UC Irvine

UC Irvine Previously Published Works

Title

Effects of age and dietary antioxidants on cerebral electron transport chain activity.

Permalink

<https://escholarship.org/uc/item/15s3r593>

Journal

Neurobiology of aging, 22(4)

ISSN

0197-4580

Authors

Sharman, EH

Bondy, SC

Publication Date

2001-07-01

DOI

10.1016/s0197-4580(01)00226-3

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Effects of age and dietary antioxidants on cerebral electron transport chain activity

Edward H. Sharman*, Stephen C. Bondy

Center for Occupational and Environmental Health, Department of Community and Environmental Medicine, University of California, Irvine, Irvine, CA, 92697-1825, USA

Received 30 November 2000; received in revised form 12 February 2001; accepted 12 February 2001

Abstract

Aging is a pleiotropic process involving genetic and environmental factors. Recently it has been demonstrated that dietary constituents may affect senescence. In the present study, adult (3 month-old) mice were fed diets supplemented with ubiquinone (coenzyme Q₁₀), α -lipoic acid, melatonin or α -tocopherol for a six-month period to determine if antioxidants may reverse or inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain (ETS) enzyme activities. The control consisted of a group of mice maintained on a basal diet for the same period. The activity of cytochrome c oxidase (Complex IV) increased with age but melatonin supplementation restored the activity to levels of 3 month-old animals. The activity of succinate dehydrogenase (Complex II) showed no age-related changes. However, this enzyme complex was elevated, in animals supplemented with coenzyme Q₁₀, α -lipoic acid and α -tocopherol, above corresponding values obtained with basal diet. NADH-ubiquinone oxidoreductase (Complex I) and ubiquinol: ferricytochrome-c oxidoreductase (Complex III) activities remained unchanged. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Diet; Aging; Brain; Melatonin; Lipoic acid; Tocopherol; Ubiquinone; Coenzyme-Q₁₀

1. Introduction

The mitochondrion, where aerobic eukaryotes generate most of their energy, has been reported to undergo significant functional changes with aging [4,13,36]. Of particular interest are the changes in activity of the enzymes constituting the mitochondrion's electron transport system (ETS) and the role such changes may play in the progression of neurodegenerative diseases and the overall aging process. Given the brain's high energy requirements, any decline in brain ETS enzyme activity could have a significant impact on brain function and particularly on the etiology of neurodegenerative disease. It has been shown that ETS enzymatic activity is reduced by excess levels of reactive oxygen species (ROS) [36]. Recent studies have also shown that oxidatively modified proteins are found not only in the CNS of aged subjects but also in the brains of subjects affected by a range of neurological disorders including Alzheimer's and Parkinson's diseases [5]. Long term dietary administration

of various plant extracts has been found to retard the onset of age-related deficits in neural functioning and this has been attributed to the antioxidant properties of such extracts [18,19]. Similar remediation by melatonin, lipoic acid and α -tocopherol has been proposed [17,30,33,40].

Dietary supplementation with antioxidants capable of entering brain mitochondria and reacting with the ROS generated there, may maintain the ETS enzyme activity level by preventing ROS-induced damage to these enzymes. To the extent that maintaining high ETS enzyme activity is critical for preventing age-related decline in brain function and the occurrence of neurodegenerative diseases, antioxidants may prevent or slow the development of these conditions. In order to be effective, it may be necessary to begin supplementation at a fairly young age, before substantial loss of enzyme activity has occurred. Therefore we began feeding modified diets to mice at three months of age and subsequently examined the effects on the activity of the ETS enzymes in cerebrocortical mitochondria. The antioxidants chosen were coenzyme Q₁₀ (ubiquinone), α -lipoic acid (thioctic acid), melatonin, and DL- α -tocopherol. We based the selection of these agents on their reported ability

* Corresponding author. Tel.: +1-949-824-8642; fax: +1-949-824-2793.

to produce changes in the CNS following systemic administration [3,6,7,31,32].

The supplemented animals were compared to two control groups: an older control group was fed unsupplemented minimal basal diet starting at 3 months of age for 6 months, and a younger control group was fed standard rodent lab chow until sacrifice at 3 months of age.

