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Brain mitochondrial dysfunction in aging: conditions that improve survival, neurological performance and mitochondrial function

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1. ABSTRACT

Mice with *(a)* high spontaneous neurological activity, or subjected to *(b)* moderate exercise or *(c)* dietary supplemented with high doses of vitamin E from 28 weeks of age to senescence (76 wk of age), showed an increased survival and a retardation in the development of the neurological deficits associated to aging. During aging there was an increase in dysfunctional brain mitochondria, characterized by an increased content of oxidation products and by a diminished functional activity. The mitochondrial oxidative damage observed in adult (52 wk) and senescent mice (76 wk) was partially ameliorated in the groups of animals subject to the mentioned experimental conditions, and this decrease in mitochondrial oxidative damage was related to the improvement in neurological performance. In brain mitochondria, the activities of enzymes that are critical for mitochondrial function (mtNOS, NADH-dehydrogenase, and cytochrome oxidase) decreased progressively during aging and constituted aging markers. Usual clinical recommendations for aged humans, such as increased neurological activity, moderate exercise, and vitamin E supplementation, proved to be effective in increasing mice survival and neurological performances, along with a better mitochondrial function and a lower content of oxidation products.

2. INTRODUCTION

A growing body of evidence indicates that oxidative damage plays a role in aging and in the frequently accompanying neurological diseases. In recent years, the free radical hypothesis of aging has attracted attention and collected an important body of experimental support (1). Aging in experimental animals, usually mice and rats, is associated with *(a)* an increased level of oxidation products, such as TBARS and protein carbonyls, in the organs and in isolated mitochondria, and *(b)* an impairment of the mitochondrial function, that consists in a decrease of active respiration and the electron transfer activity of the complexes of the respiratory chain (2). Recommendations that are commonly followed by a great number of aging humans, such as increased neurological activity (3), moderate exercise (4), and vitamin E supplementation (5), proved to be effective in increasing mice survival and neurological performances, along with a better mitochondrial function and a lower content of oxidation products.

The first aim of this study was to select life conditions that increase mice survival, i.e. median and maximal lifespan. Mice and rats are commonly used for the convenience of their relative short lifespan, which are, depending on the strains, in the range of 65 to 150 weeks (6,7). The mice strain used in this study, Swiss CD-1/UCadiz, belong to a senescence accelerated strain similar to AKR, SAM, NZB/Lac, and SJL/J that exhibit a median lifespan of 36-57 wk and a maximal lifespan of 52-83 wk (8). In the present study we selected three life conditions for the experimental mice: *(a)* mice with high spontaneous neurological activity; *(b)* mice subject to moderate physical exercise; and *(c)* mice with dietary supplementation of vitamin E. In the three cases, mice were monitored following physiological indicators of aging, such as survival and neurological performance, along with biochemical parameters indicators of mitochondrial function. In the latter category, the determinations included mitochondrial markers of oxidative damage, the electron transfer activities of the complexes (I to IV) of the mitochondrial respiratory chain, and the activity mitochondrial nitric oxide synthase (mtNOS) activity, which is being recognized as a sensitive aging marker (4,9). The biochemical markers of aging were assayed in mitochondria isolated from whole mouse brain with the rationale of the association of mitochondrial dysfunction with the neurological deficits developed during aging.

3. MITOCHONDRIA AND AGING

There is a current and active interest in the characteristics and mechanism of the decline in mitochondrial functions that occurs during aging (1,10-13), an interest that extends to treatments with antioxidants and to conditions that retard such loss of mitochondrial functions (4,5,14,15). The main mitochondrial alterations observed upon aging include an increased content of oxidation products and a diminished rate of electron transfer in mitochondrial complexes I and IV and in active state 3 respiration, but without uncoupling or decreased F_1-ATPase activity. Then, mitochondria isolated from aged animals exhibit a condition of functional impairment and are named “age-dependent dysfunctional mitochondria” (9).

The maintenance or loss of mitochondrial function during aging is both an interesting and a controversial issue. Mitochondria are brought to attention in aging biology due to (a) the central role of mitochondria in producing chemical energy (ATP) to meet cellular requirements, and (b) the declines of metabolic rate and of physical performance in energy-requiring tasks which are associated to the aging process (16). Mitochondrial function includes the conversion of the chemical redox energy of substrate oxidation; first, to a proton electrochemical gradient and then to high energy phosphate chemical bonds (ATP) in a highly organized and coupled process that requires the physical integrity of the mitochondrial inner membrane.

