ANTIOXIDANTS & REDOX SIGNALING
Volume 5, Number 5, 2003
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Forum Review

Mitochondrial Theory of Aging: Importance to Explain Why Females Live Longer Than Males

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ABSTRACT

Females live longer than males in many species, including humans. This can be explained on the basis of the mitochondrial theory of aging. Mitochondria from females produce significantly less hydrogen peroxide than those from males and have higher levels of mitochondrial reduced glutathione, manganese superoxide dismutase, and glutathione peroxidase than males. Oxidative damage to mitochondrial DNA is also fourfold higher in males than in females. These differences may be explained by estrogens. Ovariectomy abolishes the gender differences between males and females and estrogen replacement rescues the ovariectomy effect. The challenge for the future is to find molecules that have the beneficial effects of estradiol, but without its feminizing effects. Phytoestrogens or phytoestrogen-related molecules may be good candidates to meet this challenge. *Antioxid. Redox Signal.* 5, 549–556.

INTRODUCTION: THE MITOCHONDRIAL THEORY OF AGING

MANY THEORIES have been postulated to explain aging (29). One of the most prominent is the free radical theory of aging, which was first proposed by Harman in 1956 (21). Thus, oxygen-derived free radicals are responsible for the age-associated impairment at the cellular and tissue levels.

This theory is supported by experimental evidence such as the extension of life span obtained by increasing the antioxidant defense as well as the inverse relationship between the rate of reactive oxygen species (ROS) production and the maximum life span of species (4, 22, 42). Administration of antioxidants can increase the mean life span of flies (31, 47). Orr and Sohal have reported that simultaneous overexpression of copper-zinc superoxide dismutase and catalase genes in transgenic *Drosophila* extends their mean and maximum life span (33).

The fact that mitochondria are damaged inside intact cells was almost simultaneously reported by ourselves (39) and by Hagen and co-workers (20) (see Fig. 1).

Mitochondria are an important source of free radicals in cells. Moreover, mitochondrial components are targets of free radical damage associated with aging (19, 32, 39). More than 90% of the oxygen used by aerobic cells is consumed in mitochondria, and ~1–2% of oxygen used by mammalian mitochondria in state 4 does not form water, but superoxide anion (7, 10), which is converted to hydrogen peroxide within mitochondria either spontaneously or by manganese superoxide dismutase (Mn-SOD). Hydrogen peroxide is released to the extramitochondrial space (18). Studying different mammalian species, Sohal and co-workers found that mitochondria from shorter-lived species produce higher amounts of hydroperoxide than those from the longer-lived species (26, 43).Thus, oxygen free radicals and hydroperoxides are generated continuously in the mitochondrial respiratory chain (7, 10) and they, particularly hydroxyl radical, cause oxidative damage to proteins, lipids, and DNA (39).

The continuous generation of ROS by mitochondria throughout cell life produces an age-related "chronic" oxidative stress that plays a key role in cellular aging. It is now well established that oxidative damage to mitochondrial DNA, proteins, and lipids occurs upon aging (5, 19, 39–41).

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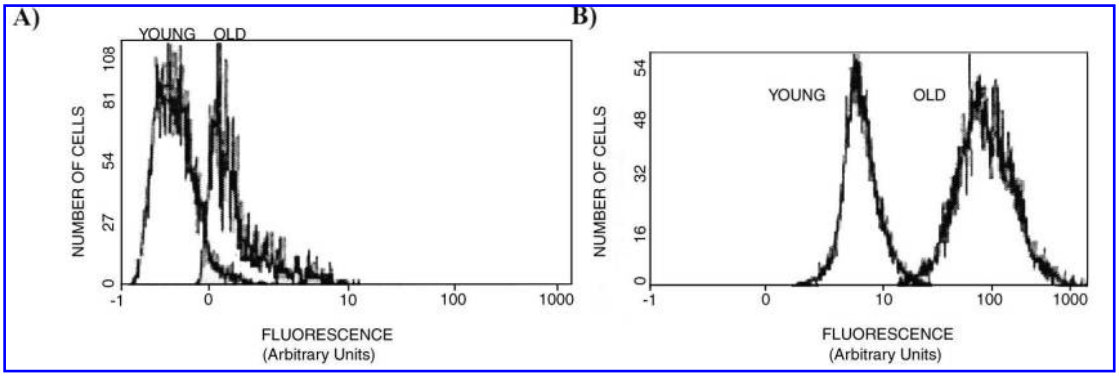

FIG. 1. Flow cytometric analysis of mitochondria and hepatocytes isolated from young and old rats. (**A**) Peroxide generation by liver mitochondria is higher in old than in young rats. It was estimated with dihydrorhodamine 123 using isolated mitochondria. (**B**) Intracellular peroxide levels are higher in hepatocytes isolated from old rats than in those from young rats. Peroxide levels were measured using dichlorodihydrofluorescein (39).

We found that late-onset administration of some sulfur-containing antioxidants protects against the age-associated glutathione (GSH) depletion in mice. It also prevented the age-related decline in neuromuscular coordination (47). These antioxidants also increased the mean life span of *Drosophila* (47).

More recently, we have investigated the protective effect of a standardized extract from dried leaves of *Ginkgo biloba* (EGb 761) on the age-associated oxidative damage to mitochondrial DNA (40). Oral administration of EGb 761 to rats for 3 months is able to prevent the oxidative damage to mitochondrial DNA that occurs in liver and brain upon aging (40). Mitochondrial GSH oxidation is also prevented. In fact, age-associated mitochondrial GSH oxidation and mitochondrial DNA damage are directly related (19) (see Fig. 2). Hence, EGb 761 or other antioxidants partially prevent the chronic oxidative stress associated with mitochondrial aging.

In conclusion, administration of certain antioxidants, such as GSH, thiazolidine carboxylate derivatives, vitamins C and E, or the *Ginkgo biloba* extract EGb 761, may prevent or delay the oxidative stress and the physiological impairment associated with aging. On the other hand, mitochondria are key mediators of apoptosis because opening the mitochondrial permeability transition pore (PT) is a critical step in apoptosis (48). Indeed, opening of the mitochondrial PT pores causes release of apoptogenic factors from the intermembrane space (12, 44). Mitochondria are at the same time the target and the source of ROS, which may induce apoptosis (37). Some common features of apoptotic cells and of cells from old animals include an increased mitochondrial hydrogen peroxide production, oxidation of GSH, and oxidation of mitochondrial DNA (13). However, a relationship between aging and apoptosis remains to be established.

