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Research Article

Does dietary restriction of amino acids other than methionine have any effect on peroxide and superoxide production rates, oxidative protein and DNA damage in the liver and heart mitochondria of aging rats?

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Abstract

Objectives: The objective of this study was to evaluate the long-term effects of amino acids other than methionine in mitotic and postmitotic tissue of rats by measuring mitochondrial peroxide and superoxide production as well as oxidative protein and DNA damage in the liver and heart of rats fed with either a normal diet (ND) or a protein-restricted diet (PREMD).

Methods: The study group comprised 4- and 12-month-old rats fed with either a ND or a PREMD for 4 months. The rate of mitochondrial peroxide and superoxide production, and the protein carbonyl (PC) and mitochondrial DNA (mtDNA) 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in the liver and heart mitochondria were measured.

Results: The mitochondrial peroxide and superoxide production rates of the liver and heart mitochondria of rats did not demonstrate any significant difference based on the diet provided. Similarly, diet did not have a significant effect on the PC level in the liver and heart mitochondria of either age group. In the 16-month-old rats, the mtDNA 8-OHdG level was significantly higher in the heart than the liver, regardless of the diet.

Conclusion: Mitochondrial reactive oxygen species production, and oxidative protein and DNA damage increased in mitotic and postmitotic tissue with age; however, the increment was more prominent in the heart than the liver. Long-term PREMD consumption did not decelerate oxidative damage in the heart or in the liver with age.

Keywords: Mitotic tissue, peroxide production, postmitotic tissue, protein carbonyl, protein restriction, superoxide production

Harman's free radical theory of aging has proposed that reactive oxygen species (ROS) produced by mitochondria have a central role in aging [1]. Further studies have provided convincing data of the role of mitochondria in the aging process [2-5]. It has been suggested that the mitochondrial respiratory chain may be responsible for most free radical generation in aerobic tissue and that an increase in the generation of oxidatively damaged biomolecules and a decrease in the antioxidant system are the main causes of the age-dependent degenerative process in biological systems [6-9]. If oxidatively damaged proteins, lipids and DNA are not effectively removed by antioxidant mechanisms or repaired by DNA repair systems, they tend to accumulate in the cell and may lead to cellular senescence [10-12]. Over the years, Harman improved his mitochondrial theory of aging and suggested that mitochondria are not only the major source, but also the target of ROS in the cells [1]. Although almost all cells are vulnerable to ROS-mediated damage, some cells and tissues are more susceptible than others. It has been suggested that this is related to mitochondrial

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DNA (mtDNA), rather than genomic DNA [13-16]. It has been shown that mtDNA damage increases with age, and that generally, the increment is more prominent in postmitotic tissue, such as those of the brain, heart, and skeletal muscle [17-19]. These results have been corroborated in studies carried out with rodents, monkeys, and humans [13, 17-19]. It has been shown that mitosis is more frequent in tissue that requires frequent renewal due to their function, such as hepatocytes and epithelial cells. Although the link between oxidative damage and aging in these actively dividing cells is less clear, it has been suggested that the cellular components of these cells are protected by frequent renewal during mitosis, and as a result, the accumulation of age-related oxidative damage in the mitochondrial genome is reduced [20, 21].

Various experimental manipulations, including dietary supplementation or restriction of some nutrients, have been performed in different species to evaluate any slowing of the aging process [22]. The most powerful and thoroughly studied manipulation to increase longevity is calorie restriction [23-25]. A 30% to 60% reduction in daily calorie intake has been shown to have a beneficial effect [26-28]. Recent studies have demonstrated that adjustments to the quantity of ingested dietary components, such as carbohydrates, lipids, and proteins, may affect longevity [29, 30]. Protein restriction alone appeared to be responsible for 50% of the life-extension effect of calorie restriction [31-33]. Other research has indicated that methionine restriction increased maximum life span in rodents [34-36]. However, reports related to the effects of amino acids other than methionine are scarce [37].