Moreover, the mitochondrial hypothesis of aging considers mitochondria as the pacemaker of tissue aging due to continuous mitochondrial production of reactive oxygen and nitrogen species. The free radical theory of aging emerged from the views of Gerschman (1954) (17) who postulated that oxygen free radicals are the common molecular mechanism of oxygen and radiation toxicity, and of Harman (1956) (18) who considered that free radicals, generated as byproducts of biological oxidations, produce random and cumulative cellular damage which leads to tissue and organ aging. Two initial free radicals, O_2^- (19,20) and NO (21,22), are continuously produced in mitochondria, and other reactive species such as H_2O_2, $ONOO^-$, HO^-, ROO^-, and $^1O_2$ are derived from the primary production of O_2^- and NO (23). Superoxide anion radical is generated as a byproduct of the respiratory chain electron transfer, mainly by the autoxidation of ubisemiquinone (24,25). Nitric oxide is the product of the enzymatic action of mitochondrial nitric oxide synthase (mtNOS) (21,22), a specialized nitric oxide synthase (26) that carries out a classical NOS reaction, requiring NADPH, arginine, O_2 and Ca^{2+}/calmodulin for enzyme activity (27).

An increase in the steady state concentration of any of the reactive oxygen or nitrogen species and the self-propagation of free radical reactions constitute the chemical basis of an increased oxidative damage to lipid and proteins, a situation that when sustained constitutes a factor that promotes a faster aging (28). The free radical chain reactions are started by O_2^- and NO and involve a series of reactive oxygen and nitrogen species which are capable of damaging mitochondrial membranes, proteins and DNA. Mitochondria, the main cellular site of oxygen uptake and oxyradical generation, are also the main target of oxyradical-mediated damage. Cumulative free radical damage contributes to cell and tissue senescence and leads mitochondria to a state of mitochondrial dysfunction with decreased organelle ability to synthesize ATP and to adapt themselves to the destabilizing effects of cellular stress (1,28). Dysfunctional mitochondria show decreased state 3 oxygen uptake (5,12) and inner membrane potential (29), and increased O_2^- and H_2O_2 production (30), as well as

increased size (29). Dysfunctional mitochondria generate intracellular signals for lysosomal digestion and for apoptosis. Thus, conditions that retard the loss of mitochondrial functions should prevent the decrease in the neurological performance associated to aging, and then increase the life span.

4. MATERIALS AND METHODS

4.1. Animals

Mice of the Swiss CD-1/UCadiz strain inbred at the Department of Experimental Animals of the University of Cadiz were housed in groups of 5 animals at 22 $\pm$ 2 °C with 12 h/12 h light/dark cycles and with full access, *ad libitum,* to food and water. Mice were weekly weighed and periodically checked to verify their pathogen-free condition. Animal experiments were carried out in accordance with the 86/609/CEE European Community regulations and the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society.

4.2. Selected conditions that increase mice survival

4.2.1. High spontaneous neurological activity

Mice were considered with high spontaneous neurological activity by selection according to their performance in two neurological tests. For selection, individual mice were subjected every 2 wk to two assays, the tightrope test (3-5,31) and the T-shaped maze test (3-5).

4.2.2. Moderate exercise

We studied the effects of moderate treadmill exercise in aging mice. Moderate exercise was imposed to a group of mice aged 28 wk, as training in a treadmill (10, 15 and 20 cm/sec, for 5 min each, every 7 days) up to 78 wk of age (4).

4.2.3. Dietary vitamin E supplementation

The control group received a standard laboratory animal food (A04 diet, Panlab LS, Barcelona, Spain) with 29 $\pm$ 1 mg *alpha*-tocopherol/kg of food, whereas vitamin E-supplemented mice received the same food added with added with 5.0 g *dl*-RRR-*alpha*-tocopherol acetate/kg of food (analyzed 4.5 $\pm$ 0.1 g *alpha* -tocopherol/kg) from 28 wk of age for their entire lives. Vitamin E supplementation was started in young adult mice to avoid effects during development and normal growth (5).

4.3. Neurological tests

Individual mice were subjected every 2 wk to the tightrope test (3-5) and to the T-shaped maze test (3-5). In the tightrope test for evaluation of neuromuscular coordination (3,31,32), mice were placed hanging from their anterior legs in the middle of a 60 cm tightrope and the test was considered successful when mice reached the column at the end of the rope in less of 60 s. In the maze test for evaluation of spontaneous exploratory and cognitive activity (3,32), mice were challenged in a T-shaped maze of 50 cm arms; the test was considered successful when mice moved towards the T- intersection in less than 60 s.