2. Materials and methods

Unless otherwise noted, all reagents were obtained from Sigma (St. Louis, MO), and optical measurements were made with a Spectronic Genesys 5 spectrophotometer.

2.1. Animals

Male B/6C3F1 mice, a hybrid between C57BL/6 and C3H (Harlan Labs, Indianapolis, IN) were housed 4–6 animals per cage at the Animal Resource Facility of the UCI College of Medicine. The animals were maintained on a 12-hour light/dark cycle in a temperature controlled ($20 \pm 1^\circ\text{C}$) room. Specified diets and water were provided *ad libitum* to 6–8 animals per group.

2.2. Diets

The minimal basal diet (#101101, Dyets Inc., Bethlehem, PA) consisted of 50% sucrose and 26% casein (w/w) as well as a minimal salt and vitamin mix including 110 ppm α -tocopheryl acetate. This was supplemented with one of the following: 0.1% DL- α -tocopheryl succinate, 0.02% co-enzyme Q₁₀ (ubiquinone), 0.165% DL-lipoic acid (thioctic acid) or 0.004% melatonin. All diets were administered to mice at 3 months of age for a further 6 months.

2.3. Preparation of the cerebrocortical synaptosomal-mitochondrial fraction

Mice were decapitated, the brains were excised quickly on ice, and the cerebrocortex was dissected out. Tissue was weighed and homogenized in 10 volumes of 0.32 M sucrose and centrifuged at $1,800 \times g$ for 10 minutes. The resulting supernatant fraction was then centrifuged at $24,000 \times g$ for 10 minutes to yield the crude cerebral mitochondrial pellet (P2). The P2 pellet was taken up in HEPES buffer to a concentration of 0.01 gEq/mL and stored at -70°C . The composition of the HEPES buffer was (mM): NaCl, 120; KCl, 2.5; NaH_2PO_4 , 1.2; NaHCO_3 , 5.0; glucose, 6.0; CaCl_2 , 1.0; and HEPES, 10, at pH 7.4.

2.4. Complex I (NADH:ubiquinone oxidoreductase) assay

The method of Shults *et al.*, [38] involving the reduction of ubiquinone-1 to the corresponding quinol with the concurrent oxidation of NADH, was utilized. However, the

assays were run at room temperature and decylubiquinone was used in place of ubiquinone-1 because the latter has higher rotenone-insensitive activity [15]. A pH 7.4 buffer consisting of 35 mM phosphate, 1.3 mM MgCl_2 , 250 μM EDTA, and 0.25 mg/ml of BSA was used. 10 μl of 0.125 M KCN solution, 10 μl of 0.1 mg/ml antimycin solution, 10 μl of decylubiquinone solution, and then 50 μl of frozen/thawed P2 fraction were added to buffer. The reaction was initiated by adding 20 μl of 20 mM NADH solution; then the mixture was shaken briefly, and the absorbance was measured immediately at 340 and 400 nm wavelengths with a Beckman DU 640B spectrophotometer. The loss in absorbance at these wavelengths was measured periodically until the rate of change was nearly zero. The mixture was shaken between measurements to facilitate continual air oxidation of decylubiquinol back to the quinone; such agitation prevented a buildup of quinol and thus assured a more accurate measure of the complex I rate. By fitting an exponential function to the (340 nm–400 nm) differential absorbance points, an initial rate could be computed that was little affected by variations in induction times. The complex I rate was calculated from the computed initial rate by utilizing an NADH differential extinction of $5.4 \text{ mM}^{-1} \text{ cm}^{-1}$ and adjusting for the amount of protein present. Rotenone-insensitive complex I activity was determined by following the above protocol, except that 1.6 μl of 6.4 mM rotenone solution was added just after adding the antimycin solution.