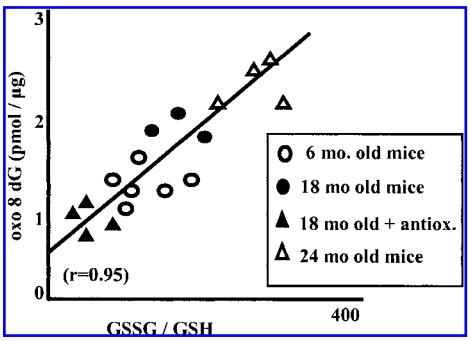

FIG. 2. Relationship between DNA damage and GSH oxidation. Age-associated mitochondrial GSH oxidation and mitochondrial DNA damage (estimated measuring the levels of 8-oxodeoxyguanosine) are directly related (19). Antioxidants given were vitamin C (50 g/kg body weight) and vitamin E (30 mg/kg body weight).

DIFFERENT LIFE EXPECTANCY BETWEEN MALES AND FEMALES OF VARIOUS SPECIES

Life expectancy over time by gender in Spain during the period 1900–1992 is shown in Table 1 (14). Two important features can be seen. On the one hand, life expectancy has increased from ~34 years to ~80 years of age during the 20th century. This is the highest increase in life expectancy in the human species in recorded history. Another increase of ~45 years is highly unlikely to occur again. Indeed, if we accept a maximal life span for humans of ~110 years, then, even if all diseases were cured and all humans reached the maximal life span, one would expect an increase of up to 30 years. Therefore, the dramatic increase in life span that has taken place in the 20th century is very unlikely to occur again. Another im-

TABLE 1. LIFE EXPECTANCY IN SPAIN BETWEEN 1900 AND 1992

Year	*Men*	*Women*	*Difference (%)*
1900	33.8	35.1	3.8
1960	67.4	72.2	7.1
1980	72.5	78.6	8.1
1992	73.7	81.0	9.9

Data are taken from reference 14.

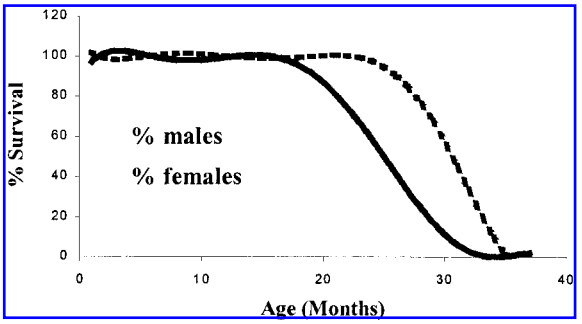

FIG. 3. Survival curve of Wistar rats. Female rats live longer than male rats. Rats were kept under standard conditions in the Faculty of Medicine, University of Valencia.

portant feature of Table 1 is that in all cases females have lived longer than males. Moreover, when, earlier in the century, both men and women died of diseases unrelated to aging, the increase of average life span of women was only 3.8% higher than in men. However, when at the end of the century, women died of diseases related to aging, their average life span was almost 10% higher than that of men. The higher longevity of females *versus* males could be attributed to social reasons. However, this is not a phenomenon specific to humans. A survival curve of Wistar rats in our laboratory is shown in Fig. 3. In this case, also, females lived considerably longer than males. Average life span for female rats was 15% higher than for males. Thus, the increase in average life span in women over men is unlikely to be attributable to social reasons, and basic biological phenomena may be underlying this fact (6).

OXIDANT PRODUCTION BY MITOCHONDRIA FROM MALES AND FEMALES

Hepatic mitochondria from females produce ~50% the amount of oxidants produced by mitochondria from males (see Fig. 4). This occurred when mitochondria were incubated either with pyruvate and malate or with succinate as respiratory substrates. When Wistar rats had been ovariectomized for 1 month, oxidant production by mitochondria was similar to that found in males. However, (see Fig. 4) when ovariectomized rats were treated with 1 mg/kg/day of estradiol, their oxidant production was similar to that of control females. Thus, ovariectomy causes an increase of oxidant production by mitochondria, which is completely prevented by estrogen replacement therapy. We conclude that estrogens are responsible for the lower production of mitochondrial oxidants in females versus males.

MITOCHONDRIA FROM POSTMITOTIC TISSUES PRODUCE MORE HYDROGEN PEROXIDE THAN THOSE FROM MITOTIC TISSUES

Miquel *et al.* have emphasized the idea (32) that aging should be studied in cells from postmitotic tissues. Mitosis causes a

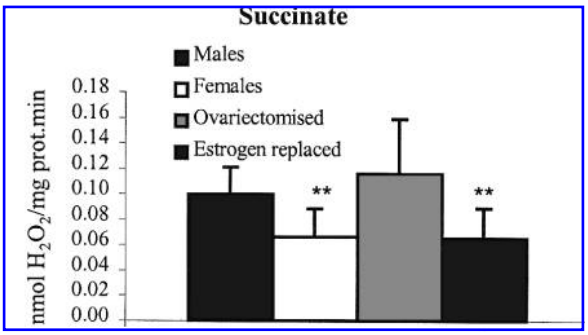

FIG. 4. Oxidant production by hepatic mitochondria from males and females: effect of ovariectomy and of estrogen replacement therapy. Hydrogen peroxide generation is higher in liver mitochondria from male rats than in mitochondria from female rats. Ovariectomy increases hydrogen peroxide production by liver mitochondria, and this effect is prevented by estrogen replacement. 17β-Estradiol was given subcutaneously at a daily dose of 1 μg/kg body weight for 1 month. The rate of hydrogen peroxide production was measured in isolated liver mitochondria from Wistar rats (3–5 months old) following the procedure described by Barja (3). Liver mitochondria were isolated as described by Rickwood *et al.* (38) and incubated with 10 m*M* succinate. The number of experiments was 12–14 for liver mitochondria. The statistical difference is indicated as follows: $^{**}p < 0.01$ versus male rats (6).