The objective of this study was to evaluate the long-term effects of amino acids other than methionine on the mitotic and postmitotic tissue of rats by measuring mitochondrial peroxide and superoxide production, as well as oxidative protein and DNA damage to the liver and heart in 16-month-old rats fed either a normal diet (ND) or a protein-restricted diet (with methionine) (PREMD) for 4 months.

Materials and Methods

Experimental design

The study design was approved by the local ethics committee for animal experiments (Animal Care and Use Committee of the University of Istanbul, Approval date and number: October 13, 2006 - 2006-53) and conducted in accordance with the Declaration of Helsinki. The subjects of this study were 4-month-old (n=15) and 12-month-old (n=30) male Wistar albino rats. All of the rats were caged individually and maintained in a 12/12-hour (light-dark) cycle at 22±20C and 50±10% relative humidity. After 1 week of acclimatization, the 12-month-old rats were divided into 2 subgroups: half (n=15) were fed ad libitum the semi-purified American Institute of Nutrition AIN-93G rodent diet (MP Biochemicals, Santa Ana, CA, USA) (ND group), and half (n=15) received a modified AIN-93G diet (MP Biochemicals, Santa Ana, CA, USA) (PREMD group) for 4 months. The PREMD feed was prepared with 40% restriction of proteins, except methionine. The 4-month-old rats also received ND for 4 months. Both diets contained the same level of methionine and caloric content. In the PREMD group, the calorie deficit due to reduced amino acid content in the diet was compensated for by adding corn starch, sucrose, and corn oil. Food consumption was measured daily throughout the study. Each day, 30 g of rat chow was provided and the mean consumption was 21-22 g/day per rat. The body weight of the rats was recorded at the beginning and the end of the experiment. The quality of their coat and the physical activity of all of the subjects was observed throughout the study.

After 4 months of dietary treatment, the animals were euthanized. The liver and heart were removed, weighed, and immediately shock-frozen in liquid nitrogen and stored at -80°C until studied.

The liver and heart mitochondria were prepared using differential centrifugation with some modifications [38]. Briefly, the tissue was minced in an ice-cold buffer solution of 10 mM Tris-hydrochloride pH 7.4, 250 mM sucrose, 1 mM Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, 0.2% bovine serum albumin (BSA), 1 mM phenylmethylsulphonyl fluoride, 1 mM dithiothreitol, 10 µg/mL aprotinin, 10 µg/mL leupeptin, and 10 µg/mL soybean trypsin inhibitor. The samples were homogenized for 1 minute on ice followed by centrifugation at 800 g for 5 minutes at 4°C. The supernatant was re-centrifuged at 10,000 g for 12 minutes at 4°C, and then the mitochondrial pellet was re-suspended in a small volume of the homogenization buffer containing no BSA. The protein content of the mitochondria samples was determined using a bicinchoninic acid assay [39].

Peroxide and superoxide production assay

Mitochondrial peroxide and superoxide production was measured with a chemiluminometric method. A Fluoroskan Ascent FL luminometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used at room temperature using either lucigenin or luminol as an enhancer. Luminol measured the total hydroxyl, hydroperoxyl, and peroxyl radical values, while lucigenin evaluated superoxide anion. Counts were obtained at 1-minute intervals for a period of 10 minutes and the area under curve was calculated. The counts were corrected for the protein content of the samples and the results were expressed as relative light units (RLU) per mg protein [40].

Protein carbonyl assay

Protein carbonyl (PC) content in liver and heart mitochondria was assessed according to spectrophotometer detection of the reaction of 2,4-dinitrophenylhydrazine with PC to form protein hydrazones [41]. The results were expressed as nanomoles of carbonyl groups per milligram of protein, with a molar extinction coefficient of 22.000 mol/L-1 cm-1 for the 2,4-dinitrophenylhydrazine derivatives.