2.5. Complex II (Succinate:ubiquinone oxidoreductase) assay

Complex II was activated by maintaining 20 μl of the P2 suspension, 100 μl of 200 mM succinate, 2 μl of 0.1 mg/ml antimycin, and 737 μl of 100 mM pH 7.4 phosphate buffer at 27°C for 20 minutes. Then 8 μl 0.125 M KCN and 100 μl of 350 μM ferricytochrome c were added; finally the reaction was initiated by addition of 33 μl of 3.3 mg/ml phenazine methosulfate. The increase in absorbance of ferrocycytochrome c at 551 nm minus the absorbance at 520 nm was measured during the first minute. Although the differential absorbance change was small, a good signal-to-noise level was obtained (Fig. 1). Taking into account that succinate is a two electron reductant, the activity in nanomoles of succinate per minute per mg of protein was determined from these absorbance data, utilizing a measured cytochrome c differential extinction of $9.0 \text{ mM}^{-1} \text{ cm}^{-1}$ [1].

2.6. Complex III (Ubiquinol:ferricytochrome-c oxidoreductase) assay

16 μl of 0.125 M KCN solution, 200 μl of 350 μM cytochrome c solution, then 50 μl of frozen/thawed P2 suspension were added to water for a final volume of 2.0 ml. 20 μl of decylubiquinol solution were then added to initiate the reaction. The reaction was followed by measuring the time-course increase in ferrocycytochrome c absorbance at

Fig. 1. Genesys 5 output graph of cytochrome c differential absorption [551 nm–520 nm] as a function of time, as used to measure complex II activity.

550 nm. Complex III activity was computed as the initial reaction rate per mg of protein [12].

Decylubiquinol was prepared by shaking a suspension of 50 μ l of alcoholic decylubiquinone (25 mg/ml in absolute ethanol), 200 μ l of n-hexane, and 1 ml of an aqueous solution of 0.25 M sucrose in 0.1 M phosphate pH 7.4 buffer with a few milligrams of sodium dithionite powder. The hexane phase was separated, the hexane was removed under vacuum, and the decylubiquinol was dissolved in 50 μ l ethanol and stored at -20°C in the dark prior to use [35].

2.7. Complex IV (Cytochrome-c oxidase) assay

20 μ l of ferrocytochrome-c solution were added to 10 mM pH 7.4 phosphate buffer to a final volume of 1.0 ml. Then 10 μ l of P2 suspension were added to initiate the reaction. The reaction was followed by measuring the time-course of the absorbance difference: 550 nm–540 nm for 30 seconds at room temperature. The complex IV activity was computed as nanomoles of cytochrome c per minute per mg of protein, utilizing a differential cytochrome c extinction of $15 \text{ mM}^{-1} \text{ cm}^{-1}$.

Ferrocytochrome-c was prepared by adding an excess of ascorbic acid to cytochrome-c and purified by using a Sephadex G-25 column. The solution was then stored at -70°C in nitrogen-purged vials [42].

2.8. Protein estimation

Total protein in the mitochondrial P2 fractions was estimated by Bradford's method [9] with a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA).

2.9. Statistical analyses

Differences between groups were assessed by one-way Analysis of Variance followed by Fisher's Least Significant Difference Test. The acceptance level of significance was $p < 0.05$ using a two-tailed distribution.

3. Results

3.1. Complex I

There were no significant differences among the cortical complex I activities of any of the groups (Table 1).

3.2. Complex II

There was no significant change between the younger and older control complex II activities. However, activities of mice supplemented with coenzyme Q_{10} , lipoic acid, and α -tocopherol were significantly higher than the level of the older mice receiving basal diet. The activity of the melatonin-supplemented mice was also higher, but this was not significant (Fig. 2).

3.3. Complex III

No significant differences were found among the complex III activities of the older control or any of the supplemented mice groups (Table 1).

3.4. Complex IV

The complex IV activity of the older mice, as judged by the rate of cytochrome c oxidation, was considerably higher than that of the younger mice (Fig. 3). Dietary exposure to

Table 1
Respiratory chain specific enzyme activities from brains of mice fed basal or supplemented diets

Age at sacrifice (mo)	Diet	Complex I	Complex II	Complex III	Complex IV
Younger (3)	Basal	183 $\pm$ 22	52 $\pm$ 5	850 $\pm$ 139	*237 $\pm$ 31
Older (9)	Basal	221 $\pm$ 18	45 $\pm$ 3	675 $\pm$ 78	383 $\pm$ 44
Older (9)	Ubiquinone	207 $\pm$ 33	*63 $\pm$ 8	690 $\pm$ 102	435 $\pm$ 71
Older (9)	Lipoate	233 $\pm$ 40	*58 $\pm$ 7	692 $\pm$ 88	346 $\pm$ 42
Older (9)	Tocopherol	231 $\pm$ 22	*59 $\pm$ 9	656 $\pm$ 86	434 $\pm$ 40
Older (9)	Melatonin	249 $\pm$ 8	52 $\pm$ 6	767 $\pm$ 117	*195 $\pm$ 25

Activities are in units of nmol/min/mg mitochondrial protein $\pm$ SE (n = 6).