renewal of cell components, and therefore cell senescence is better studied in postmitotic tissues. The brain provides a unique model in which postmitotic cells (neurons) and mitotic cells (glia) coexist. Synaptic (neuronal) and nonsynaptic (glial) mitochondria may be isolated by differential centrifugation (27). Hydrogen peroxide production from synaptic mitochondria was more than fivefold higher than that from nonsynaptic mitochondria (see Fig. 5). In both cases, mitochon-

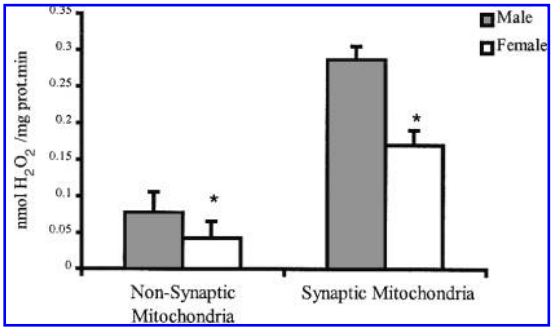

FIG. 5. Oxidant production by synaptic and nonsynaptic mitochondria. Hydrogen peroxide generation is higher in brain mitochondria from male rats than in mitochondria from female rats. The rate of hydrogen peroxide production was measured in synaptic and nonsynaptic mitochondria from Wistar rats (3–5 months old) following the procedure described by Barja (3). Synaptic and nonsynaptic mitochondria were isolated following the method described by Lai and Clark (27). Synaptic mitochondria were incubated with 10 m*M* succinate. The number of experiments was three to five for synaptic mitochondria and seven to nine for nonsynaptic mitochondria. The statistical difference is indicated as follows: $^{*}p < 0.05$ versus male rats (6).

dria from males always produced a consistently and significantly higher level of oxidants than those from females. This occurred when mitochondria were incubated either with succinate or with pyruvate and malate as respiratory substrate (see Fig. 5). In a similar fashion, when we compared hydrogen peroxide production by hepatic mitochondria with that from synaptic mitochondria, we found that the latter had a much higher rate of hydrogen peroxide production than the former (Figs. 4 and 5).

MITOCHONDRIAL GSH, BUT NOT TOTAL CELLULAR GSH IS HIGHER IN FEMALES THAN IN MALES

The GSH redox system constitutes a major intracellular endogenous defense mechanism against oxidation (45). Indeed, intracellular GSH levels in hepatocytes are ~ 5 µmol/g of tissue, *i.e.,* a value similar to that of glucose (46). Early studies by Jocelyn (25) and by Meister (30) showed that mitochondrial GSH is critical in the maintenance of normal cell function. This has also been emphasized by Fernández Checa *et al.* (15). We found that aging causes a significant decrease in GSH levels in mitochondria. In fact, most of the oxidation of GSH that can be observed in cells is due to oxidation of the mitochondrial compartment (19). Levels of mitochondrial GSH in males are 40% lower than those of females (see Fig. 6). However, when female Wistar rats had been ovariectomized for 1 month, their mitochondrial GSH levels fell to values similar to those found in males. Moreover, when ovariectomized rats were treated with estrogen replacement therapy (1 mg/kg /day of 17β-estradiol injected subcutaneously for 1 month), GSH levels returned to the high values found in females. This, together with the facts reported in Fig. 4, *i.e.,* that estrogen therapy decreases hydrogen peroxide production by mitochondria, emphasizes the protective role of estrogens against mitochondrial free radical damage associated with aging.

OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IS MUCH HIGHER IN MALES THAN IN FEMALES

In our previous aging studies, we observed that oxidation of mitochondrial GSH correlates with oxidative damage to mitochondrial DNA (see Fig. 2) (19). The same occurs in apoptosis (13). Thus, we checked whether males had a higher level of oxidative damage to mitochondrial DNA than females. Indeed this is the case and the level of 8-oxo-deoxyguanosine in hepatic mitochondrial DNA is fourfold higher than that found in females (see Fig. 7). Recent work by Loft and colleagues showed that urinary excretion of 8-oxo-deoxyguanosine is higher in males than in females (28). In a similar fashion, Proteggente *et al.* reported that oxidative damage to DNA is higher in males than in females; however, damage to mitochondrial DNA was not measured (35). The group of Ames was first to report that mitochondrial DNA oxidation is associated with aging (1). We confirmed these findings and, as mentioned earlier, observed that there is a direct relationship between oxidation of GSH and oxidation of DNA in mitochondria (19). More recently, Barja and his co-workers have found an increase in mitochondrial, but not in nuclear, DNA damage inversely associated with longevity (4). Long-lived species show a lower level of oxidation of mitochondrial DNA than short-lived ones (36).

ESTRADIOL INCREASES THE EXPRESSION OF MITOCHONDRIAL ANTIOXIDANT AND LONGEVITY GENES

The beneficial effects of estradiol have been widely documented. In particular, estradiol has been shown to have a cardioprotective role (2). The possible importance of estradiol in the prevention of Alzheimer's disease has been proposed, but is not widely accepted (24). Moreover, estrogens have a powerful *in vitro* antioxidant effect. However, the possible effects

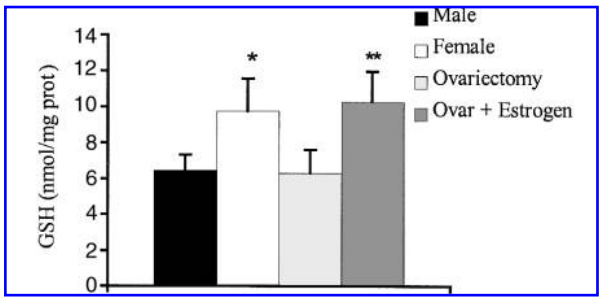

FIG. 6. GSH levels in hepatic mitochondria from males and females: effect of ovariectomy and of estrogen replacement therapy. GSH levels are lower in liver mitochondria from male rats than in those from females. Ovariectomy decreases GSH, and estrogen replacement therapy prevents this decrease. Animal experimental conditions are as in Fig. 5. GSH levels were determined as described by Brigelius *et al.* (8). The statistical difference is indicated as follows: $*p < 0.05$, $**p < 0.01$ versus male rats.