4.4. Isolation of mitochondria

Brain and liver mitochondria were isolated from whole organs homogenized in 0.23 M mannitol, 0.07 M sucrose, EDTA 1 mM, and Tris HCl 10 mM, pH 7.4, at a ratio of 9 ml of homogenization medium/1 g of tissue in a Potter homogenizer with a Teflon pestle. The homogenate was centrifuged at 700 *g* for 10 min and the supernatant at 8000 *g* for 10 min to precipitate mitochondria that were washed in the same conditions(4,5,9). Mitochondrial suspensions, containing about 20 mg protein/ml, were used immediately after isolation for oxygen uptake determination or frozen in liquid N_2 and kept at – 80 °C. Mitochondrial samples were twice frozen and thawed and homogenized each time by passage through a tuberculin needle. The procedure yielded a preparation with disrupted mitochondrial membranes that had 0.18-0.25 nmol cytochrome aa_3/mg protein that was used for determination of enzyme activities and oxidative stress markers. The protein content of the samples was determined using the Folin reagent and bovine serum albumin as standard.

4.5. Biochemical markers of oxidative damage

The mitochondrial content of thiobarbituric acid-reactive substances (TBARS) and protein carbonyls were determined in submitochondrial membranes by the original assays of Fraga et al. (33) and of Oliver et al. (34), modified as previously described (9). Protein carbonyls are expressed in pmol/mg of mitochondrial protein.

4.6. Mitochondrial electron transfer activities

The membrane-bound activities of Complexes I-III, II-III, and IV were determined spectrophotometrically at 30 °C with the mitochondrial membranes suspended in 100 mM phosphate buffer, pH 7.4 (3-5). For NADH-cytochrome c reductase (Complexes I-III) and succinate-cytochrome c reductase (Complexes II-III) activities, mitochondrial membranes were added with 0.2 mM NADH or with 20 mM succinate as substrates, 0.1 mM cytochrome c^{3+} and 1 mM KCN and the enzymatic activity determined at 550 nm (E = 19 mM^{-1} cm^{-1}) and expressed as nmol cytochrome c reduced/mg protein. Cytochrome oxidase (Complex IV) activity was determined in the same phosphate buffer added with 0.1 mM cytochrome c^{2+} which was prepared by reduction with $NaBH_4$ and HCl. The rate of cytochrome c oxidation was calculated as the first order reaction constant (k') /mg protein and expressed as nmol cytochrome c oxidized at 10 microM cytochrome c/mg protein, which gives rates of the order of mitochondrial electron transfer activities.

4.7. Mitochondrial nitric oxide synthase (mtNOS) activity

Mitochondrial NO production was determined by the oxyhemoglobin (HbO_2) oxidation assay at 30 °C, as previously described (35). The reaction medium consisted of 0.1 mM NADPH, 0.2 mM arginine, 1 mM $CaCl_2$, 4 microM Cu,Zn-SOD, 0.1 microM catalase and 25 microM HbO_2 heme, in 50 mM phosphate, and 0.5-0.7 mg protein/ml, pH 7.4. A diode array sensitive spectrophotometer (model 8453 Agilent Corp., Palo Alto, California, USA) was used to follow the absorbance change at 577 nm with a reference wavelength at the isosbestic point of 591 nm ($E_{577-591}$ = 11.2 mM^{-1} cm^{-1}). Production of NO was calculated from the absorbance change that was

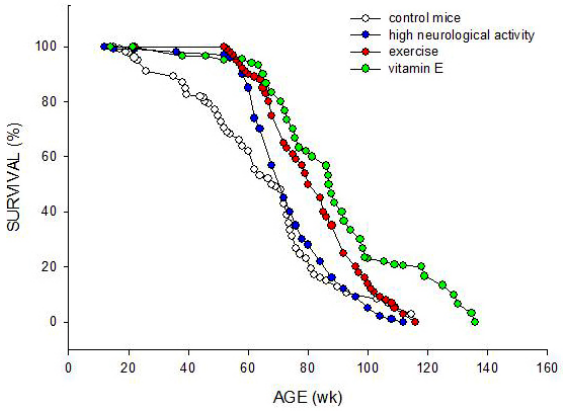

Figure 1. Mice survival curves. Control mice (n = 50): median lifespan, 61 ± 4 wk; maximal lifespan, 116 ± 4 wk. High spontaneous neurological activity (n= 40): median lifespan 68 ± 4 wk; maximal lifespan, 112 ± 4 wk Physical exercise (n = 40): median lifespan, 77 ± 4 wk; maximal lifespan, 109 ± 4 wk. Vitamin E-supplemented mice (n = 40): median lifespan, 85 ± 4 wk; maximal lifespan, 136 ± 4 wk.