*: Differs significantly from corresponding value for older animals fed basal diet.

Fig. 2. Activity of complex II in cortical mitochondria of mice treated for 6 months with various diets. Each value represents the mean of 6 animals $\pm$ SE. †: differs significantly from 9 month-old (older control) mice fed basal diet ($p < 0.05$).

coenzyme Q₁₀, lipoic acid, and α -tocopherol did not modify the complex IV activities of older mice. However, the complex IV activity of the melatonin-supplemented mice was restored from levels consonant with those of older controls, to values statistically indistinguishable from younger mice.

Fig. 3. Activity of complex IV in cortical mitochondria of mice treated for 6 months with various diets. Each value represents the mean of 6 animals $\pm$ SE. †: differs significantly from 3 month-old (younger control) mice fed basal diet ($p < 0.05$).

4. Discussion

In order to have a direct effect on ETS enzyme activity within the brain, an orally-administered substance must enter the circulatory system, cross the blood-brain barrier, and be transported into the mitochondrial inner membrane. Several reports show that the penetrance of coenzyme Q into the rodent brain is very marginal [23,26]. In contrast, one study showed that feeding 12 month-old rats 200 mg/kg/day of coenzyme Q for 2 months increases brain mitochondrial content of this cofactor by over 50% [28]. Regardless of whether measurable increases of coenzyme Q are produced by supplementation, sufficient increments appear to be achieved for physiological action within the CNS. In Huntington's disease patients, increased occipital cortex lactate levels are an indication of compromised oxidative metabolism; following oral coenzyme Q₁₀ administration, lactate fell toward normal levels and increased again following cessation of supplementation [20]. Moreover, supplementation of parkinsonian patient diets with 400 mg/day of coenzyme Q₁₀ increased complex I activity of platelets [39].

The ability of α -tocopherol to cross into the brain is similarly limited and only follows a linear dose/response characteristic under conditions of deficiency [27]. We have

found that repeated daily dosing of rats for 14 days with vitamin E (200 mg/kg body weight) has a minor effect on cortical levels of α -tocopherol. However, this is sufficient to effect significant biochemical changes [6]. Even 2 days of α -tocopherol injection have been reported to be protective against oxidative injury within the CNS [43].

Lipoic acid is readily absorbed from the diet and crosses the blood-brain barrier [31]. Supplementation of rat diets with lipoate has been shown to cause behavioral changes as demonstrated by an increase in ambulatory activity [17].

In the current study, melatonin had the most pronounced effect in preventing an age-related increase in complex IV activity. This agent can readily pass into the CNS and improve motor activity in aged C57BL/6 mice [2]. Melatonin can also suppress iron or paraquat-induced neurodegeneration in rat brain [25]. Its mechanism of action for protecting against such oxidant events may be indirect and involve promotion of antioxidant enzyme expression [21].

A number of studies have measured the change in activity of the ETS enzymes within cerebral tissue with age. The current work was designed to avoid age-related extremes but rather to investigate the changes associated with transition from full maturity to early aging. Thus, comparisons were made between 3 and 9 month-old mice. These ages constitute neither the very young, nor the very old state. Rather they essentially represent the transition from younger to older middle age. It is the differences that emerge during this period that may lay the early foundations underlying senescence. This may account for the lack of significant change in several mitochondrial complex activities in the present study. We found no changes in complex I despite the fact that seven subunits of this complex are encoded by mammalian mitochondrial DNA. However, the enzyme activity of this complex is the most difficult to accurately quantitate [24]. The increase in complex IV reported in the current study, is consistent with increases reported for both ROS generation [16], and state 3 glutamate/malate respiration in cardiac muscle of middle-aged rats [11], and in skeletal muscle [22]. In addition, mitochondria from the senescence-accelerated mouse strain (SAMP8) possess higher respiratory rates and redox state than the corresponding senescence-resistant strain (SAMR1) [29]. These data were obtained from 2 month-old mice prior to any observable age-related deficits. However, parallel changes have not been found in human neurodegenerative disorders. In fact, decreased complex IV levels have been reported in brains of Alzheimer's disease patients [8,10]. While extreme old age may be characterized by sluggish cerebral mitochondrial respiration accompanied by reduced overall metabolic rates [14], there is evidence both from the current study and reports of others, that this may be preceded by a mitochondrial state which may be hyperactive yet inefficient. Future study of the effects of melatonin supplementation on complex IV activities in more aged animals would be useful to elucidate these possibilities.