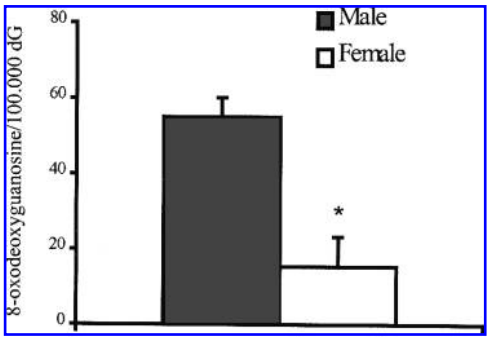

FIG. 7. Mitochondrial DNA damage in liver from males and females. Mitochondrial DNA oxidative damage is higher in liver from male rats than in liver from female rats. Isolation of mitochondrial DNA and measurement of 8-oxodeoxyguanosine levels (as an index of oxidative damage) were performed as described by García de la Asunción *et al.* (19). The statistical difference is indicated as follows: $*p < 0.05$ versus male rats.

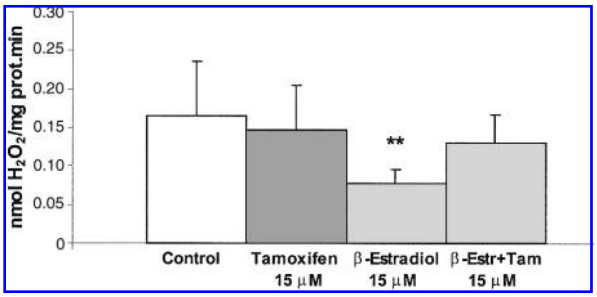

FIG. 8. Hydrogen peroxide levels in mammary gland tumor cells (MCF-7) treated with estradiol and/or tamoxifen. Hydrogen peroxide levels are lower in cells preincubated for 1 h with 15 μ*M* estradiol. The addition of 15 μ*M* tamoxifen prevents this effect. Hydrogen peroxide levels were measured in MCF-7 cells following the procedure described by Barja (3). The statistical difference is indicated as follows: $^{**}p < 0.01$ versus male rats.

of estradiol on longevity *in vivo* are unlikely to be due to its chemical antioxidant properties. In fact, the recommended dose of estradiol as part of estrogen replacement therapy is 50 μg/day. However, a widely recommended dose of vitamin E as an antioxidant supplement is 400 mg/day, *i.e.,* 8,000 times more than that of estradiol. Therefore, to have significant antioxidant properties due to its chemical structure, estradiol should be 8,000 times more potent an antioxidant than vitamin E. This is clearly not the case. Therefore, we reasoned that the beneficial effects of estrogens in preventing oxidant production by mitochondria might not be due to their chemical properties, but to the induction of the expression of antioxidant genes. To check this hypothesis, we tested whether the effect of estradiol on hydrogen peroxide levels in cells is mediated by receptors. To this end, we used tamoxifen (the estrogen receptor antagonist) and checked its effects on hydrogen peroxide levels in a mammary tumor gland cell line (MCF-7 cells). Estradiol caused a significant decrease in the levels of hydrogen peroxide in cells (in agreement with our findings *in vivo*). Tamoxifen significantly blocked this effect (see Fig. 8).

Thus, we checked whether estradiol, acting via its interaction with estrogen receptors, increased the expression of the antioxidant enzymes superoxide dismutase and glutathione peroxidase.

The fact that glutathione peroxidase activity was higher in females than in males was first reported by Pinto and Bartley in the late 1960s (34). However, these authors did not relate this fact to the different longevity between males and females. Glutathione peroxidase activity in males is ~50% of the values found in females (see Fig. 9). Furthermore, we checked whether this was due to a different gene expression in females and males. Molecular expression of the glutathione peroxidase gene is significantly lower in males than in females (see Fig. 9).

