was negatively correlated with maximum longevity (Fig. 1A) and the same was true for the sensitivity to lipid peroxidation (not shown). Similar results were obtained in liver tissue [77] and liver mitochondria and their phospholipid classes [75,80] in different mammalian species, as well as in birds (longer-lived) compared to mammals of similarly body size. Long-lived animals obtain a low DBI mainly by avoiding highly unsaturated fatty acids like 22:6n-3, and sometimes 20:4n-6, substituting them mainly for 18:3n-3 and 18:2n-6. This lowers their degree of unsaturation without changing the total amount of polyunsaturated fatty acids, allowing a strong decrease in sensitivity to lipid peroxidation probably without major changes in membrane fluidity, a "homeoviscous-longevity adaptation" [78]. On the other hand, lipid peroxidation generates aldehydic products, like malondialdehyde (MDA) and others, that covalently attach to protein lysine residues. Recently we found that the level of MDAL protein adducts negatively correlates with maximum longevity in the heart of different mammalian species (Fig. 1B) [81]. Thus, the longer the life span of a species, the lower is its fatty acid double bond content, its sensitivity to lipid peroxidation and its level of lipoxidation-derived protein modification. Bird (long-lived) species also

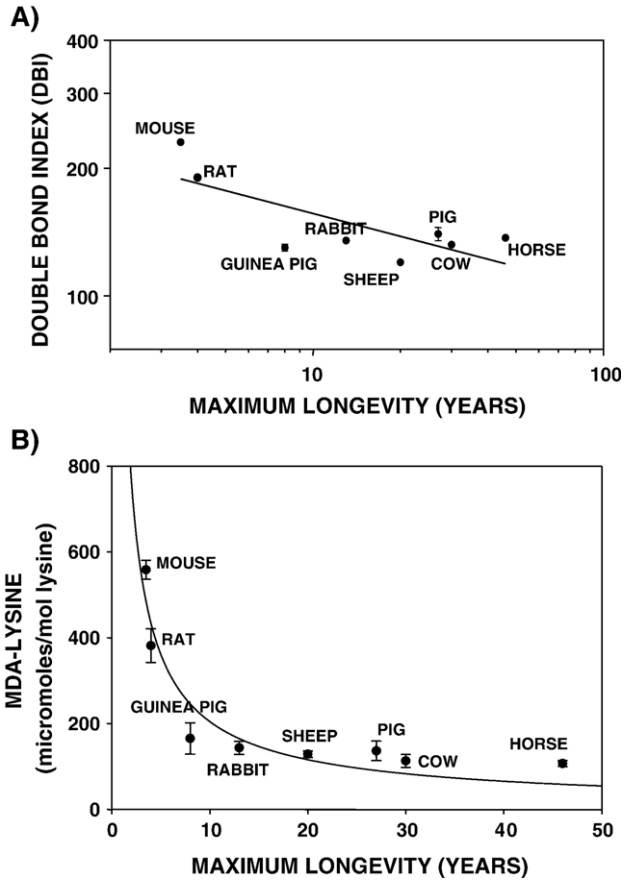

Fig. 1. (A) Correlation between phospholipid membrane fatty acid unsaturation and maximum longevity in the heart of mammals. The total number of fatty acid double bonds (DBI=double bond index) was calculated from the abundance of each fatty acid. The DBI of heart phospholipid fatty acids negatively correlates with MLSP of different mammalian species ($r=-0.78$; $P<0.02$; $n=5–7$ different animals per species except for sheep: $n=3$) (data come from ref. [76]). (B) Correlation between MDA-lysine (MDAL) protein adducts and maximum longevity in the heart of mammals. MDAL adducts in heart proteins negatively correlate with MLSP in mammals ($r=-0.92$; $P<0.001$; $n=7$ different animals per species) (data come from ref. [81]).

show lower fatty acid unsaturation and sensitivity to lipid peroxidation in liver and heart mitochondria (reviewed in [78]), lower levels of aminoadipic and glutamic semialdehydes (specific protein carbonyls) and lower levels of CML, CEL and MDAL in brain proteins than the corresponding comparable mammals [82]. The relationship between fatty acid unsaturation and oxidative damage is also observed in experimental studies in vivo. We have recently observed that treating rats with dietary oils with a low number of double bonds lowers the degree of fatty acid unsaturation of brain cellular membranes and lowers brain lipoxidation-derived protein modification and 8-oxodG levels in brain mtDNA [83]. This makes sense because lowering fatty acid unsaturation decreases the susceptibility of membranes to lipid peroxidation. Lipid peroxidation-derived end-products ("enals") can also react at the exocyclic amino groups of dG, dA, and dC to form various alkylated products [84]. Some common enals that cause DNA damage, analogously to proteins, are MDA, acrolein, and 4-hydroxynonenal, among others. Common adducts arising from enals are exocyclic adducts such

as etheno adducts, and M1dG. These DNA damage markers are mutagenic, carcinogenic, and have powerful effects on signal transduction pathways. Furthermore, they (a) are present in the genome of healthy humans and other animal species; (b) are efficient premutagenic lesions that induce mutations frequently detected in oncogenes or tumor suppressor genes from human tumors; (c) show increased levels in aged animals; and (d) increase nearly 20 fold with a high PUFA diet. Thus, lipid peroxidation may be a significant endogenous source of DNA damage and mutations.

5. Caloric restriction, mitochondrial ROS generation, and oxidative stress

The results of many studies concerning the mitochondrial free radical theory of aging can explain in part the wide variation in MLSP among different animal species. However, correlation with MLSP (in the appropriate direction) is necessary but not sufficient to validate a theory of aging, because correlation does not necessarily means that a cause–effect relationship exists. Thus, experimental studies in which the rate of aging is modified are needed to clarify if MitROS production and oxidative stress also change in the expected direction. Caloric restriction (CR) is the best known experimental manipulation that decreases the rate of aging and increases MLSP in many species including laboratory rodents, and it has many beneficial effects for health in mice, rats, other animals, and possibly primates including humans [85], although demographic studies indicate that dietary restriction in *Drosophila* only decreases short-term mortality [86]. The benefits induced by CR in rodents include improvements in mitochondrial oxidative stress [87,88] and increases in longevity even in long-lived mutant dwarf mice [89]. There is now highly consistent evidence that MitROS production and oxidative damage to mitDNA and other intracellular macromolecules are lowered by this antiaging manipulation in mammals, although a study in *Drosophila* did not found changes in MitROS generation after dietary restriction [90]. The effect of CR on MitROS production was first investigated in mice [91]. More recently, it has been intensively studied in detail in many rat tissues [92–99]. These investigations, usually applying 40% CR, consistently demonstrate that long-term CR significantly decreases the rate of MitROS generation in rat tissues including skeletal muscle, kidney, liver, heart and brain (Table 1), whereas after short-term CR (a few weeks or even 4 months), those decreases can only sometimes be detected (see [100] for review). It was found that 6–7 weeks of restriction are enough to decrease MitROS production and 8-oxodG in mtDNA and nDNA in rat liver [92], which is very useful for further experimental studies concerning CR and mitochondrial oxidative stress. It was also found that the decrease in ROS generation in CR rats specifically occurs at complex I in all the organs studied up to date (heart, liver and brain), and that it occurs together with a decrease in % FRL, indicating that the efficiency of the mitochondrial respiratory chain in avoiding ROS production is increased in CR animals [92–94,98]. These changes are strikingly similar to those described above when comparing animals with different longevities, suggesting that they can be a highly conserved mechanism of life span extension both within and between species. Interestingly, the decrease in MitROS generation observed in CR rats is accompanied by significant decreases in 8-oxodG levels in mtDNA alone, or in mtDNA and nDNA, depending on the tissue studied [92–95,98]; reviewed in [100], as well as by decreases in oxidation- (glutamic semialdehyde, a protein carbonyl), glycoxidation- (CML and CEL), and lipoxidation-derived (CML and MDAL) damage to heart mitochondrial proteins [17,101], although in some cases (e.g., in rat liver) only small changes in protein markers were observed [102]. These findings led to suggest that lowering the rate of ROS production is a common mechanism used by both long-lived and CR animals to decrease steady-state oxidative damage to lipids, proteins, and especially to mtDNA, and thereby mtDNA mutations and aging rate [7,8,39,93,100]. That increasing mtDNA mutation increases aging rate has been recently shown in mtDNA polymerase γ mutant mice [42].