DNA isolation and 8-hydroxy-2'-deoxyguanosine assay

Liver and heart mtDNA was isolated using the method described by Latorre et al. [41] as adapted for mammals [42]. Briefly, the isolated mtDNA was incubated at 37°C for 1 hour with nuclease P1 (5U/L) and for another 1 hour with alkaline phosphatase (2U/L). Following digestion, the samples were filtered through Microcon YM-10 filters (cat. no 42407; MilliporeSigma, St. Louis, MO, USA) at 14,000 rpm for 10 minutes to remove enzymes and other macromolecules. The mtDNA 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations of digested samples were determined in duplicate using a commercially available enzyme-linked immunosorbent assay kit (cat. no NWK-8OHDG02; Northwest Life Science Specialties LLC, Vancouver, WA, USA).

Statistical analysis

The results were analyzed using SPSS for Windows, Version 15.0 software (SPSS Inc. Chicago, IL, USA). The results were presented as mean±SD. Comparisons within independent groups were performed with one-way analysis of variance when numerical variables demonstrated normal distribution (liver superoxide and peroxide production rates and protein carbonyl [PCO] levels, heart peroxide production rate, and PCO and mtDNA 8-OHdG levels). Otherwise, the Kruskal-Wallis test was performed (liver mtDNA 8-OHdG, heart superoxide production rate). Between-group differences were compared with the Tukey test or the Mann-Whitney U test and interpreted with Bonferroni's test. Comparisons between dependent groups (liver vs. heart) were performed with a paired sample t-test when differences between means showed normal distribution (superoxide and peroxide production rates and PCO level), and if not, the Wilcoxon test (mtDNA 8-OHd level) was performed. P<0.05 was considered significant.

Results

Measurements recorded at the conclusion of the study period indicated that the different diets did not result in any significant differences in the total body weight (Table 1) or liver and heart weights (data not shown).

The superoxide and peroxide production rates of liver (p<0.01 and p<0.001; Tukey test) and heart (p<0.01 for both; Mann-Whitney U test for superoxide production, Tukey test for peroxide production) mitochondria indicated that 16-month-old rats receiving ND had elevated superoxide and peroxide production rates in comparison with the 8-month-old rats (Table 2). The 16-month-old PREMD rats had a significantly higher rate of peroxide production in the liver and heart mitochondria (liver: 6.48±2.05, heart: 6.41±0.85) than the 8-month-old rats (liver: p<0.05, heart: p<0.01; Tukey test) (Table 2).

Although the PC levels of the liver and heart mitochondria were significantly elevated as the rats grew older (p<0.001 for both; Tukey test), comparison of the 16-month-old rats showed that the ND and PREMD did not have any beneficial

Table 1. Body weight of rats given a normal diet (ND) or a protein-restricted diet (PREMD) at the beginning and end of the study

Body weight (g)	ND aged 8 months (n=15)	ND aged 16 months (n=15)	PREMD aged 16 months (n=15)
Baseline (aged 4 and 12 months)	280±25.8	350±39.8	355±34.7
Conclusion (4th month)	300±29.5	387±40.9	378±28.3

Results are presented as mean±SD.

Table 2. Liver and heart mitochondria superoxide and peroxide production rates and protein carbonyl and 8-OHdG levels of 4- and 12-month-old rats after 4-month ND or PREMD consumption

	Superoxide production (RLU/mg protein)	Peroxide production (RLU/mg protein)	Protein carbonyl (nmol/mg protein)	mtDNA 8-OHdG (ng/mL)
Liver mitochondria				
8-m ND (n=15)	0.59±0.19	4.70±1.26	3.25±0.69	0.35±0.26
16-m ND (n=15)	0.90±0.33**	7.34±1.22*	6.66±1.29*	0.81±0.66***
16-m PREMD (n=15)	0.67±0.29	6.48±2.05***	5.06±1.77**,##	0.36±0.26###
Heart mitochondria				
8-m ND (n=15)	0.72±0.18	5.52±0.78	4.10±1.39	0.38±0.18
16-m ND (n=15)	0.96±0.23**	7.08±1.84**	8.09±3.75*	0.93±0.44*
16-m PREMD (n=15)	0.54±0.18**,#	6.41±0.85**	6.16±2.27**	0.87±0.28*