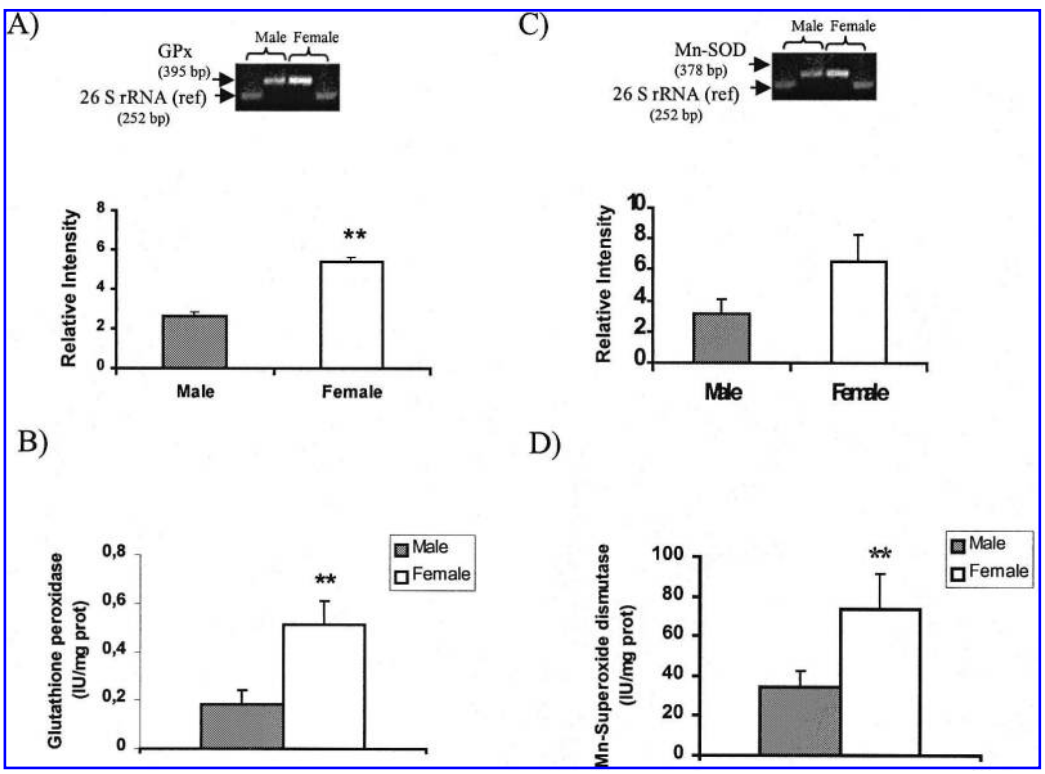

FIG. 9. Expression and activity of glutathione peroxidase (GPx) and Mn-SOD in liver from male and female rats. Antioxidant defense is higher in liver mitochondria from female rats than in those from males (6). Expression of antioxidant enzymes was studied by RT-PCR using specific oligonucleotides. (**A**) Glutathione peroxidase mRNA expression. (**B**) Glutathione peroxidase activity measured as described by Flohe and Gunzler (16). (**C**) Mn-SOD mRNA expression. (**D**) Mn-SOD activity was measured as described by Flohe and Otting (17). The statistical difference is indicated as follows: $^{**}p < 0.01$ versus male rats.

In a similar fashion, we tested the effect of gender on enzyme activity and on gene expression of mitochondrial superoxide dismutase. Mn-SOD activity in hepatic mitochondria from males is ~40% of the value found in females (see Fig. 9). This is due to a higher expression of the Mn-SOD gene in females than in males (see Fig. 9). Thus, the expression of both superoxide dismutase and glutathione peroxidase is significantly lower in males than in females. This is important in the context of previous work by Orr and Sohal (33) who showed, in *Drosophila,* that overexpression of superoxide dismutase or catalase alone did not increase average life span. However, *Drosophila* overexpressing both superoxide dismutase and catalase did indeed have a higher average life span than the controls. In this sense, female rats behave as double transgenics overexpressing both superoxide dismutase and glutathione peroxidase when compared with males. This in itself may explain why the average life span of female rats is higher than that of males.

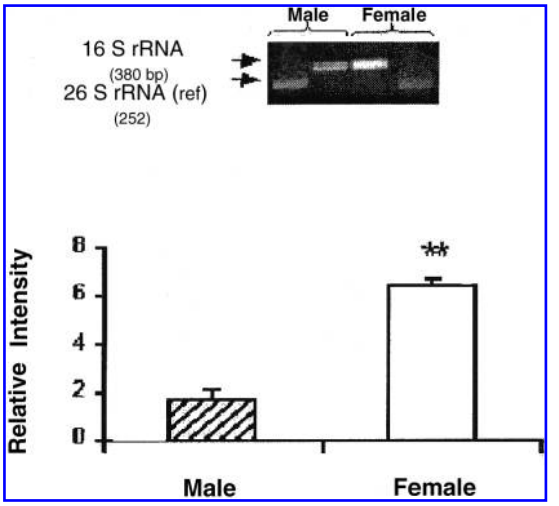

FIG. 10. Expression of the biological marker 16S rRNA in liver from male and female rats. Expression of the biological marker 16S rRNA is higher in liver from female rats than in liver from male rats. The mRNA expression was studied by RT-PCR using specific oligonucleotides (6). The statistical difference is indicated as follows: $^{**}p < 0.01$ versus male rats.

BIOLOGICAL MARKERS OF AGING INDICATE THAT FEMALES ARE BIOLOGICALLY YOUNGER THAN MALES OF THE SAME CHRONOLOGICAL AGE

A major challenge of gerontology is to find reliable markers of biological, as opposed to chronological, aging. In many cases, the search for reliable parameters of biological aging has been sterile. Work by Lang and co-workers has established GSH levels as a reliable marker of biological aging (23). GSH levels are significantly higher in females than in males of the same chronological age (see Fig. 6). Thus, from this point of view, females behave as younger than males of the same age. Another interesting marker of aging is 16S rRNA. Work by the group of Marco (9) showed an important decrease in mitochondrial transcripts of 16S rRNA with aging. Moreover, they reported that these changes correlate with the shape of the life span curve. Work by the group of Davies showed that 16S rRNA degradation is associated with oxidative stress (11). Thus, we checked the hypothesis that mitochondria from cells of higher biological age should have lower expression of 16S rRNA. If this is the case, mitochondria from female animals should have a higher expression of 16S rRNA than those from males. Indeed, this is the case and 16S rRNA expression was >700% higher in females than in males (see Fig. 10). Thus, we can conclude that biological markers of aging indicate that females behave as if they were younger than males of the same chronological age.

CONCLUDING REMARKS

Females live longer than males in many species, including humans. This can be explained on the basis of the mitochondrial theory of aging. Hepatic or brain mitochondria from females produce significantly less hydrogen peroxide than those from males. On the other hand, they have a higher level of mitochondrial GSH than those from males. Oxidative damage to mitochondrial DNA, which is related to aging, is fourfold higher in males than in females. This difference between females and males may be explained by the beneficial effects of estrogens: ovariectomy abolishes the gender differences between males and females, and estrogen replacement therapy prevents the effects of ovariectomy. The beneficial effects of estrogens are due, not to their chemical antioxidant properties, but rather to their receptor-mediated effects on the genome. Expression of antioxidant enzymes such as Mn-SOD and glutathione peroxidase is higher in females than in males. In this respect, females behave as double transgenics overexpressing superoxide dismutase and glutathione peroxidase. The challenge for the future in this field is to find molecules that have the beneficial effects of estradiol, but without its feminizing effects. Phytoestrogens or phytoestrogen-related molecules may be good candidates to meet this challenge.

ABBREVIATIONS

GSH, glutathione; Mn-SOD, manganese superoxide dismutase; PT, transition pore; ROS, reactive oxygen species.