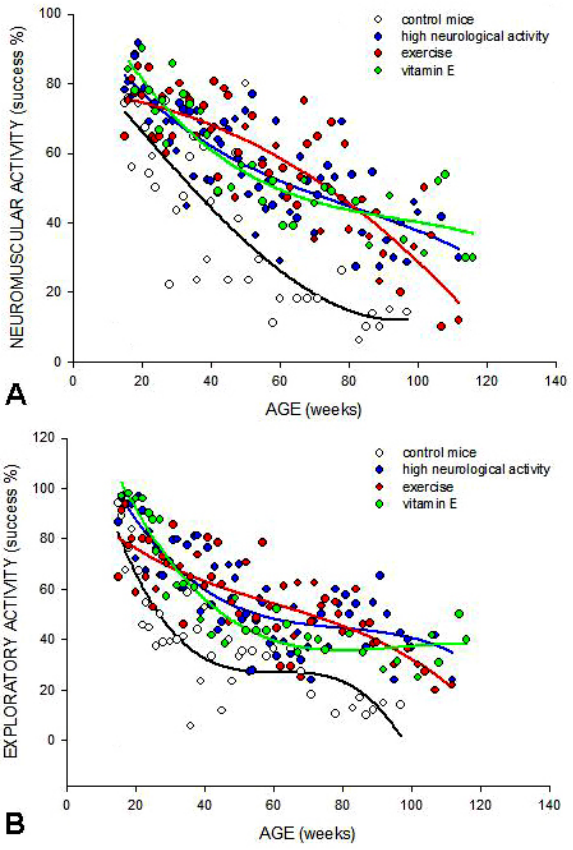

Figure 2. Effect of selected conditions on neuromuscular and exploratory activities of mice as a function of age. A. Tightrope test: control, r^2 = 0.66; high spontaneous neurological activity, r^2 = 0.64; physical exercise, r^2 = 0.71; vitamin E supplemented, r^2 = 0.80; *t*-test for control mice *vs*. selected conditions $p < 0.05$. B. T-maze test: control, r^2 = 0.76; high spontaneous neurological activity, r^2 = 0.68; physical exercise, r^2 = 0.62; vitamin E supplemented, r^2 = 0.89; *t*-test for control mice *vs*. selected conditions $p < 0.05$.

inhibited by 2 mM N^G-methyl-L-arginine, usually 92-96 %, and expressed in nmol NO/min.mg protein.

4.8. Statistics

The survival curves were analyzed by the Kaplan-Meier test. Numbers in tables and figures are mean values ± SEM. The differences between groups were analyzed by the Student-Newman-Keuls as post hoc test after significant one-way ANOVA. A *p* value of < 0.05 was considered statistically significant. Statistical analyses were carried out using a statistical package (SPSS 11.5 for Windows).

5. EFFECTS OF SELECTED CONDITIONS ON SURVIVAL AND NEUROLOGICAL PERFORMANCE IN AGING MICE

The kinetics of aging are well described by the survival curves (6). Figure 1 shows that male mice, either with high spontaneous neurological activity; or subjected to moderate exercise; or supplemented with vitamin E, increased their median life span. The group of mice supplemented with vitamin E also showed a marked increased in maximal lifespan. Male mice showed 10-15 % more effect than females for the same treatments and conditions (data not showed) (3-5).

Mice were individually tested for neurological performance every 2 weeks, starting at 28 weeks of age and for their entire lifespan. Neuromuscular coordination was assayed with the tightrope test and the exploratory and cognitive function were tested in the T-shaped maze (3,4,31). Success in both tests decreased continuously upon mice aging, however, the decline was less marked in mice with either high neurological spontaneous activity, or trained with moderate physical exercise or supplemented with vitamin E (Figure 2). Success in the tightrope and T-maze tests, considering as reference the performance at 28 weeks of age (young mice), was decreased by 40% at 52 weeks (adult mice) and by 80% at 76 weeks of age (senescent mice). This loss in motor coordination was ameliorated by 15% and 40% at 52- and 78 weeks in mice subject to selected conditions (3-5).