Results are presented as mean±SD. *p<0.001; **p<0.01; *** p<0.05. 8- vs. 16-month-old rats receiving either ND or PREMD; Comparisons were performed with Kruskal-Wallis test for liver mtDNA 8-OHdG level and heart superoxide production rate, and one-way analysis of variance for the rest of the parameters. #p<0.001; ##p<0.01; ###p<0.05 ND vs. PREMD in 16-month-old rats; Comparisons of liver vs. heart tissue were performed with the Wilcoxon test for mtDNA 8-OHd level and paired sample t-test for the rest of the parameters. 8-OHdG mt: 8-hydroxy-2'-deoxyguanosine; mtDNA: Mitochonrial DNA; ND: Normal diet; PREMD: Protein-restricted diet.

effect on PC formation rate in heart. The PC level in the liver of the PREMD fed 16-month-old rats was significantly lower than that of the ND group rats of the same age ($p<0.01$; Tukey test).

As the rats grew older, the difference in the mtDNA 8-OHdG level in the liver mitochondria were statistically significant in the ND rats ($p<0.001$; Mann-Whitney U test), but not in the PREMD group subjects. The mtDNA 8-OHdG content was significantly elevated in the heart mitochondria of both the ND and PREMD groups ($p<0.001$ for both; Tukey test). The comparison of 16-month-old rats fed with ND and PREMD revealed that the PREMD 16-month-old rats had a significantly lower mtDNA 8-OHdG level in the liver ($p<0.05$; Mann Whitney U test), but not the heart (Table 2).

When the findings of the liver and heart mitochondria of rats from the same age group and the same diet were compared, it was observed that both the superoxide and peroxide production rate was significantly higher in the heart of the 8-month-old ND fed rats ($p<0.05$ for both; Wilcoxon test). Such a difference was not observed in the liver and heart mitochondria of the 16-month-old rats of either diet group (Fig. 1). The 8-OHdG level of the heart mtDNA was significantly higher than that of the liver mitochondria in the 16-month-old rats ($p<0.05$; Student t-test) (Fig. 1).

Comparison of liver and heart mitochondria showed no significant difference in the PC level at any age (Fig. 1). In contrast, the mtDNA 8-OHdG level of liver and heart exhibited significant differences with age: the mtDNA 8-OHdG level of the 16-month-old rats was significantly higher in the heart than the liver, regardless of the diet (ND: $p<0.05$, PREMD: $p<0.001$; Student t-test) (Fig. 1).

Discussion

Our findings indicated that although postmitotic tissue mitochondria produced significantly higher levels of superoxide and peroxide than the mitotic tissue, there was no significant difference in the level of oxidative protein and mtDNA damage in the 8-month-old rats. As the rats grew older, the ROS production rate and the level of oxidatively damaged macro-