Table 1
Summary of investigations about the effect of long-term caloric restriction on the mitochondrial rate of ROS generation

Tissue	Species	CR effect on MitROS generation	Reference
Heart	Rat, Mouse	↓	[91,93]
Brain	Rat, Mouse	↓	[91,98]
Liver	Rat	↓	[94]
Skeletal Muscle	Mouse, Rat	↓	[95,99]
Kidney	Mouse	↓	[91]

The time period of caloric restriction was at least 1 year in all cases.

6. Mechanism of caloric restriction effect on mitochondrial oxidative stress: the protein and methionine connection

Although numerous studies have documented the decrease in MitROS production and oxidative damage to mitochondrial macromolecules in CR, the dietary factor that causes these beneficial changes is unknown. We are presently undertaking investigations aimed to clarify this subject. Clarifying what factors cause the decrease in MitROS production and oxidative stress and the increase in MLSP in CR is a promising way to uncover some of the fundamental mechanisms of aging in mammals. In addition, such kinds of studies can suggest interventions that are more easily practicable for human beings than caloric restriction. CR is a difficult manipulation for human populations due to: (a) the strong difficulty of modifying acquired nutritional habits in humans; (b) the high risk of malnutrition; and (c) possible decreases in acute resistance to the normally stressful human living conditions, the three reasons being even more relevant problems in the case of children and old individuals.

It is commonly believed that the antiaging effect of CR is due to the decreased intake of calories themselves rather than to decreases in specific dietary components. However, some recent findings question this classical consensus [103] and it is known that variations in the proportions of some of the main

Table 2
Effects of protein restriction (PR) on maximum longevity

PR	Strain	(*N*/group)	Maximum life span	Reference
85%PR	mice (C57BL/6J)	140	38.9%↑	[107]
83%PR	mice (C57; A/J; F1)	50	20%↑; 6.8%↑; 24%↑	[108]
66%; 83%PR	mice (BALB/c)	200	6.5%↑; 6.5%↑	[109]
72%PR	mice (DBA/2f; B/W)	–	16%↑; 44%↑; 33%↑	[110]
50%PR	rat Wistar	44	25%↑	[111]
40%PR	rat Fisher 344	40	3%↑	[112]
40%PR	rat Fisher 344	60	5%↑	[113]
40%; 57%PR	rat Wistar	36	38.7%↓; 6.1%↓	[114]
PR (self determined)	rat Ch. River COBS	121	↑(after 7 months age)	[115]
50%PR	rat	10	11%↑	[116]
63%; 73%PR	rat Sprague–Dawley	210	43%↑; 11%↑	[117]
Mean increase of 15 studies			19.6% increase in MLSP	

dietary components can also affect longevity [104,105], reviewed in [106]. Concerning the effects of protein restriction (PR) on aging rate, reconsideration of classic studies performed in rodents shows that increases in MLSP have been found in almost every investigation. Ten out of eleven published studies (and 16 out of 18 different life-long survival experiments in these studies) in rats or mice found that protein restriction increases maximum life span (Table 2) [107–117], although the magnitude of this increase (ca. 20% increase in MLSP taking into account all the 16 positive studies) was usually lower than that typically found in CR (ca., 40%). This suggests that protein restriction can be responsible for around 50% of the life-prolonging effects of CR. Recent studies in *Drosophila* also indicate that the life-extension effect of calorie restriction is not due to calories themselves [103], while they are compatible with a specific role of decreased protein intake in life-extension. This possibility would be also consistent with the well-described fact that 80% methionine restriction (MetR) increases maximum longevity in rats independently of energy restriction [118–122] although the molecular mechanisms responsible are unknown.

Concerning oxidative stress, changes in liver and blood GSH have been studied in MetR [119], but the possible role of MitROS production and macromolecular damage has never been investigated. The increase in MLSP in Fisher 344 rats was observed in at least two independent studies [118,119,121], and there is preliminary evidence [120] indicating that MetR also extends lifespan in at least three other different rat strains (Brown Norway, Sprague–Dawley and Wistar rats). A MetR-induced increase in MLSP in mice was also recently observed in BALB cJ×C57BL/6 mice [122]. These life span extensions were accompanied by lower liver GSH and probably increased hepatic GSH export to blood, causing higher blood GSH, increases in resistance to oxidatively-induced liver injury, slower cataract development, smaller age-related changes in T cell subsets (CD8, CD8M, CD4P and CD8P), higher levels of MIF (macrophage migration inhibition factor, known to be higher in other rodent models of extended longevity), and lower serum glucose, insulin, IGF-I and T_4 levels [118,119,121,122]. Interestingly, the magnitude of a putative 40% MetR-induced increase in MLSP (as calculated from that found in 80% CR) is likely similar (22% increase) to that observed in 40% protein restriction (ca., 20% increase in MLSP). Furthermore, CR and protein restriction (PR) share many common effects in addition to life prolongation, including delays in puberty, decreases in growth, transitory decreases in metabolic rate, boosting of cell-mediated immunity, or decreases in preneoplastic lesions and tumors [123]. Low protein diets, like CR, also decelerate glomerulosclerosis in mice [124], delay the occurrence of chronic nephropathy and cardiomyopathy in rats [125], and protect the rat liver against exposure to toxic chemicals [126]. A lower but significant life extension effect in PR than in CR would also agree with the widely accepted notion that aging has more than one single main cause. CR could decrease aging rate through PR-induced decreases in MitROS production and oxidative stress, as well as through other unknown mechanisms possibly induced by the calories themselves or by other dietary components. It was recently found that 6–7 weeks of 40% PR without strong restriction of calories (Table 3) decreases MitROS production specifically at complex I, lowers the % FRL and decreases 8-oxodG in mtDNA in rat liver mitochondria [106], and decreases specific markers of protein oxidative modification in rat liver mitochondria and hepatic tissue

Table 3
Summary of effects of caloric (CR), protein (PR) and methionine restriction (MetR) on oxidative stress and maximum longevity (MLSP)

	MitROSp	MitVO2	FRL(%)	ox.mtDNA	ox.Proteins	MLSP
CR (40%)	↓ (cx I)	=	↓	↓	↓	↑ (40%)
PR (40%)	↓ (cx I)	=	↓	↓	↓	↑ (20%)
MetR (80%)	↓ (cx I and III)	↑	↓	↓	↓	↑ (40%)

MitROSp=mitochondrial ROS production; MitVO2=mitochondrial oxygen consumption; FRL(%)=% free radical leak at the respiratory chain; ox.mtDNA=oxidative DNA damage to mtDNA (8-oxodG); ox.Proteins=oxidative damage to proteins (specific protein carbonyls, CML, CEL and MDAL). Complex I (cxI) and III (cxIII) are the sites were ROS production decreases in each intervention.