REFERENCES

1. Ames BN, Shigenaga M, and Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 90: 7915–7922, 1993
2. Arnal JF, Clamens S, Pechet C, Negre-Salvayre A, Allera C, Girolami J-P, Salvayre R, and Bayard F. Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. *Proc Natl Acad Sci U S A* 93: 4108–4113, 1996.
3. Barja G. Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity and

relation to aging and longevity. *J Bioenerg Biomembr* 31: 347–366, 1999.
4. Barja G and Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J* 14: 312–318, 2000.
5. Benzi G and Moretti A. Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system. *Free Radic Biol Med* 19: 77–101, 1995.
6. Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo FV, and Vina J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic Biol Med* 34: 546–552, 2003.
7. Boveris A and Chance B. The mitochondrial generation of hydrogen peroxide. *Biochem J* 134: 707–716, 1973.
8. Brigelius R, Muckel C, Akerboom TPM, and Sies H. Identification and quantitation of glutathione in hepatic protein mixed disulfides and its relationship to glutathione disulfide. *Biochem Pharmacol* 32: 2529–2534, 1983.
9. Calleja M, Peña P, Ugalde C, Ferreiro C, Marco R, and Garesse R. Mitochondrial DNA remains intact during *Drosophila* aging, but the levels of mitochondrial transcripts are significantly reduced. *J Biol Chem* 268: 18891–18897, 1993.
10. Chance B, Sies H, and Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527–604, 1979.
11. Crawford DR, Wang Y, Schools GP, Kochheiser J, Davies KJA. Down regulation of mammalian mitochondrial RNAs during oxidative stress. *Free Radic Biol Med* 22: 551–559, 1997.
12. Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N, Prévost MC, Leber B, Andrews D, Penninger J, and Kroemer G. Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. *FASEB J* 14: 729–739, 2000.
13. Esteve J, Mompó J, García de la Asunción J, Sastre J, Asensi M, Boix J, Viña JR, Viña J, and Pallardó FV. Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies in vivo and in vitro. *FASEB J* 13: 1055–1064, 1999.
14. Fernández Ballesteros R. Diez-Nicolas J, and Ruiz-Torres A. Spain. In: *Aging in Europe,* edited by Schroots J. Fernandez-Ballesteros R, and Rudinger G. Amsterdam: IOS Press, 1999, pp. 107–121.
15. Fernandez-Checa JC, Kaplowitz N, Garcia-Ruiz C, and Colell A. Mitochondrial glutathione: importance and transport. *Semin Liver Dis* 18: 389–401, 1998.
16. Flohe L and Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 105: 114–121, 1984.
17. Flohe L and Otting F. Superoxide dismutase assays. *Methods Enzymol* 105: 93–104, 1984.
18. Forman HJ and Azzi A. On the virtual existence of superoxide anions in mitochondria: thoughts regarding its role in pathophysiology. *FASEB J* 11: 374–375, 1997.
19. Garcia de la Asunción J, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, Sastre J, and Viña J. Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. *FASEB J* 10: 333–338, 1996.
20. Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, and Ames BN. Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. *Proc Natl Acad Sci U S A* 94: 3064–3069, 1997.
21. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11: 298–300, 1956.
22. Harman D. Free radical theory of aging: role of free radicals in the origination and evolution of life, aging and disease processes. In: *Free Radicals, Aging and Degenerative Diseases,* edited by Johnson JE Jr, Walford R, Harman D, and Miquel J. New York: Alan R. Liss, 1986, pp. 3–49.
23. Hazelton GA and Lang CA. Glutathione levels during the mosquito life span with emphasis on senescence. *Proc Soc Exp Biol Med* 176: 249–256, 1984.
24. Henderson VW, Paganini-Hill A, Miller BL, Elble RJ, Reyes PF, Shoupe D, McCleary CA, Klein RA, Hake AM, and Farlow MR. Estrogen for Alzheimer's disease in women. Randomized, double-blind, placebo-controlled trial. *Neurology* 54: 295–301, 2000.
25. Jocelyn PC. Some properties of mitochondrial glutathione. *Biochim Biophys Acta* 396: 427–436, 1975.
26. Ku H, Brunk UT, and Sohal RS. Relationship between mitochondrial superoxide and hydroperoxide production and longevity of mammalian species. *Free Radic Biol Med* 15: 621–627, 1993.
27. Lai JCK and Clark JB. Preparation of synaptic and nonsynaptic mitochondria from mammalian brain. *Methods Enzymol* 55: 51–60, 1979.
28. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, and Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* 13: 2241–2247, 1992.
29. Medvedev Z. An attempt at a rational classification of theories of ageing. *Biol Rev Camb Philos Soc* 65: 375–398, 1990.
30. Meister A. Mitochondrial changes associated with glutathione deficiency. *Biochim Biophys Acta* 1271: 35–42, 1995.
31. Miquel J and Economos AC. Favorable effects of the antioxidants sodium and magnesium thiazolidine carboxylate on the vitality and life span of *Drosophila* and mice. *Exp Gerontol* 14: 279–285, 1979.
32. Miquel J, Economos AC, Fleming J, and Johnson JE Jr. Mitochondrial role in cell aging. *Exp Gerontol* 15: 575–591, 1980.
33. Orr WC and Sohal R. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster. Science* 263: 1128–1130, 1994.
34. Pinto RE and Bartley W. The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenates. *Biochem J* 112: 109–115, 1969.
35. Proteggente AR, England TG, Rehman A, Rice-Evans CA, and Halliwell B. Gender differences in steady-state levels of oxidative damage to DNA in healthy individuals. *Free Radic Res* 36: 157–162, 2002.
36. Richter C, Park JW, and Ames B. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A* 85: 6465–6467, 1988.
37. Richter C, Schweizer M, Cossarizza A, and Franceschi C. Control of apoptosis by the cellular ATP level. *FEBS Lett* 378: 107–110, 1996.