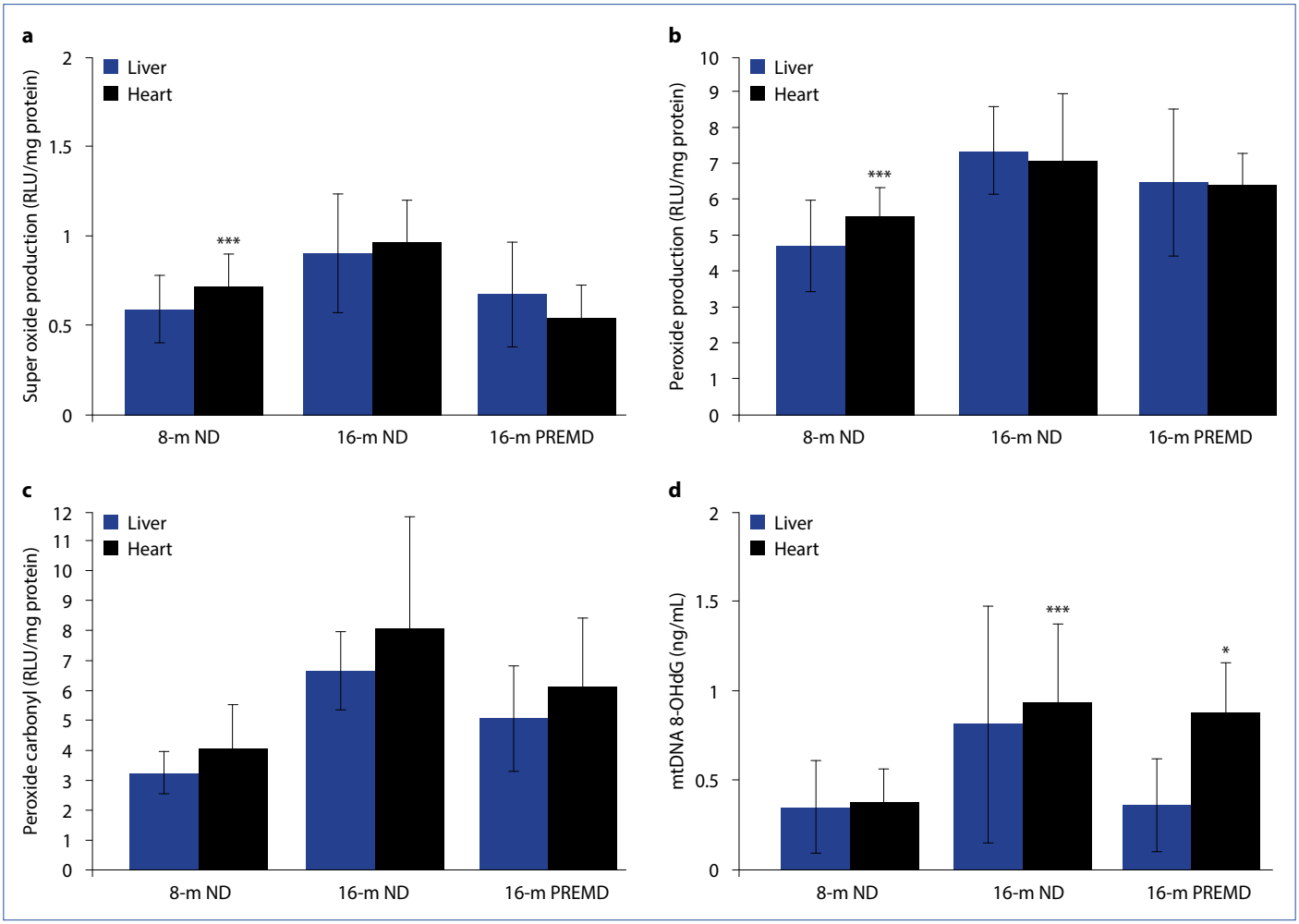

Figure 1. Effects of aging on (a) mitochondirial superoxide and (b) peroxide production rates, (c) protein carbonyl, and (d) and mtDNA 8-OHdG levels in the liver and heart of rats receiving either ND or PREMD feed for 4 months. *$p<0.001$; **$p<0.01$; *** $p<0.05$. Comparisons of liver vs. heart tissue were performed with the Wilcoxon test for mtDNA 8-OHd level and a paired sample t-test for the rest of the parameters.