5.1. High spontaneous neurological activity

Mice with high spontaneous neurological activity showed an increased in median lifespan by 11% (3) and a marked improve in the performance of behavioral tests. In comparing rodent strains, lifespan is directly related to the quality of neurological and endocrine responses and to the performance in mazes and behavioral tests (36-38), which seems to reflect the fact that aging is characterized by a general decline of physiological functions with a more marked effect in the ones that depend on central nervous system functions. It is apparent that a better behavioral response, both in terms of neuromuscular function and of exploratory activity is linked to a longer life span and to a decreased age-dependent neurodegeneration (38).

5.2. Moderate exercise

The group of animals trained with a moderate exercise showed an increased median life span by about

Table 1. Effect of selected conditions on protein and lipid oxidation products in brain mitochondria of aging mice

Age Organ/Marker/Group	28 wk	52 wk	76 wk
PROTEIN CARBONYLS			
Control mice	50 ± 4	69 ± 4[1]	88 ± 5[1]
High spontaneous neurological activity		70 ± 5	77 ± 5[2]
Moderate physical exercise		59 ± 4[2]	75 ± 4
Dietary vitamin E supplementation		55 ± 5[2]	63 ± 5[2]
TBARS			
Control mice	5.2 ± 0.4	7.4 ± 0.4[1]	8.7 ± 0.4[1]
High spontaneous neurological activity		7.4 ± 0.5	7.6 ± 0.4[2]
Moderate physical exercise		5.7 ± 0.4[2]	7.9 ± 0.4
Dietary vitamin E supplementation		6.7 ± 0.4	7.1 ± 0.4[2]

Values in pmol/mg mitochondrial protein. 12 mice in each group. [1] $p < 0.05$ for aging, compared with 28 wk old mice. [2] $p < 0.05$ for high spontaneous neurological activity, physical exercise or vitamin E-supplemented compared with control mice.

19% (4), thus, the mice presented a beneficial effect of the moderate exercise in the success of neurological tests. Regular physical exercise seems to retard the accumulation of cell damage and physiological dysfunction that is characteristic of the aging process (39,40). There is ample evidence of the reduction of skeletal muscle mass associated to aging and also of the beneficial effects of regular exercise in increasing muscle mass and strength in elderly individuals (41). The available evidence extends from experimental animals to humans and from biochemical markers to physiological parameters and behavioral performances (42-46). A series of reports documented that the beneficial effects of exercise are not restricted to skeletal muscle but extended to other organs, as mouse (47,48) and human heart (49,50) and human brain (51). However, the beneficial effects of physical exercise are conditioned by the type, intensity and duration of the exercise (52,53).

5.3. Dietary vitamin E supplementation

The mice dietary supplemented with vitamin E showed the highest effect in survival, with a 40% increase in median life span, and a 17% increase in maximal life span (5). Vitamin E also retarded the neurological deficits associated to aging, since mice that received vitamin E exhibited better performances in the tightrope and the T-maze tests. Vitamin E has been extensively assayed in experimental animal diseases and in the protection and treatment of human diseases. It has been claimed that vitamin E supplementation prevents or ameliorates chronic and age-associated diseases such as cardiovascular disease, chronic inflammation, and neurological disorders (54,55). However, there is no consistent information concerning vitamin E effects on the median and maximal lifespan of experimental animals and on the decline of physiological functions associated with aging. Morley and Trainor (56) reported that vitamin E at 400 mg/kg throughout mice life had no effect on median lifespan (about 116 wk), whereas Blackett and Hall (57) using 2500 mg/kg reported an increase in rat median lifespan but not in maximal lifespan. On the other hand, Reckelhoff et al. (58) giving 5000 mg/kg from 52 to 88 wk of rat age observed a prevention in the decline of renal function upon aging. The mechanism of action of vitamin E on survival could be explained by their combined effects as chain-breaker antioxidant and as a regulator of cell signaling and gene expression (59,60).

6. EFFECTS OF SELECTED CONDITION ON MITOCHONDRIAL FUNCTION IN AGING MICE

6.1. Mitochondrial oxidative damage

The mitochondrial content of lipid and protein oxidation products, an indication of free-radical mediated reactions and oxidative damage, was increased in the brain and liver of aging mice (Table 1). In a mechanistic approach, the decreases in neuromuscular coordination and maze performance upon aging were found directly related to the brain content of lipid and protein oxidation products (3-5,37). Prevention of the age-associated decline in mouse neurological functions has been also observed after supplementation with acetyl-carnitine and lipoic acid (14,61) and with flavonoid-rich vegetable extracts (62), effects that were interpreted as due to protection or remediation of an oxidative damage.