(unpublished results). The decreases in MitROS generation, % FRL, 8-oxodG and protein damage also occur in rat liver mitochondria after 6–7 weeks of 40% CR [92,97]; reviewed in [100]. Strikingly, the magnitude, kind of changes, mechanisms and site of action of those decreases are similar in both PR and CR, while lipid restriction without CR does not cause such decreases [127]. This suggests that restriction of protein intake can be responsible for the well-known decreases in MitROS production and oxidative stress that take place in CR and thus for part of the increase in MLSP. In agreement with this possibility, it is known that progressively decreasing the level of dietary protein in mice, from 24% to 12% and from 12% to 6%, decreases liver lipid peroxidation, lowers free activities of lysosomal enzymes in brain, liver and intestine, and decreases lipofuscin accumulation [128]. Concerning possible alternative mechanisms of life extension in CR, although available studies are still scarce and open to debate [129], it has been found that the decrease in cardiac MitROS production with pyruvate/malate as substrates induced by CR in Wistar rats is not reversed by either insulin or GH, and that these two hormones do not increase MitROS generation in liver mitochondria from CR rats [130]. These last results do not support the idea that the lowering of MitROS generation in CR could be due to a lowered insulin/IGF-1 like signalling. On the other hand, we have recently found that 6–7 weeks of methionine restriction without caloric restriction also decreases MitROS generation, % FRL, 8-oxodG in mtDNA, and specific markers of protein oxidative modification, in rat heart and liver mitochondria (Table 3). This, together with the well-known fact that MetR increases MLSP in rats and mice [118–122], and with the CR and PR-induced decreases in MitROS generation, 8-oxodG in mtDNA, and mitochondrial protein damage, strongly suggests that restriction of methionine intake can be responsible for the decreases in MitROS production and oxidative stress that take place in CR and thus for part of the increase in MLSP induced by this dietary manipulation.

Interestingly, in the classic studies showing that MetR without CR increases rat MLSP it was found that MetR changes hepatic levels of thiol-containing compounds like GSH as early (at least) as at 8 weeks of MetR [119], a time window similar to the one at which we observe decreases in MitROS generation (6–7 weeks). The study of the MetR effect on ROS production was performed using exactly the same dietary protocol that increases maximum longevity in rats [119,121]: 80% methionine restriction compensated by corresponding increases in dietary L-glutamate. These results strongly suggest that MetR may be the common factor responsible for the lowering of MitROS production and oxidative stress both in PR and CR. Further experiments are needed to clarify: (a) if these changes also occur at 40% MetR; (b) if they take place when other dietary components are not modified.

Various lines of evidence also suggest that methionine or its related metabolites may be involved in aging and longevity. Interestingly, we have recently found that methionine is the only amino acid present in heart intracellular proteins that strongly correlates with mammalian MLSP, and that this correlation is negative [81] (Fig. 2). Moreover, protein methionine content is also lower in tissues of long-lived birds than in short-lived mammals of similar body size [82,131]. Thus, the longer the life span of a species, the lower is its tissue protein methionine content. Many other recent investigations also point to a relationship between methionine and aging [132–135]. In addition, excessive methionine dietary supplementation damages many vital organ systems and increases tissue oxidative stress. Thus, methionine supplementation increases plasma hydroperoxides and LDL-cholesterol [136], raises iron and lipid peroxidation, conjugated dienes, and cholesterol in rat liver [137,138], is hepatotoxic and alters liver antioxidants like SOD, catalase, GSH-peroxidase and GSH in rats [137,139,140], increases liver oxidative stress [140], raises plasma, heart and aortic homocysteine levels leading to angiotoxicity and mitochondrial degeneration in arterial smooth muscle cells [136,140,141] and accelerated aging of rat vascular system [142], induces hypertension and coronary disease [140,143], decreases vitamin E levels in liver and heart [136], and possibly speeds up brain aging [139]. The high methionine content of the Western diet may also predispose human beings to cardiovascular disease [139]. Interestingly, similar negative effects of methionine supplementation have been described in rats fed high protein (50%) and high methionine (2%) diets for 2 years [142]. High protein diets (50% protein for 1 week), and casein-rich compared to soybean-rich diets, increase plasma protein carbonyls and are cholesterolemic and atherogenic in rats [136,144], which is interesting because casein has higher methionine content than soybean protein, and because protein oxidation seems to play a role in atherosclerosis and other degenerative diseases [145].

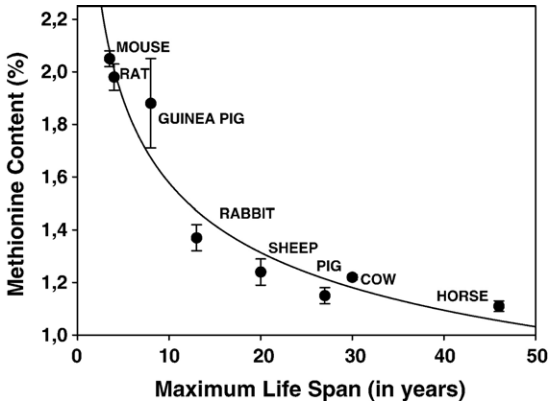

Fig. 2. Correlation between methionine content of heart proteins and maximum longevity in mammals. Methionine protein content negatively correlates with maximum longevity in the heart of eight mammalian species strongly differing in MLSP ($r=-0.96$; $P<0.001$; $n=7$ different animals per species).

Methionine could induce damage through various possible mechanisms. Methionine residues of proteins are among the amino acids most susceptible to oxidation by ROS [132,146] and the sensitivity of proteins to oxidative stress increases as a function of their number of methionine residues [135]. Oxidation of methionine residues generates methionine sulfoxide in proteins, which deprives them of their function as methyl donors, and may lead to loss of their biological activity [147,148]. This modification can be repaired by methionine sulfoxide reductase in a thioredoxin-dependent reaction. In this

context, it is very illustrative that knocking out methionine sulfoxide reductase-A lowers MLSP and increases protein carbonyls and sensitivity to hyperoxia in mice [132]. The opposite manipulation, overexpression of methionine sulfoxide reductase A, increases life span and delays the aging process in *Drosophila* [133]. On the other hand, the oxidized form of thioredoxin, produced during the reduction of methionine sulfoxide, can be converted back to reduced thioredoxin by the enzyme thioredoxin reductase. In agreement with the methionine oxidation hypothesis of aging, it has been reported that overexpression of thioredoxin reductase increases longevity in mice [149,150].

A high methionine dietary content could also be detrimental due to its conversion to homocysteine. It has been proposed that the increase in plasma homocysteine induced by methionine supplementation increases ROS production, and this leads to LDL oxidation and atherosclerosis [136]. Homocysteine levels increase with age in humans and represent a risk factor for aging and free radical-associated degenerative diseases including at least atherosclerosis, cognitive decline, thrombosis, cancer, stroke, wasting, chronic kidney disease and Parkinson's disease [151–154]. Homocysteine has a free thiol group that can be readily oxidized leading to the generation of protein mixed disulfides or disulfide bridges between different proteins or between subunits of the same protein. It has been observed that addition of oxidized glutathione (GSSG) to mitochondrial complex I increases its rate of superoxide radical generation [155]. Thus, MitROS production can be regulated by thiol agents including homocysteine, offering a plausible molecular mechanism for the effects of MetR and supplementation on mitochondrial oxidative stress, tissue damage and longevity. That effect of GSSG can possibly explain the direct correlation found between GSSG/GSH ratio and 8-oxodG in mtDNA in tissues of aging mice [28]. Similarly, recent studies suggest that treating rat heart mitochondria with homocysteine in vitro increases MitROS production (estimated from luminol enhanced chemiluminescence) [156], although these experiments should be repeated using more specific techniques before firm conclusions can be drawn. In any case, there is a strong need for further experimental work about the lowering effects of methionine restriction on mitochondrial oxidative stress and on the mechanisms by which MetR leads to slower aging.