8-OHdG mt: 8-hydroxy-2'-deoxyguanosine; mtDNA: Mitochonrial DNA; ND: Normal diet; PREMD: Protein-restricted diet.

molecules maintained an increasing trend in both the mitotic and postmitotic tissue, but the only significant difference was observed in mtDNA damage. The 8-OHdG level of heart mtDNA was significantly higher than that of the liver mitochondria in the 16-month-old rats. These results may support Harman's [1] theory of aging, which states that the progressive accumulation of oxidatively damaged cellular macromolecules within the cells, such as proteins and mtDNA, is responsible for the aging process. It has been shown that mtDNA, and mitochondrial lipids and proteins are continuously exposed to ROS generated by the respiratory chain due to their close proximity [12, 43]. mtDNA is associated with some proteins, and these mtDNA-protein assemblies (mitochondrial nucleoid) coordinate the relationship between mtDNA and cellular metabolism [44]. The proteins that constitute the nucleoid primarily play a role in mtDNA maintenance, mitochondrial biogenesis, metabolism, and mitochondria-to-nucleus signaling [44-46]. Though mtDNA is packed with proteins, like genomic DNA, its circular structure without histones, as in bacteria, means that the protective effect of nucleoid proteins is not as efficient as that seen in genomic DNA [47]. It is also believed that the DNA repair capacity of mtDNA is not as efficient as that of genomic DNA; thus, causing a tendency for the accumulation of oxidative damage. However, only about 1% of mitochondrial proteins are encoded by mtDNA, while the remainder are encoded by nuclear genes and transported into the matrix by transport systems located in mitochondrial membranes [14, 48]. Mitochondria use some control mechanisms to eliminate oxidatively damaged proteins, including degradation by proteases, the ubiquitin-proteasome pathway, and induction of protein expression [48, 49]. In spite of these cellular defense and repair mechanisms, oxidatively damaged molecules accumulate in the mitochondria and cells with age, which subsequently lead to mitochondrial malfunction and cell death [50]. It has been suggested that although both mitotic and postmitotic cells are subject to age-related oxidative damage, it is more prominent in postmitotic cells, such as neurons and myocardial and other muscle cells [47, 50]. The limited or lack of division of postmitotic cells as a result of age-related oxidative damage leads to a slow but progressive accumulation over time.

It has been shown that some dietary or pharmacological manipulations, such as caloric restriction and reduced insulin or insulin-like growth factor 1-receptor signaling may reduce the level of oxidatively damaged biomolecules and delay aging [26-29]. Recent studies of dietary manipulations have suggested that limitation of dietary components, like proteins or some individual amino acids, may also have beneficial effects on aging [28-31]. Some of these studies have demonstrated that the restriction of methionine alone increased the maximum life span in rats [34] and mice [35, 36]. However, the effects of amino acids other than methionine have not yet been fully studied. This study was an examination of the effects of long-term feeding with a PREMD. Our results indicated that long-term PREMD use had distinctive effects on mitotic and postmitotic tissue. While the liver mtDNA 8-OHdG level increased 131% in the ND rats, the increment was only 2% in the PREMD rats. This effect was not as significant on the heart mitochondria; the increase in the heart mtDNA 8-OHdG level was 144% in the ND group and 128% in the PREMD group.

Conclusion

In conclusion, the results of this study indicated that mitochondrial ROS production, as well as oxidative protein and DNA damage increased in both mitotic and postmitotic tissue with age; however, the increment was more prominent in the heart than the liver. Long-term feeding with a PREMD did not decelerate oxidative damage with aging in either the heart or the liver. As a result, although postmitotic tissue has less ability to up-regulate antioxidant defenses and/or to repair accumulated oxidative damage than tissues with greater proliferation capacity, dietary restriction of amino acids other than methionine did not produce any reduction of the adverse effects of the aging process.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Harman D. The biologic clock: the mitochondria? J Am Geriatr Soc 1972;20(4):145–7. [CrossRef]
2. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998;78(2):547–81. [CrossRef]
3. Genova ML, Pich MM, Bernacchia A, Bianchi C, Biondi A, Bovina C, et al. The mitochondrial production of reactive oxygen species in relation to aging and pathology. Ann N Y Acad Sci 2004;1011:86–100. [CrossRef]
4. Pallardó FV, Lloret A, Lebel M, d'Ischia M, Cogger VC, Le Couteur DG, et al. Mitochondrial dysfunction in some oxidative stress-related genetic diseases: Ataxia-Telangiectasia, Down Syndrome, Fanconi Anaemia and Werner Syndrome. Biogerontology 2010;11(4):401–19. [CrossRef]
5. Lee HC, Wei YH. Mitochondria and aging. Adv Exp Med Biol