The content of protein carbonyls in brain mitochondria increased by about 38 % and by 76 % in adults (52 wk) and senescent mice (76 wk), respectively. Mice with high spontaneous neurological activity, trained with moderate exercise, or supplemented with vitamin E from 28 wk of age exhibited a marked improvement in mitochondrial oxidative damage (Table 1).

Aging and age-associated neurodegeneration in mice are related to marked decreases in neuromuscular and exploratory functions with inverse statistical relationships between mice performance in behavioral tests and indicators of cellular brain oxidative stress (3,37).

6.2. Mitochondrial inner membrane enzyme activities

The number of dysfunctional mitochondria increases in the brain during the senescence. The activities of a few enzymes that can be considered as critical for mitochondrial function, such as NADH-ubiquinone reductase (complex I), cytchrome oxidase (complex IV) and mtNOS decreased progressively during aging (Table 2) (2,5,9). Brain mitochondria isolated from aged rats showed increased mitochondrial fragility (9). Two other enzyme activities that are essential for mitochondrial function have also been reported selectively decreased on aging: adenine nucleotide translocase, that catalyzes the fast ADP/ATP exchange between cytosol and mitochondria (63); and acyl carnitine transferase, that catalyzes fatty acid transport to the mitochondrial matrix (14).

Table 2. Effect of selected condition on enzymatic activities of brain mitochondrial membranes in aging mice

Age Enzyme activity/Group	28 wk	52 wk	76 wk
NADH-cytochrome c reductase			
Control mice	330 ± 10	273 ± 10[1]	212 ± 10[1]
High spontaneous neurological activity		285 ± 10	250 ± 10[2]
Moderate physical exercise		291 ± 10	275 ± 10[2]
Dietary vitamin E supplementation		290 ± 10	283 ± 11[2]
Succinate-cytochrome c reductase			
Control mice	127 ± 9	125 ± 9	131 ± 9
High spontaneous neurological activity		131 ± 9	127 ± 9
Moderate physical exercise		127 ± 9	132 ± 9
Dietary vitamin E supplementation		129 ± 9	128 ± 9
Cytochrome oxidase			
Control mice	124 ± 8	96 ± 8[1]	79 ± 8[1]
High spontaneous neurological activity		105 ± 8[2]	95 ± 8[2]
…Moderate physical exercise		112 ± 9[2]	100 ± 8[2]
Dietary vitamin E supplementation		107 ± 8	102 ± 8[2]
mtNOS			
Control mice	0.65 ± 0.05	0.36 ± 0.04[1]	0.20 ± 0.03[1]
High spontaneous neurological activity		0.50 ± 0.05[2]	0.33 ± 0.03[2]
Moderate physical exercise		0.53 ± 0.04[2]	0.31 ± 0.04[2]
Dietary vitamin E supplementation		0.54 ± 0.05[2]	0.41 ± 0.05[2]

NADH- and succinate-cytochrome c reductase, and cytochrome oxidase activities are expressed in nmol cytochrome c (reduced or oxidized)/min. mg protein; and mtNOS in nmol NO/min. mg protein. 12 mice in each group. [1] $p < 0.05$ for aging, compared with 28 wk old mice. [2] $p < 0.05$ for high spontaneous neurological activity, physical exercise or vitamin E-supplemented compared with control mice.

Table 2 illustrates about the occurrence of such changes in male mice brain mitochondria. Inner membrane mitochondrial enzyme activities: (i) NADH-cytochrome c reductase, (ii) cytochrome oxidase and (iii) mtNOS were markedly decreased upon aging (Table 2). The 17-36 % decrease of NADH-cytochrome c reductase activity (complexes I + III) at 52-76 wk of age, is attributed to Complex I decreased activity, since succinate-cytochrome c reductase activity (complex I + II) was not affected by aging (3-5). Cytochrome oxidase activity decreased by 23-36% at 52-78 wk of age. NADH-dehydrogenase activity was 12-20 % protected at 52-78 wk of age by the conditions of high spontaneous neurological activity, moderate exercise, and supplementation with vitamin E. Cytochrome oxidase activities was 9-17% protected at 52-78 wk by the three experimental conditions. The activity of mtNOS decreased by 44-70 % at 52-78 wk of age, and this decrease was prevented by 15-34 % by the three experimental conditions.