7. Conclusions

Most of the available data indicate that 40% caloric restriction decreases the rate of generation of mitochondrial reactive oxygen species and lowers oxidative damage to mitochondrial DNA and proteins in rodent organs [100]. Long-lived species also have lower levels of these parameters than short-lived ones, suggesting the existence of at least one common mechanism of longevity extension in both cases [7]. However, it was not known what specific dietary components, if any, mechanistically cause those decreases during CR. It is known that 40% protein restriction (PR) increases maximum longevity (Table 2) and that this increase is smaller (around 20%) than that observed in 40% CR (around 40% increase). In the case of methionine restriction (MetR), a 44% increase in MLSP has been observed [119], but this increase was found after restricting methionine by 80%, not by 40%. Thus, it is

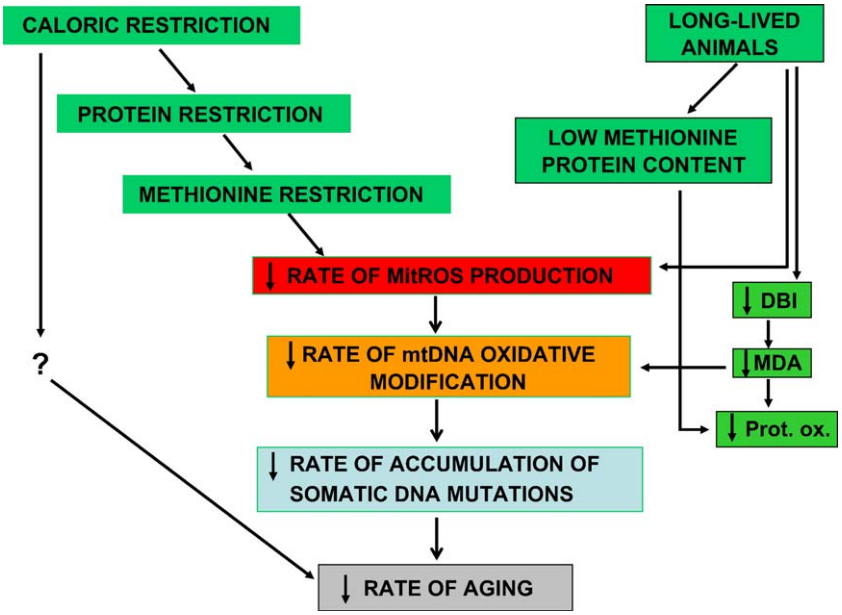

Fig. 3. Model of likely cause–effect relationships concerning the mitochondrial free radical theory of aging. This model further delineates recent views (8) on the mechanistic connection between CR, MitROS production and oxidative stress, and aging, taking into account two relevant recent findings: (a) the methionine content of tissue proteins negatively correlates with maximum longevity in mammals and birds; (b) 80% methionine restriction, like 40% CR and 40% PR, significantly decreases the rate of ROS generation at complex I and mtDNA 8-oxodG in rat heart and liver mitochondria. It is known that CR, PR and MetR significantly increase maximum longevity in rats (Tables 2 and 3). The low MitROS production of CR and PR animals seems to be due to their low methionine ingestion. Other unknown mechanisms different from MitROS production can contribute to decrease aging rate in CR. DBI (double bond index) indicates membrane fatty acid unsaturation. MDA = malondialdehyde. "Prot.ox." represents various markers of protein oxidative modification.

most likely that 40% MetR increases MLSP to a smaller extent, similar to the one observed in PR (around 20% increase in MLSP). Therefore, the decrease in methionine intake could be responsible for all or most of the increase in MLSP observed in PR as well as for around 50% of the increase in MLSP observed in CR. On the other hand, it was recently found that 40% protein restriction without CR decreases MitROS generation and oxidative damage to mtDNA (8-oxodG), PR totally mimicking CR quantitatively and qualitatively in this respect [106], whereas lipid restriction does not change those parameters [127]. Furthermore, it was recently found that MetR also decreases ROS generation in rat liver and heart mitochondria. All these suggest that the reduction in methionine intake during CR and PR can be the cause of the decrease in MitROS production and oxidative damage to mtDNA, and can be responsible for around 50% of the increase in MLSP observed in caloric restriction (Fig. 3). Moreover, it was found that methionine is the only amino acid whose abundance in tissues strongly (and negatively) correlates with MLSP: the longer the life span, the smaller the level of methionine in intracellular proteins. Many other kinds of studies also connect methionine and its metabolites, including homocysteine, to aging.

Finally, it is commonly considered that maintenance of the organism is the key for a high longevity. But what are the fundamental mechanisms of maintenance in long-lived species? Concerning oxidative stress, available evidence points to two general mechanisms: (1) decreasing the rate of generation of endogenous damage (e.g., the rate of MitROS production) and (2) possession of macromolecules less sensitive to oxidative damage; this is obtained in the case of lipids by decreasing the degree of fatty acid unsaturation, and in the proteins at least by decreasing the number of methionine residues. Of these two general longevity mechanisms, caloric restriction uses the first one, decreasing the rate of MitROS generation. This seems to be due to a great extent to the decrease in methionine intake of the caloric restricted animals.

Acknowledgements

The results from the authors described in this review have been supported by grant nos. SAF2002-01635 and BFU2005-02584 from the Spanish Ministry of Science and Education to G. Barja, and from the Spanish Ministry of Science and Technology (BFI2003-01287) and the Generalitat of Catalunya (Departament d'Universitats, Recerca, i Societat de la Informació, DURSI) to R.Pamplona.