2012;942:311–27.
6. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000;29(3–4):222–30. [CrossRef]
7. Ademoglu E, Ozcan K, Tanrikulu-Kucuk S, Gurdol F. Age-related changes in the activity and expression of manganese superoxide dismutase, and mitochondrial oxidant generation in female and male rats. Turk J Biochem 2013;38(4);445–50.
8. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation and impairment of antioxidant enzymes in aging. Exp Biol Med 2002;227(9):671–82. [CrossRef]
9. Kang D, Hamasaki N. Mitochondrial oxidative stress and mitochondrial DNA. Clin Chem Lab Med 2003;41(10):1281–8.
10. Greaves LC, Turnbull DM. Mitochondrial DNA mutations and aging. Biochim Biophys Acta 2009;1790(10):1015–20.
11. Vatner SF, Zhang J, Oydanich M, Berkman T, Naftalovich R, Vatner DE. Healthful aging mediated by inhibition of oxidative stress. Ageing Res Rev 2020;64:101194. [CrossRef]
12. Wallace DC. Mitochondrial DNA in aging and disease. Sci Am 1997;277(2):40–7. [CrossRef]
13. Sastre J, Pallardó FV, Viña J. The role of mitochondrial oxidative stress in aging. Free Radic Biol Med 2003;35(1):1–8. [CrossRef]
14. Alexeyev MF, Ledoux SP, Wilson GL. Mitochondrial DNA and aging. Clinical Science 2004;107(4):355–64. [CrossRef]
15. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 2005;309(5733):481–4.
16. Lim KS, Jeyaseelan K, Whiteman M, Jenner A, Halliwell B. Oxidative damage in mitochondrial DNA is not extensive. Ann N Y Acad Sci 2005;1042:210–20. [CrossRef]
17. Bejma J, Ramires P, Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver. Acta Physiol Scand 2000;169(4):343–51.
18. Spindler SR. Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction. Mech Ageing Dev 2005;126(9):960–6.
19. Phaneuf S, Leeuwenburgh C. Cytochrome c release from mitochondria in the aging heart: a possible mechanism for apoptosis with age. Am J Physiol Regul Integr Comp Physiol 2002;282(2):R423–30. [CrossRef]
20. Sawada M, Sester U, Carlson JC. Superoxide radical formation and associated biochemical alterations in the plasma membrane of brain, heart, and liver during the lifetime of the rat. J Cell Biochem 1992;48(3):296–304.
21. Ji LL, Dillon D, Wu E. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am J Physiol 1990;258(4 Pt 2):R918–23. [CrossRef]
22. Warner HR. Twenty years of progress in biogerontology research. Age (Dordr) 2005;27(4):321–8. [CrossRef]
23. Liang Y, Liu C, Lu M, Dong Q, Wang Z, Wang Z, et al. Calorie restriction is the most reasonable anti-ageing intervention: a meta-analysis of survival curves. Sci Rep 2018;8(1):5779.
24. Page MM, Robb EL, Salway KD, Stuart JA. Mitochondrial redox metabolism: aging, longevity and dietary effects. Mech Ageing Dev 2010;131(4):242–52.
25. McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. Nutrition 1989;5(3):155–71; discussion 172.
26. Gredilla R, Sanz A, Lopez-Torres M, Barja G. Caloric restriction decreases mitochondrial free radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat heart. FASEB J 2001;15(9):1589–91. [CrossRef]
27. López-Torres M, Gredilla R, Sanz A, Barja G. Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. Free Radic Biol Med 2002;32(9):882–9. [CrossRef]
28. Mota-Martorell N, Jove M, Pradas I, Berdún R, Sanchez I, Naudi A, et al. Gene expression and regulatory factors of the mechanistic target of rapamycin (mTOR) complex 1 predict mammalian longevity. Geroscience 2020;42:1157–73. [CrossRef]
29. Mair W, Piper MD, Partridge L. Calories do not explain extension of life span by dietary restriction in Drosophila. PLoS Biol 2005;3(7):e223. [CrossRef]
30. Pamplona R, Barja G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. Biochim Biophys Acta 2006;1757(5–6):496–508.
31. Sanz A, Caro P, Barja G. Protein restriction without strong caloric restriction decreases mitochondrial oxygen radical production and oxidative DNA damage in rat liver. J Bioenerg Biomembr 2004;36(6):545–52. [CrossRef]
32. Youngman LD, Park JY, Ames BN. Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. Proc Natl Acad Sci U S A 1992;89(19):9112–6. [CrossRef]
33. Richie JP Jr, Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N, Zimmerman JA. Methionine restriction increases blood glutathione and longevity in F344 rats. FASEB J 1994;8(15):1302–7.
34. Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. 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 2005;4(3):119–25. [CrossRef]
35. Troen AM, French EE, Roberts JF, Selhub J, Ordovas JM, Parnell LD, et al. Lifespan modification by glucose and methionine in Drosophila melanogaster fed a chemically defined diet. Age (Dordr) 2007;29(1):29–39. [CrossRef]
36. Tanrikulu-Kucuk S, Ademoglu E. Dietary restriction of amino acids other than methionine prevents oxidative damage during aging: involvement of telomerase activity and telomere length. Life Sci 2012;90(23–24):924–8.
37. Jacobus WE, Saks VA. Creatine kinase of heart mitochondria: changes in its kinetic properties induced by coupling to oxidative phosphorylation. Arch Biochem Biophys 1982;219(1):167–78. [CrossRef]
38. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150(1):76–85. [CrossRef]
39. Haklar G, Sayin-Ozveri E, Yuksel M, Aktan AO, Yalcin AS. Different kinds of reactive oxygen and nitrogen species were detected in colon and breast tumors. Cancer Letters 2001;165(2):219–24.

40. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 1994;233:357–63. [CrossRef]
41. Latorre A, Moya A, Ayala FJ. Evolution of mitochondrial DNA in Drosophila subobscura. Proc Natl Acad Sci U S A 1986;83(22):8649–53. [CrossRef]
42. de la Asuncion JG, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, et al. Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J 1996;10(2):333–8. [CrossRef]
43. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J 1996;10(13):1532–8.
44. Garrido N, Griparic L, Jokitalo E, Wartiovaara J, van der Bliek AM, Spelbrink JN. Composition and dynamics of human mitochondrial nucleoids. Mol Biol Cell 2003;14(4):1583–96.
45. Ekstrand MI, Falkenberg M, Rantanen A, Park CB, Gaspari M, Hultenby K, et al. Mitochondrial transcription factor A regulates mtDNA copy number in mammals. Hum Mol Genet 2004;13(9):935–44.
46. Kukat C, Wurm CA, Spåhr H, Falkenberg M, Larsson NG, Jakobs S. Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. Proc Natl Acad Sci U S A. 2011;108(33):13534–9. [CrossRef]
47. Pinto M, Moraes CT. Mechanisms linking mtDNA damage and aging. Free Radic Biol Med 2015;85:250–8. [CrossRef]
48. Wiedemann N, Frazier AE, Pfanner N. The protein import machinery of mitochondria. J Biol Chem 2004;279(15):14473–6.
49. Käser M, Langer T. Protein degradation in mitochondria. Semin Cell Dev Biol 2000;11(3):181–90. [CrossRef]
50. Weber TA, Reichert AS. Impaired quality control of mitochondria: aging from a new perspective. Exp Gerontol 2010;45(7–8):503–11. [CrossRef]