38. Rickwood D, Wilson MT, and Darley-Usmar VM. Isolation and characteristics of intact mitochondria. In: *Mitochodria: A Practical Approach,* edited by Darley-Usmar VM, Wilson MT, and Rickwood D. Oxford: IRL Publisher Press Limited, 1987, pp. 1–16.
39. Sastre J, Pallardó VC, Plá R, Pellín A, Juan G, O'Connor E, Estrela JM, Miquel J, and Viña J. Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. *Hepatology* 24: 1199–1205, 1996.
40. Sastre J, Millán A, García de la Asunción J, Plá R, Juan G, Pallardó FV, O'Connor E, Martín JA, Droy-Lefaix MT, and Viña J. A *Ginkgo biloba* extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. *Free Radic Biol Med* 24: 298–304, 1998.
41. Shigenaga MK, Hagen TM, and Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Nat Acad Sci U S A* 91: 10771–10778, 1994.
42. Sohal RS and Weindruch R. Oxidative stress, caloric restriction and aging. *Science* 273: 59–63, 1996.
43. Sohal RS, Svensson I, and Brunk UT. Hydrogen peroxide production by liver mitochondria in different species. *Mech Ageing Dev* 53: 209–215, 1990.
44. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Constantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, and Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441–446, 1999.
45. Viña J (Ed.). *Glutathione: Metabolism and Physiological Functions.* Boston, MA: CRC Press, 1990.
46. Vina J, Hems R, and Krebs HA. Reaction of formiminoglutamate with liver glutamate dehydrogenase. *Biochem J* 170: 711–713, 1978.
47. Viña J, Sastre J, Anton V, Bruseghini L, Esteras A, and Asensi M. Effect of aging on glutathione metabolism. Protection by antioxidants. In: *Free Radicals and Aging,* edited by Emerit I. and Chance B. Basel, Switzerland: Birkhauser Verlag, 1992, pp. 136–144.
48. Zamzami NS, Susin A, Marchetti P, Hirsch T, Gómez-Monterrey I, Castedo M, and Kroemer G. Mitochondrial control of nuclear apoptosis. *J Exp Med* 183: 1533–1544, 1996.

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1. Zhen Pan, Christopher Chang. 2012. Gender and the regulation of longevity: Implications for autoimmunity. *Autoimmunity Reviews* **11**:6-7, A393-A403. [CrossRef]
2. Nobuyuki Kimura, Sachi Okabayashi, Fumiko Ono. 2012. Dynein Dysfunction Disrupts Intracellular Vesicle Trafficking Bidirectionally and Perturbs Synaptic Vesicle Docking via Endocytic Disturbances. *The American Journal of Pathology* **180**:2, 550-561. [CrossRef]
3. P. Pascua, C. Camello-Almaraz, M. J. Pozo, F. E. Martin-Cano, E. Vara, J. A. Fernández-Tresguerres, P. J. Camello. 2011. Aging-induced alterations in female rat colon smooth muscle: the protective effects of hormonal therapy. *Journal of Physiology and Biochemistry* . [CrossRef]
4. Rocío Guevara, Magdalena Gianotti, Jordi Oliver, Pilar Roca. 2011. Age and sex-related changes in rat brain mitochondrial oxidative status. *Experimental Gerontology* . [CrossRef]
5. Yusra A Al-Yafee, Laila Y Al- Ayadhi, Samina H Haq, Afaf K El-Ansary. 2011. Novel metabolic biomarkers related to sulfur-dependent detoxification pathways in autistic patients of Saudi Arabia. *BMC Neurology* **11**:1, 139. [CrossRef]
6. James W. Simpkins, Kun Don Yi, Shao-Hua Yang, James A. Dykens. 2010. Mitochondrial mechanisms of estrogen neuroprotection. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1800**:10, 1113-1120. [CrossRef]
7. Jose Viña , Consuelo Borrás . 2010. Women Live Longer than Men: Understanding Molecular Mechanisms Offers Opportunities to Intervene by Using Estrogenic Compounds. *Antioxidants & Redox Signaling* **13**:3, 269-278. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Arjen Joosse, Esther De Vries, Casper H. Van Eijck, Alexander M. M. Eggermont, Tamar Nijsten, Jan Willem W. Coebergh. 2010. Reactive oxygen species and melanoma: an explanation for gender differences in survival?. *Pigment Cell & Melanoma Research* **23**:3, 352-364. [CrossRef]
9. Raúl López-Grueso, Consuelo Borrás, Juan Gambini, José Viña. 2010. El envejecimiento y la ovariectomía causan una disminución del consumo cerebral de glucosa in vivo en ratas Wistar. *Revista Española de Geriatría y Gerontología* **45**:3, 136-140. [CrossRef]
10. Irina I. Rudneva, Ekaterina N. Skuratovskaya, Natalya S. Kuzminova, Tatyana B. Kovyrshina. 2010. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **151**:2, 229-239. [CrossRef]
11. Xing-Tai Li, Hong-Cheng Li, Chun-Bin Li, De-Qiang Dou, Ming-Bo Gao. 2010. Protective Effects on Mitochondria and Anti-Aging Activity of Polysaccharides from Cultivated Fruiting Bodies of Cordyceps militaris. *The American Journal of Chinese Medicine* **38**:06, 1093-1106. [CrossRef]
12. Fernanda Schäfer Hackenhaar, Tiago Boeira Salomon, Paulo V. Gil Alabarse, Guilherme Ehrenbrink, Mara Silveira Benfato. 2009. Pulmonary antioxidant defences and protein damage during the ageing process of both sexes. *Cell Biochemistry and Function* **27**:6, 378-382. [CrossRef]
13. Rocío Guevara, Francisca M. Santandreu, Adamo Valle, Magdalena Gianotti, Jordi Oliver, Pilar Roca. 2009. Sex-dependent differences in aged rat brain mitochondrial function and oxidative stress. *Free Radical Biology and Medicine* **46**:2, 169-175. [CrossRef]
14. T. Jursa, D. R. Smith. 2008. Ceruloplasmin Alters the Tissue Disposition and Neurotoxicity of Manganese, but not its Loading onto Transferrin. *Toxicological Sciences* **107**:1, 182-193. [CrossRef]
15. Agata Karowicz-Bilinska, Magdalena Plodzidym, Joanna Krol, Anna Lewinska, Grzegorz Bartosz. 2008. Changes of markers of oxidative stress during menstrual cycle. *Redox Report* **13**:5, 237-240. [CrossRef]
16. J TRESGUERRES, R KIREEV, A TRESGUERRES, C BORRAS, E VARA, C ARIZNAVARRETA. 2008. Molecular mechanisms involved in the hormonal prevention of aging in the rat#. *The Journal of Steroid Biochemistry and Molecular Biology* **108**:3-5, 318-326. [CrossRef]