References

[1] B.L. Strehler, Time, Cells and Aging, Academic Press, New York, 1962, p. 456.
[2] D. Harman, Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11 (1956) 298–300.
[3] D. Harman, The biological clock: the mitochondria? J. Am. Geriatr. Soc. 20 (1972) 145–147.
[4] B.P. Yu, J.J. Chen, C.M. Kang, M. Choe, Y.S. Maeng, B.J. Kristal, Mitochondrial aging and lipoperoxidative products, Ann. N. Y. Acad. Sci. 786 (1996) 44–56.
[5] R. Weindruch, R.S. Sohal, Caloric intake and aging, N. Engl. J. Med. 337 (1997) 986–994.
[6] K.B. Beckman, B. Ames, The free radical theory of aging matures, Physiol. Reviews 78 (1998) 547–581.
[7] G. Barja, Aging in vertebrates and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? Biol. Rev. 79 (2004) 235–251.
[8] G. Barja, Free radicals and aging, Trends Neurosci. 27 (2004) 595–600.
[9] E.R. Stadtman, Role of oxidant species in aging, Curr. Med. Chem. 11 (2004) 1105–1112.
[10] R.S. Sohal, I. Svensson, U.T. Brunk, Hydrogen peroxide production by liver mitochondria in different species, Mech. Ageing Dev. 53 (1990) 209–215.
[11] H.H. Ku, U.T. Brunk, R.S. Sohal, Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species, Free Radic. Biol. Med. 15 (1993) 621–627.
[12] M. López-Torres, R. Pérez-Campo, C. Rojas, S. Cadenas, G. Barja, Maximum life span in vertebrates: correlation with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H2O2, and basal and maximum aerobic capacity, Mech. Ageing Dev. 70 (1993) 177–199.
[13] G. Barja, S. Cadenas, C. Rojas, M. López-Torres, R. Pérez-Campo, A decrease of free radical production near critical targets as a cause of maximum longevity, Comp. Biochem. Physiol. 108B (1994) 501–512.
[14] G. Barja, S. Cadenas, C. Rojas, R. Pérez-Campo, M. López-Torres, Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds, Free Radic. Res. 21 (1994) 317–328.
[15] J.M. Carney, P.E. Starke-Reed, C.N. Oliver, R.W. Landm, M.S. Cheng, J.F. Wu, R.A. Floyd, Reversal of age-related increase in brain protein oxidation, decrease in enzymatic activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-*tert*-butyl-alpha-phenylnitrone, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 3633–3636.
[16] C. Leeuwenburgh, P. Wagner, J.O. Holloszy, R.S. Sohal, J.W. Heinecke, Caloric restriction attenuates dityrosine cross-linking of cardiac and skeletal muscle proteins in aging mice, Arch. Biochem. Biophys. 346 (1997) 74–80.
[17] R. Pamplona, M. Portero-Otín, J.M. Bellmunt, R. Gredilla, G. Barja, Aging increases $N^{epsilon}$-(Carboxymethyl)lysine and caloric restriction decreases $N^{epsilon}$-(Carboxyethyl)lysine and $N^{epsilon}$-(Malondialdehyde) lysine in rat heart mitochondrial proteins, Free Radic. Res. 36 (2002) 47–54.
[18] S. Tahara, M. Matsuo, T. Kaneko, Age-related changes in oxidative damage to lipids and DNA in rat skin, Mech. Ageing Dev. 122 (2001) 415–426.
[19] T. Miyazawa, T. Suzuki, K. Fujimoto, M. Kinoshita, Age-related change of phosphatidylcholine hydroperoxide and phosphatidylethanolamine hydroperoxide levels in normal human red blood cells, Mech. Ageing Dev. 86 (1996) 145–150.
[20] M. Kinoshita, S. Oikawa, K. Hayasaka, A. Sekikawa, T. Nagashima, K. Toyota, T. Miyazawa, Age-related increases in plasma phosphatidylcholine hydroperoxide concentrations in control subjects and patients with hyperlipidaemia, Clin. Chem. 46 (2000) 822–828.
[21] S.M.K. Davies, A. Poljak, M.W. Duncan, G.A. Smythe, M.P. Murphy, Measurements of protein carbonyls, ortho- and meta-tyrosine and oxidative phosphorylation complex activity in mitochondria from young and old rats, Free Radic. Biol. Med. 31 (2001) 181–190.
[22] J.L. Quiles, J.J. Ochoa, J.R. Huertas, J. Mataix, Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet, Exp. Gerontol. 39 (2004) 189–194.
[23] J.L. Quiles, J.J. Ochoa, C. Ramirez-Tortosa, M. Battino, J.R. Huertas, Y. Martín, J. Mataix, Dietary fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats, Exp. Gerontol. 39 (2004) 1189–1198.
[24] M.C. Ramirez-Tortosa, J. García-Alonso, M.L. Vidal-Guevara, J.L. Quiles, M.J. Periago, J. Linde, M.D. Mesa, G. Ros, P. Abellá, A. Gil,

Oxidative stress status in an institutionalised elderly group after the intake of a phenolic-rich dessert, Br. J. Nutr. 91 (2004) 943–950.
[25] C.G. Fraga, M.K. Shigenaga, J.W. Park, P. Degan, B.N. Ames, Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine, Proc. Natl. Acad. Sci. U.S. A. 87 (1990) 4533–4537.
[26] P. Mecocci, U. MacGarvey, A.E. Kaufman, D. Koontz, J.M. Shoffner, D.C. Wallace, F. Beal, Oxidative damage to mitochondrial DNA shows age-dependent increases in human brain, Ann. Neurol. 34 (1993) 609–616.
[27] R.S. Sohal, S. Agarwal, M. Candas, M.J. Forster, H. Lal, Effect of age on DNA oxidative damage in different tissues of C57BL/6 mice, Mech. Ageing Dev. 76 (1994) 215–224.
[28] J.G. Asunción, A. Millan, R. Pla, L. Bruseghini, A. Esteras, F.V. Pallardo, J. Sastre, J. Viña, Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA, FASEB J. 10 (1996) 333–338.
[29] A. Herrero, G. Barja, Effect of aging on mitochondrial and nuclear DNA oxidative damage in the heart and brain throughout the life-span of the rat, J. Am. Aging Assoc. 24 (2001) 45–50.
[30] M. Hamilton, H. Van Remmen, J.A. Drake, H. Yang, Z.M. Guo, K. Kewitt, C.A. Walter, A. Richardson, Does oxidative damage to DNA increase with age? Proc. Natl. Acad. Sci. 98 (2001) 10469–10474.
[31] K.R. Short, M.L. Bigelow, J. Kahl, R. Singh, J. Coenen-Schimke, S. Raghavakaimal, K.S. Nair, Decline in skeletal muscle mitochondrial function with aging in humans, Proc. Natl. Acad. Sci. 102 (2005) 5618–5623.
[32] T. Hirano, R. Yamaguchi, S. Asami, N. Iwamoto, H. Kasai, 8-hydroxyguanine levels in nuclear DNA and its repair in rat organs associated with age, J. Gerontol. 51A (1996) B303–B307.
[33] G. Barja, The flux of free radical attack through mitochondrial DNA is related to aging rate, Aging Clin. Exp. Res. 12 (2000) 342–355.
[34] S. Melov, D. Hinerfeld, L. Esposito, D.C. Wallace, Multi-organ characterization of mitochondrial genomic rearrangements in ad libitum and caloric restricted mice show striking somatic mitochondrial DNA rearrangements with age, Nucleic Acids Res. 25 (1997) 974–982.
[35] K. Khrapko, K. Ebralidse, Y. Kraytsberg, Where and when do somatic mtDNA mutations occur? Ann. N.Y. Acad. Sci. 1019 (2004) 240–244.
[36] T. Ozawa, Mitochondrial gene mutation in cell death and aging, J. Bioenerg. Biomembr. 31 (1999) 377–390.
[37] A.D.N.J. de Grey, The reductive hot spot hypothesis: an update, Arch. Biochem. Biophys. 373 (2000) 295–301.
[38] C. Richter, J.W. Park, B.N. Ames, Normal oxidative damage to mitochondrial and nuclear DNA is extensive, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 6465–6467.
[39] G. Barja, A. Herrero, Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals, FASEB J. 14 (2000) 312–318.
[40] J.L. Blanchard, M. Lynch, Trends Genet. 16 (2000) 315–320.
[41] V.A. Bohr, Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells, Free Radic. Biol. Med. 32 (2002) 804–812.
[42] A. Trifunovic, A. Wredenberg, M. Falkenberg, J.N. Spelbrink, A.T. Rovio, C.E. Bruder, Y.M. Bohlooly, S. Gidlof, A. Oldfors, R. Wiborn, J. Tornell, H.T. Jacobs, N.G. Larsson, Premature aging in mice expressing defective mitochondrial DNA polymerase, Nature 429 (2004) 417–423.
[43] S.R. Thorpe, J.W. Baynes, Maillard reaction products in tissue proteins: new products and new perspectives, Amino Acids 25 (2003) 275–281.
[44] A. Boveris, E. Cadenas, Mitochondrial production of hydrogen peroxide regulation by nitric oxide and the role of ubisemiquinone, IUBMB Life 50 (2000) 245–250.
[45] G. Benzi, A. Moretti, Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system, Free Radic. Biol. Med. 19 (1995) 77–101.
[46] R. Pérez-Campo, M. López-Torres, S. Cadenas, C. Rojas, G. Barja, The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach, J. Comp. Physiol., B 168 (1998) 149–158.
[47] A. Sanz, R. Pamplona, G. Barja, Is the mitochondrial free radical theory of aging intact? Antioxid. Redox Signal. (in press).
[48] S.E. Schriner, N.L. Linford, G.M. Martin, P. Treuting, C.E. Ogburn, M. Emond, P.E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D.C. Wallace, P.S. Rabinovitch, Extension of murine life span by over-expression of catalase targeted to mitochondria, Science 308 (2005) 1909–1911.
[49] F.L. Muller, J. Mele, V. Van Remmen, A. Richardson, Proving the in vivo relevance of oxidative stress in aging using knockout and transgenic mice, in: T. Von Zglinicki (Ed.), Aging at Molecular Level, Kluwer, Boston, 2003, pp. 131–144.
[50] R. Pérez-Campo, M. López-Torres, C. Rojas, S. Cadenas, G. Barja, Longevity and antioxidant enzymes, non-enzymatic antioxidants, and oxidative stress levels in the vertebrate lung: a comparative study, J. Comp. Physiol. 163 (1994) 682–689.
[51] G. Barja, A. Herrero, Localization at complex I and mechanism of the higher free radical production of brain non-synaptic mitochondria in the short-lived rat than in the longevous pigeon, J. Bioenerg. Biomembr. 30 (1998) 235–243.
[52] A. Herrero, G. Barja, Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon, Mech. Ageing Dev. 98 (1997) 95–111.
[53] A. Herrero, G. Barja, H_2O_2 production of heart mitochondria and aging rate are slower in canaries and parakeets than in mice: sites of free radical generation and mechanisms involved, Mech. Ageing Dev. 103 (1998) 133–146.
[54] H.H. Ku, R.S. Sohal, Comparison of mitochondrial pro-oxidant generation and antioxidant defenses between rat and pigeon: possible basis of variation in longevity and metabolic potential, Mech. Ageing Dev. 72 (1993) 67–76.
[55] J. St Pierre, J.A. Buckingham, S.J. Roebuck, M.D. Brand, Topology of superoxide production from different sites in the respiratory chain, J. Biol. Chem. 277 (2002) 44784–44904.
[56] S. Hekimi, L. Guarente, Genetics and the specificity of the aging process, Science 299 (2003) 1351–1354.
[57] F. Krause, C.Q. Scheckhuber, A. Werner, S. Rexroth, N.H. Reifschneider, N.A. Dencher, H.D. Osiewacz, Supramolecular organization of cytochrome c oxidase- and alternative oxidase-dependent respiratory chains in the filamentous fungus Podospora anserine, J. Biol. Chem. 279 (2004) 26453–26461.
[58] Y. Stroikin, H. Dalen, U.T. Brunk, A. Terman, Testing the "garbage" accumulation theory of ageing: mitotic activity protects cells from death induced by inhibition of autophagy, Biogerontology 6 (2005) 39–47.
[59] K. Takeshige, S. Minakami, NADH- and NADPH-dependent formation of superoxide anions by bovine heart submitochondrial particles and NAD-ubiquinone reductase preparation, Biochem. J. 180 (1979) 129–135.
[60] J.F. Turrens, A. Boveris, Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria, Biochem. J. 191 (1980) 421–427.
[61] M.L. Genova, B. Ventura, G. Giulano, C. Bovina, G. Formiggini, G. Parenti Castelli, G. Lenaz, The site of production of superoxide radical in mitochondrial Complex I is not a bound ubisemiquinone but presumably iron–sulphur cluster N2, FEBS Lett. 505 (2001) 364–368.
[62] Y. Kushnareva, A.N. Murphy, A. Andreyev, Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state, Biochem. J. 368 (2002) 545–553.
[63] A.P. Kudin, G. Debska-Vielhaber, W.S. Kunz, Characterization of superoxide-producing sites in isolated brain mitochondria, J. Biol. Chem. 279 (2004) 4127–4135.
[64] A.J. Lambert, M.D. Brand, Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Biol. Chem. 279 (2004) 39414–39420.
[65] A. Galkin, U. Brandt, Superoxide radical formation by pure complex I (NADH: ubiquinone oxidoreductase) from Yarrowia lipolyitica, J. Biol. Chem. 280 (2005) 30129–30135.
[66] T. Ohnishi, J.E. Johnson Jr., T. Yano, R. Lobrutto, W.R. Widger, Thermodynamic and EPR studies of slowly relaxing ubisemiquinone