The association between mtNOS activity and cellular homeostasis has been called the "pleiotropic effect of mtNOS" and was it was suggested that the effect is exerted through NO and H_2O_2 signaling from mitochondria to the cytosol, a process that indicates a high mitochondrial energy charge (64). We have observed increases in NO production by mtNOS, in cytochrome oxidase activity and in mitochondrial mass in the ovary proliferative phase (65). In such way that our results are consistent with the recently reported role of endogenous NO in the mitochondrial biogenesis in mammals (66). Brain mtNOS has also been reported increased during synaptogenesis (67) and spatial memory development (68). A decrease in the mitochondrial production of NO by mtNOS could be involved as a decrease in mitochondria-cytosol signaling in the brain mitochondrial dysfunction of aged mammals.

Oxidative damage negatively correlated with mitochondrial enzyme activities (Figure 3), which supports the view that intermediates and oxidation products of the lipoperoxidation process constitute the molecular mechanism of the decreased enzymatic activity.

A cumulative oxidative damage and a reduction of the mitochondrial capacity to produce ATP in the organs and tissues of aged mammals are the two main concepts of the mitochondrial hypothesis of aging (1,69,70).

Reduced ATP production can occur through reduction of either mitochondrial mass or the specific rate of ATP synthesis. Recently, we reported that the mitochondrial content of rat brain and liver are not reduced in aging (9). Mitochondrial function encompasses electron transfer in the mitochondrial respiratory chain with H^+ release to the intermembrane space, and H^+ re-entry to the matrix through F_0 with ATP synthesis. An age-dependent impairment of mitochondrial function may comprise: (a) decreased electron transfer rates, (b) failure in maintaining of the H^+ electrochemical gradient, and (c) impairment of the H^+ driven ATP synthesis. The effects of aging on mitochondrial O_2 uptake and oxidative phosphorylation were determined in mice brain with malate-glutamate and succinate as substrates (5). The effects of aging on state 3 respiration, the active state, agree with the effects on electron transfer; the rates of mitochondrial O_2 uptake correlated with the complex I and IV activities in brain. Interestingly, aging from 28 to 52 wk did not produced uncoupling or decrease in the ADP:O ratios in brain and

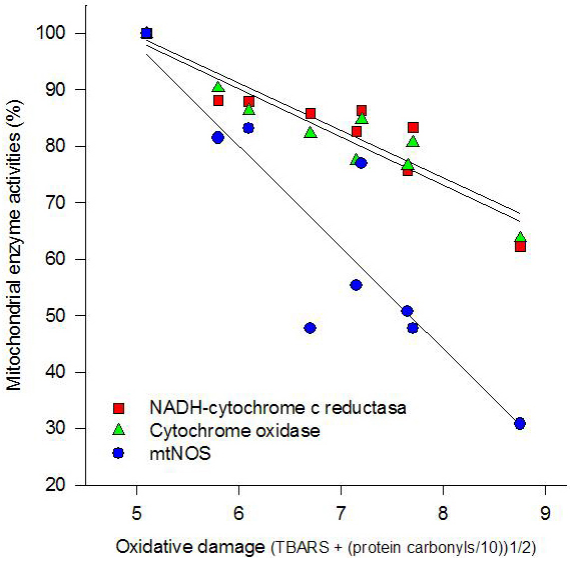

Figure 3. Correlation between (a) oxidative damage (0.5 x [TBARS + (protein carbonyls/10)]), and (b) mitochondrial enzyme activities: NADH-cytochrome c reductase, cytochrome oxidase and mitochondrial nitric oxide synthase (mtNOS) (Activities expressed as percentage). Oxidative damage, vs. NADH cytochrome c reductase activity $r^2 = 0.84$, $p < 0.05$; id. vs. cytochrome oxidase activity, $r^2 = 0.89$, $p < 0.05$; id. vs. mtNOS, $r^2 = 0.80$, $p < 0.05$.