17. Walter Malorni , Ilaria Campesi , Elisabetta Straface , Stefano Vella , Flavia Franconi . 2007. Redox Features of the Cell: A Gender Perspective. *Antioxidants & Redox Signaling* **9**:11, 1779-1802. [Citation] [Full Text PDF] [Full Text PDF with Links]
18. Ali Razmara, Sue P. Duckles, Diana N. Krause, Vincent Procaccio. 2007. Estrogen suppresses brain mitochondrial oxidative stress in female and male rats. *Brain Research* **1176**, 71-81. [CrossRef]
19. R. A. Kireev, A. F. Tresguerres, E. Vara, C. Ariznavarreta, J. A. F. Tresguerres. 2007. Effect of chronic treatments with GH, melatonin, estrogens, and phytoestrogens on oxidative stress parameters in liver from aged female rats. *Biogerontology* **8**:5, 469-482. [CrossRef]
20. Wulf Dröge, Hyman M. Schipper. 2007. Oxidative stress and aberrant signaling in aging and cognitive decline. *Aging Cell* **6**:3, 361-370. [CrossRef]
21. Ana Navarro , Maria Jesús Sánchez-Pino , Carmen Gómez , Manuel J. Bández , Enrique Cadenas , Alberto Boveris . 2007. Dietary Thioproline Decreases Spontaneous Food Intake and Increases Survival and Neurological Function in Mice. *Antioxidants & Redox Signaling* **9**:1, 131-141. [Abstract] [Full Text PDF] [Full Text PDF with Links]
22. J. William O. Ballard, Richard G. Melvin, Joseph T. Miller, Subhash D. Katewa. 2007. Sex differences in survival and mitochondrial bioenergetics during aging in Drosophila. *Aging Cell* **6**:5, 699. [CrossRef]
23. Evelyn Perez, Xiaofei Wang, James W. SimpkinsRole of antioxidant activity of estrogens in their potent neuroprotection 503-524. [CrossRef]
24. Sameh S. Ali, Chengjie Xiong, Jacinta Lucero, M. Margarita Behrens, Laura L. Dugan, Kevin L. Quick. 2006. Gender differences in free radical homeostasis during aging: shorter-lived female C57BL6 mice have increased oxidative stress. *Aging Cell* **5**:6, 565-574. [CrossRef]
25. Tapiwanashe Magwere, Melanie West, Kumars Riyahi, Michael P. Murphy, Robin A.J. Smith, Linda Partridge. 2006. The effects of exogenous antioxidants on lifespan and oxidative stress resistance in Drosophila melanogaster. *Mechanisms of Ageing and Development* **127**:4, 356-370. [CrossRef]
26. C ALVARADOFRADUA. 2005. El daño peroxidativo en leucocitos peritoneales de ratones viejos disminuye si se suplementa la dieta con galletas enriquecidas con antioxidantes. Relación con la supervivencia#. *Revista Española de Geriatría y Gerontología* **40**:6, 351-356. [CrossRef]
27. Bradford Frank, Sanjay Gupta. 2005. A Review of Antioxidants and Alzheimer's Disease. *Annals of Clinical Psychiatry* **17**:4, 269-286. [CrossRef]
28. A. Navarro, M.J. Bández, C. Gómez, H. González, N. Escudero, J.C. García-Ortiz, J.F. Carrión, M.J. Sánchez-Pino, J.M. López-Cepero. 2005. Efecto de dos antioxidantes en la supervivencia, las actividades neurológicas y la función mitocondrial de ratones senescentes#. *Revista Española de Geriatría y Gerontología* **40**:4, 235-242. [CrossRef]
29. Consuelo Borras, Juan Gambini, Ma Carmen Gomez-Cabrera, Juan Sastre, Federico V. Pallardo, Giovanni E. Mann, Jose Vina. 2005. 17beta-oestradiol up-regulates longevity-related, antioxidant enzyme expression via the ERK1 and ERK2[MAPK]/NFkappaB cascade. *Aging Cell* **4**:3, 113-118. [CrossRef]
30. Scott W. Ballinger. 2005. Mitochondrial dysfunction in cardiovascular disease. *Free Radical Biology and Medicine* **38**:10, 1278-1295. [CrossRef]
31. Jose Viña, Consuelo Borrás, Juan Gambini, Juan Sastre, Federico V. Pallardó. 2005. Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. *FEBS Letters* **579**:12, 2541-2545. [CrossRef]
32. Hea Young Oh , Sung Soo Kim , Hae-Yun Chung , Sun Yoon . 2005. Isoflavone Supplements Exert Hormonal and Antioxidant Effects in Postmenopausal Korean Women with Diabetic Retinopathy. *Journal of Medicinal Food* **8**:1, 1-7. [Abstract] [Full Text PDF] [Full Text PDF with Links]
33. V KATALINIC, D MODUN, I MUSIC, M BOBAN. 2005. Gender differences in antioxidant capacity of rat tissues determined by 2,2#-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric

reducing antioxidant power (FRAP) assays. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **140**:1, 47-52. [CrossRef]

34. D. J. Marcinek. 2004. Mitochondrial dysfunction measured in vivo. *Acta Physiologica Scandinavica* **182**:4, 343-352. [CrossRef]
35. A Navarro. 2004. Mitochondrial enzyme activities as biochemical markers of aging. *Molecular Aspects of Medicine* **25**:1-2, 37-48. [CrossRef]
36. Tim S Nawrot, Jan A Staessen, Jeffrey P Gardner, Abraham Aviv. 2004. Telomere length and possible link to X chromosome. *The Lancet* **363**:9408, 507-510. [CrossRef]
37. 2003. Anti-Aging Medicine LiteratureWatch. *Journal of Anti-Aging Medicine* **6**:4, 341-347. [Citation] [Full Text PDF] [Full Text PDF with Links]
38. Tory M. Hagen . 2003. Oxidative Stress, Redox Imbalance, and the Aging Process. *Antioxidants & Redox Signaling* **5**:5, 503-506. [Citation] [Full Text PDF] [Full Text PDF with Links]