species in the isolated bovine heart complex I, FEBS Lett. 579 (2005) 500–506.
[67] A.L. Lehninger, Principles of Biochemistry, 4th ed., Freeman and Co, New York, 2005, pp. 721–722.
[68] Y. Liu, G. Fiskum, D. Schubert, Generation of reactive oxygen species by the mitochondrial electron transport chain, J. Neurochem. 80 (2002) 780–787.
[69] A. Herrero, G. Barja, Localization of the site of oxygen radical generation inside the Complex I of heart and nonsynaptic brain mammalian mitochondria, J. Bioenerg. Biomembr. 32 (2000) 609–615.
[70] M. Foksinski, R. Rozalski, J. Guz, B. Ruszowska, P. Sztukowska, M. Ptwowarski, A. Klungland, R. Olinski, Urinary excretion of DNA repair products correlates with metabolic rates as well as with maximum life pans of different mammalian species, Free Radic. Biol. Med. 37 (2004) 1449–1454.
[71] A. Herrero, G. Barja, 8-oxodeoxyguanosine levels in heart and brain mitochondrial and nuclear DNA of two mammals and three birds in relation to their different rates of aging, Aging Clin. Exp. Res. 11 (1999) 294–300.
[72] S. Melov, P.E. Coskun, D.C. Wallace, Mouse models of mitochondrial disease, oxidative stress, and senescence, Mutat. Res. 434 (1999) 233–242.
[73] E. Wang, A. Wonq, G. Cortopassi, The rate of mitochondrial mutagenesis is faster in mice than in humans, Mutat. Res. 377 (1997) 157–166.
[74] R. Pamplona, J. Prat, S. Cadenas, C. Rojas, R. Pérez-Campo, M. López-Torres, G. Barja, Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from longevous species: the pigeon and human case, Mech. Ageing Dev. 86 (1996) 53–66.
[75] R. Pamplona, M. Portero-Otín, C. Ruiz, J. Prat, M.J. Bellmunt, G. Barja, Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals, J. Lipid Res. 39 (1998) 1989–1994.
[76] R. Pamplona, M. Portero-Otín, C. Ruiz, R. Gredilla, A. Herrero, G. Barja, Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals, Mech. Ageing Dev. 112 (1999) 169–183.
[77] R. Pamplona, M. Portero-Otín, D. Riba, J.R. Requena, S.R. Thorpe, M. López-Torres, G. Barja, Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life span, J. Gerontol. 55A (2000) B286–B289.
[78] R. Pamplona, G. Barja, M. Portero-Otín, Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation, Ann. N. Y. Acad. Sci. 959 (2002) 475–490.
[79] A.J. Hulbert, On the importance of fatty acid composition of membranes for aging, J. Theor. Biol. 234 (2005) 277–288.
[80] M. Portero-Otín, M.J. Bellmunt, M.C. Ruiz, G. Barja, R. Pamplona, Correlation of fatty acid unsaturation of the major liver mitochondrial phospholipid classes in mammals to their maximum life span potential, Lipids 36 (2001) 491–498.
[81] M.C. Ruiz, V. Ayala, M. Portero-Otín, J.R. Requena, G. Barja, R. Pamplona, Protein methionine content and MDA-lysine protein adducts are inversely related to maximum life span in the heart of mammals, Mech. Ageing Dev. 126 (2005) 1106–1114.
[82] R. Pamplona, M. Portero-Otín, A. Sanz, V. Ayala, E. Vasileva, E. Barja, Protein and lipid oxidative damage and complex I content are lower in the brain of parakeets and canaries than in mice. Relation to aging rate, Age 27 (2005) 267–280.
[83] R. Pamplona, M. Portero-Otín, A. Sanz, J. Requena, G. Barja, Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain, Exp. Gerontol. 39 (2004) 725–733.
[84] L.J. Marnett, Oxy radicals, lipid peroxidation and DNA damage, Toxicology 181 (2002) 219–222.
[85] R. Weindruch, Caloric restriction: life span extension and retardation of brain aging, Clin. Neurosci. Res. 2 (2003) 279–284.
[86] W. Mair, P. Goymer, S.D. Pletcher, L. Partridge, Demography of dietary restriction and death in *Drosophila*, Science 301 (2003) 1731–1733.
[87] B.P. Yu, E.A. Suescun, S.Y. Yan, Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: modulation by dietary restriction, Mech. Ageing Dev. 65 (1992) 17–33.
[88] T. Armeni, G. Principato, J.L. Quiles, C. Pieri, S. Bompadre, M. Battino, Mitochondrial dysfunctions during aging: vitamin E deficiency or caloric restriction. Two different ways of modulating stress, J. Bioenerg. Biomembr. 35 (2003) 181–191.
[89] A. Bartke, J.C. Wright, J.A. Mattison, D.K. Ingram, R.A. Miller, G.S. Roth, Extending the lifespan of long-lived mice, Nature 414 (2001) 412.
[90] S. Miwa, K. Riyahi, L. Partridge, M.D. Brand, Lack of correlation between mitochondrial reactive oxygen species production and life span in *Drosophila*, Ann. N. Y. Acad. Sci. 1019 (2004) 388–391.
[91] R.S. Sohal, H.H. Ku, S. Agarwal, M.J. Forster, H. Lal, Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse, Mech. Ageing Dev. 74 (1994) 121–133.
[92] R. Gredilla, G. Barja, M. López-Torres, Effect of short-term caloric restriction on H_2O_2 production and oxidative DNA damage in rat liver mitochondria, and location of the free radical source, J. Bioenerg. Biomembr. 33 (2001) 279–287.
[93] R. Gredilla, A. Sanz, M. López-Torres, G. Barja, Caloric restriction decreases mitochondrial free radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat heart, FASEB J. 15 (2001) 1589–1591.
[94] M. López-Torres, R. Gredilla, A. Sanz, G. Barja, Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria, Free Radic. Biol. Med. 32 (2002) 882–889.
[95] B. Drew, S. Phaneuf, A. Dirks, C. Selman, R. Gredilla, A. Lezza, G. Barja, C. Leeuwenburgh, Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart, Am. J. Physiol. 284 (2003) R474–R480.
[96] S. Judge, A. Judge, T. Grune, C. Leeuwenburgh, Short-term CR decreases cardiac mitochondrial oxidant production but increases carbonyl content, Am. J. Physiol. 286 (2004) R254–R259.
[97] J.J. Ramsey, K. Hagopian, T.M. Kenny, E.K. Koomson, L. Bevilacqua, R. Weindruch, M.E. Harper, Proton leak and hydrogen peroxide production in liver mitochondria from energy restricted rats, Am. J. Physiol. 286 (2004) E31–E40.
[98] A. Sanz, P. Caro, J. Ibáñez, J. Gómez, R. Gredilla, G. Barja, Dietary restriction at old age lowers mitochondrial oxygen radical production and leak at complex I and oxidative DNA damage in rat brain, J. Bioenerg. Biomembr. 37 (2005) 83–90.
[99] L. Bevilacqua, J.J. Ramsey, K. Hagopian, R. Weindruch, M.E. Harper, Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle, Am. J. Physiol. 289 (2005) 429–438.
[100] R. Gredilla, G. Barja, Caloric restriction, aging and oxidative stress, Endocrinology 146 (2005) 3713–3717.
[101] R. Pamplona, M. Portero-Otín, J. Requena, R. Gredilla, G. Barja, Oxidative, glycoxidative and lipoxidative damage to rat heart mitochondrial proteins is lower after four months of caloric restriction than in age-matched controls, Mech. Ageing Dev. 123 (2002) 1437–1446.
[102] A.J. Lambert, M. Portero-Otín, R. Pamplona, B.J. Merry, Effect of ageing and caloric restriction on specific markers of protein oxidative damage and membrane peroxidizability in rat liver mitochondria, Mech. Ageing Dev. 125 (2004) 529–538.
[103] W. Mair, M.D.W. Piper, L. Partridge, Calories do not explain extension of life span by dietary restriction in *Drosophila*, PLOS Biol. 3 (2005) 1305–1311.
[104] V.E. Archer, Does dietary sugar and fat influence longevity? Med. Hypoteses 60 (2003) 924–929.
[105] M.C.M. Mark, K.M. Reiser, R. Harris Jr., R.B. McDonald, Source of dietary carbohydrate affects life span of Fischer 344 rats independent of caloric restriction, J. Gerontol. 50A (1995) B148–B154.
[106] A. Sanz, P. Caro, G. Barja, Protein restriction without strong caloric restriction decreases mitochondrial oxygen radical production and oxidative DNA damage in rat liver, J. Bioenerg. Biomembr. 36 (2004) 545–552.