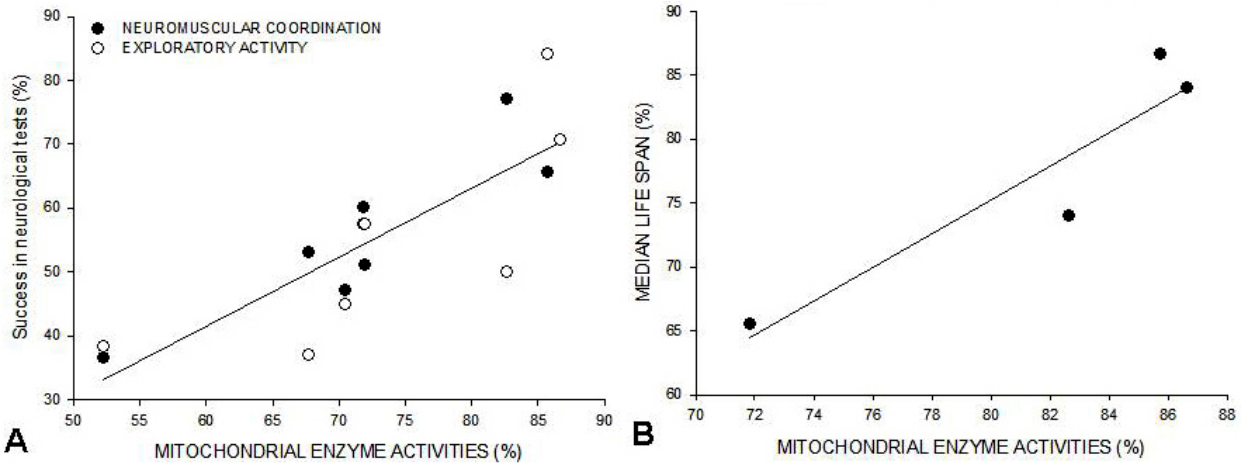

Figure 4. Correlation between mitochondrial inner membrane enzymatic activities (mean value of NADH-cytochrome c reductase, cytochrome oxidase and mtNOS) at 28, 52 and 76 wk of age with: (A) success in the T-maze and in the tightrope tests at the same age points. $r^2 = 0.68$ $p < 0.05$ and; (B) mediam lifespan, $r^2 = 0.87$ $p < 0.05$.

liver mitochondria, indicating that energy conservation and ATP synthesis were unaffected by aging (5). The loss in electron transfer and respiration rates was markedly prevented by vitamin E supplementation (5). Both, complex I and complex IV, are then to be considered as markers of aging (1,3,4,9,23).

7. SURVIVAL, BEHAVIOR AND BRAIN MITOCHONDRIAL ENZYME ACTIVITIES

High spontaneous neurological activity, moderate physical exercise and high-dose vitamin supplementation E retarded the neurological deficits associated to age in aging mice. The neurological activity, as measured in the behavioral tests, correlates with the mitochondrial enzyme activities, NADH-cytochrome c reductase, cytochrome oxidase and mtNOS (Figure 4 A). Moreover, the retard in the decline of brain mitochondrial enzyme activities observed in the spontaneous high neurological activity mice, in the moderate exercise group, and in the mice supplemented with vitamin E correlated with an increased mice survival (Figure 4 B).

In mice, spontaneous high neurological activity, moderate exercise, and high-dose vitamin E supplementation increase lifespan, likely by a decrease in cellular oxidative damage and by preventing the decreased mitochondrial functions that accompany the age-associated decline of physiological functions. It seems that these three conditions are

able to trigger regulatory responses that retard age-dependent processes, such as the impairment of behavioral performances, the development of cellular oxidative stress, and the decrease of mitochondrial enzymatic activities.

8. PERSPECTIVE

The mitochondrial enzyme activities of inner membrane, NADH dehydrogenase, cytochrome oxidase, and mtNOS, are to be taken as markers of brain aging, with the decreases in enzymatic activity are directly related to the loss of neurological function in aged mice. Thus, mice with spontaneous high neurological activity, subject to moderate physical exercise, or supplemented with vitamin E, show a protection in the decline of NADH dehydrogenase, cytochrome oxidase and mtNOS activities in aged mice. The degrees of retard in the decrease in mitochondrial activities and in the decrease in neurological performances correlated with an increased life span. The marked decrease in mtNOS activity in aged brain mitochondria may constitute a decrease in mitochondrial signaling that does not favor a sustained neuronal homeostasis.

In summary, high spontaneous neurological activity, moderate exercise and supplementation with vitamin E are three experimental conditions that partially prevent the loss of brain mitochondrial enzyme activities and neurological function which are characteristics of aging, and, at the same time, extended mice lifespan.

9. ACKNOWLEDGMENTS

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