[107] S. Leto, G.C. Kokkonen, C.H. Barrows Jr., Dietary protein, life-span, and biochemical variables in female mice, J. Gerontol. 31 (1976) 144–148.
[108] C.L. Goodrick, Body weight increment and length of life: the effect of genetic constitution and dietary protein, J. Gerontol. 33 (1978) 184–190.
[109] G. Stoltzner, Effects of life-long dietary protein restriction on mortality, growth, organ weights, blood counts, liver aldolase and kidney catalase in Balb/C mice, Growth 41 (1977) 337–348.
[110] G. Fernandes, E.J. Yunis, R.A. Good, Influence of diet on survival of mice, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 1279–1283.
[111] C.H. Barrows Jr., G. Kokkonen, Protein synthesis, development, growth and life span, Growth 39 (1975) 525–533.
[112] B.P. Yu, E.J. Masoro, C.A. Maman, Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics, J. Gerontol. 40 (1985) 657–670.
[113] M. Horakova, Z. Deyl, J. Hausmann, K. Macek, The effect of low protein-high dextrin diet and subsequent food restriction upon life prolongation in Fischer 244 male rats, Mech. Ageing Dev. 45 (1988) 1–7.
[114] T.A. Davis, C.W. Bales, R.E. Beauchene, Differential effects of caloric and protein restriction in the aging rat, Exp. Gerontol. 18 (1983) 427–435.
[115] M.H. Ross, G. Bras, Food preference and length of life, Science 190 (1975) 165–167.
[116] D.S. Miller, P.R. Payne, Longevity and protein intake, Exp. Gerontol. 3 (1968) 231–234.
[117] M.H. Ross, Length of life and nutrition in the rat, J. Nutr. 75 (1961) 197–210.
[118] N. Orentreich, J.R. Matias, A. DeFelice, J.A. Zimmerman, Low methionine ingestion by rats extends life span, J. Nutr. 123 (1993) 269–274.
[119] J.P. Richie Jr., Y. Leutzinger, S. Parthasarathy, V. Malloy, N. Orentreich, J.A. Zimmerman, Methionine restriction increases blood glutathione and longevity in F344 rats, FASEB J. 8 (1994) 1302–1307.
[120] J.A. Zimmerman, V. Malloy, R. Krajcik, N. Orentreich, Nutritional control of aging, Exp. Gerontol. 38 (2003) 47–52.
[121] J.P. Richie Jr., D. Komninou, Y. Leutzinger, W. Kleinman, N. Orentreich, V. Malloy, J.A. Zimmerman, Tissue glutathione and cysteine levels in methionine-restricted rats, Nutrition 20 (2004) 800–805.
[122] R.A. Miller, G. Buehner, Y. Chang, J.M. Harper, R. Sigler, Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance, Aging Cell 4 (2005) 119–125.
[123] L.D. Youngman, J.Y.K. Park, B.N. Ames, Protein oxidation associated with aging is reduced by dietary restriction of protein or calories, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 9112–9116.
[124] S.Q. Doi, S. Rasaiah, I. Tack, J. Mysore, J.J. Kopchick, J. Moore, P. Hirszel, L.J. Striker, G.E. Striker, Low-protein diet suppresses serum insulin-like growth factor-1 and decelerates the progression of growth hormone-induced glomerulosclerosis, Am. J. Nephrol. 21 (2001) 331–339.
[125] H. Maeda, C.A. Gleiser, E.J. Masoro, I. Murata, C.A. McMahan, B.P. Yu, Nutritional influences on aging of Fischer 344 rats: II Pathology, J. Gerontol. 40 (1985) 671–688.
[126] M.A.M. Rodrigues, M. Sanchez-Negrette, M.S. Mantovani, L.S. Sant'ana, A.Y.O. Angeleli, M.R. Montenegro, J.L.V. de Camargo, Liver response to low-hexachlorobenzene exposure in protein- or energy-restricted rats, Food Chem. Toxicol. 29 (1991) 757–764.
[127] A. Sanz, P. Caro, G. Barja, Effect of lipid restriction on mitochondrial free radical production and oxidative DNA damage, Ann. New York Acad. Sci. (2005).
[128] A.K. De, S. Chipalkatri, A.S. Aiyar, Some parameters of ageing in relation to dietary protein, Mech. Ageing Dev. 21 (1983) 37–48.
[129] A.J. Lambert, B.J. Merry, Effect of caloric restriction on mitochondrial reactive oxygen species production and bioenergetics: reversal by insulin, Am. J. Physiol. 286 (2004) R71–R79.
[130] A. Sanz, R. Gredilla, R. Pamplona, M. Portero-Otín, E. Vara, J.A.F. Tresguerres, G. Barja, Effect of insulin and growth hormone on rat heart and liver oxidative stress in control and caloric restricted animals, Biogerontology 6 (2005) 15–26.
[131] M. Portero-Otín, J.R. Requena, M.J. Bellmunt, V. Ayala, R. Pamplona, Protein nonenzymatic modifications and proteasome activity in skeletal muscle from the short-lived rat and long-lived pigeon, Exp. Gerontol. 39 (2004) 1527–1535.
[132] J. Moskovitz, S. Bar-Noy, W.M. Williams, J. Requena, B.S. Berlett, E.R. Stadtman, Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12920–12925.
[133] H. Ruan, X.D. Tang, M.L. Chen, M.L. Joiner, G. Sun, N. Brot, H. Weisssbach, S.H. Heinemann, L. Iverson, C.F. Wu, T. Hoshi, High-quality life extension by the enzyme peptide methionine sulfoxide reductase, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2748–2753.
[134] E.R. Stadtman, J. Moskovitz, R.L. Levine, Oxidation of methionine residues in proteins: biological consequences, Antioxid. Redox Signal. 5 (2003) 577–582.
[135] E.R. Stadtman, H. Van Remmen, A. Richardson, N.B. Wehr, R.L. Levine, Methionine oxidation and aging, Biochim. Biophys. Acta 1703 (2005) 135–140.
[136] N. Hidiroglou, G. Sarwar Gilani, L. Long, X. Zhao, R. Madere, K. Cockell, B. Belonge, W.M.N. Ratnayake, R. Peace, The influence of dietary vitamin E, fat, and methionine on blood cholesterol profile, homocysteine levels, and oxidizability of low density lipoprotein in the gerbil, J. Nutr. Biochem. 15 (2004) 730–740.
[137] S.M. Lynch, J.J. Strain, Increased hepatic lipid peroxidation with methionine toxicity in the rat, Free Radic. Res. Commun. 5 (1989) 221–226.
[138] N. Mori, K. Hirayama, Long-term consumption of a methionine-supplemented diet increases iron and lipid peroxide levels in rat liver, J. Nutr. 130 (2000) 2349–2355.
[139] N. Mori, K. Hirayama, Effect of long-term excessive L-methionine consumption on transferring receptor abundance and mitochondrial H2O2 generation in rat liver, J. Health Sci. 50 (2004) 277–285.
[140] S. Robin, V. Maupoil, P. Laurant, A. Jackeson, A. Berthelot, Effect of methionine-supplemented diet on the blood pressure of Sprague Dawley and deoxycorticosterone acetate-salt hypertensive rats, Br. J. Nutr. 91 (2004) 857–865.
[141] D. Matthias, C.H. Becker, R. Riezler, P.H. Kindling, Homocysteine induced arteriosclerosis-like alterations of the aorta in normotensive and hypertensive rats following application of high doses of methionine, Atherosclerosis 122 (1996) 201–216.
[142] D. Fau, J. Peret, P. Hadjiliskym, Effects of ingestion of high protein or excess methionine diets by rats for two years, J. Nutr. 118 (1988) 128–133.
[143] M. Toborek, E. Kopieczna-Grzebienak, M. Drózdz, M. Wieczorek, Increased lipid peroxidation as a mechanism of methionine-induced atheriosclerosis in rabbits, Atheriosclerosis 115 (1995) 217–224.
[144] K.J. Petzke, J. Proll, J. Brükner, C.C. Metges, Plasma protein carbonyl concentration is not enhanced by chronic intake of high-protein diets in adult rats, J. Nutr. Biochem. 10 (1999) 268–273.
[145] R.T. Dean, S. Fu, R. Stocker, M.J. Davies, Biochemistry and pathology of radical-mediated protein oxidation, Biochem. J. 324 (1997) 1–18.
[146] V.S. Slyshenkov, A.A. Shevalye, A.V. Liopo, L. Wojtczak, Protective role of methionine against free radical damage of rat brain synaptosomes, Acta Biochim. Pol. 49 (2002) 907–916.
[147] H. Carp, F. Miller, J.R. Hoidal, A. Janoff, Potential mechanism of emphysema: a1-proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 2041–2045.
[148] M.A. Ciorba, S.H. Heinemann, H. Weissbach, N. Brot, T. Hoshi, Modulation of potassium channel function by methionine oxidation and reduction, PNAS USA 94 (1997) 9932–9937.
[149] H. Nakamura, A. Mitsui, J. Yodoi, Thioredoxin overexpression in transgenic mice, Methods Enzymol. 347 (2002) 436–440.
[150] A. Mitsui, J. Hamuro, H. Nakamura, N. Kondo, Y. Hirabayashi, S. Ishizaki-Koizumi, T. Hirakawa, T. Inoue, J. Yodoi, Overexpression of human thioredoxin in transgenic mice controls oxidative stress and lifespan, Antioxid. Redox Signal. 4 (2002) 693–696.

[151] W. Drögue, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (2001) 47–95.
[152] P. Durand, M. Prost, N. Loreau, S. Lussier-Cacan, D. Blacke, Impaired homocysteine metabolism and atherothrombotic disease, Lab. Invest. 81 (2001) 645–672.
[153] C.K.B. Ferrari, Functional foods, herbs and nutraceuticals: towards biochemical mechanisms of healthy aging, Biogerontology 5 (2004) 275–289.
[154] T. Ninomiya, Y. Kiyohara, M. Kubo, Y. Tanizaki, K. Tanak, K. Okubo, H. Nakamura, J. Hata, Y. Oishi, I. Kato, H. Hirakata, M. Lida, Hyperomocysteinemia and the development of chronic kidney disease in a general population: the Hisayama study, Am. J. Kidney Dis. 44 (2004) 437–445.
[155] E.R. Taylor, F. Hurrell, R.J. Shannon, T.K. Lin, J. Hirst, M.P. Murphy, Reversible glutathionylation of complex I increases mitochondrial superoxide formation, J. Biol. Chem. 278 (2003) 19603–19610.
[156] L. Chang, J. Zhao, J. Xu, W. Jiang, C.S. Tang, Y.F. Qi, Effects of taurine and homocysteine on calcium homeostasis and hydrogen peroxide and superoxide anions in rat myocardial mitochondria, Clin. Exp. Pharmacol. Physiol. 31 (2004) 237–243.