The results reported here demonstrate that reversal of

age-related changes in ETS can be enhanced by melatonin and not by the other agents tested. This is in agreement with our findings that only melatonin can prevent age-related changes in cortical reactive oxygen species and nitric oxide synthase (Bondy *et al.*, submitted for publication), and that only the melatonin-enriched diet can prevent the enhanced responsiveness of cerebral cytokine mRNA to lipopolysaccharide found in aged animals (manuscript in preparation). Thus melatonin appears to possess unique properties not shared by other antioxidants or mitochondrial cofactors. This may have implications for the utility of this agent in slowing progression of age-related neurodegenerative changes, although demonstration of any neuroprotective benefits in truly aged animals requires further investigation.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (ES 7992 and AG 16794 and AG 14882). We are grateful to Ms. Ellen Y. Yang for technical assistance.

References

- [1] Ackrell BA, Kearney EB, Singer TP. Mammalian succinate dehydrogenase. *Methods in Enzymology* 1978;53:466–83.
- [2] Araghi-Niknam M, Lane L, Watson RR. Modification of physical movement in old C57BL/6 mice by DHEA and melatonin supplementation. *Proc Soc Exptl Med Biol* 1999;221:193–7.
- [3] Beal MF, Matthews RT, Tielman A, Shults CW. Coenzyme Q₁₀ attenuates the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced loss of striatal dopaminergic neurons in aged mice. *Brain Res* 1998;783:109–14.
- [4] Benzi G, Moretti A. Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system. *Free Radical Biology and Medicine* 1995;19:77–101.
- [5] Bondy SC. Free radicals in neurodegenerative disease. In: *Advances in Neurodegenerative Disorders Vol. 2*, Marwah J, Teitelbaum H, editors. Prominent Press, Scottsdale, AZ 1999. pp. 1–23.
- [6] Bondy SC, Guo SX, Adams JD, Klaidman LK, Edwards J. Dietary α -tocopherol content modulates responses to moderate ethanol consumption. *Env Tox Pharm* 1996;1:45–9.
- [7] Bondy SC, Guo SX, Adams JD. Prevention of ethanol-induced changes in reactive oxygen parameters by α -tocopherol. *Alc Alcohol* 1996;31:403–10.
- [8] Bowling AC, Beal MF. Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sciences* 1995;56:1151–71.
- [9] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye-binding. *Analytical Biochemistry* 1976;72:248–54.
- [10] Chagnon P, Betard C, Robitaille Y, Cholette A, Gauvreau D. Distribution of brain cytochrome oxidase activity in various neurodegenerative diseases. *Neuroreport* 1995;6:711–15.
- [11] Chen JC, Warshaw JB, Sanadi DR. Regulation of mitochondrial respiration in senescence. *Journal of Cell Physiology* 1972;80:141–8.
- [12] Cocco T, Di Paola M, Papa S, Lorusso M. Arachidonic acid interaction with the mitochondrial electron chain promotes reactive oxygen species generation. *Free Radical Biology and Medicine* 1999;27:51–9.

- [13] Cooper JM, Mann VM, Schapira AHV. Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *Journal of the Neurological Sciences* 1992;113:91–8.
- [14] Davis M, Whitely T, Turnbull DM, Mendelow AD. Selective impairments of mitochondrial respiratory chain activity during aging and ischemic brain damage. *Acta Neurochirurg Suppl* 1997;70:56–8.
- [15] Genova ML, Castelluccio C, Fato R, Parenti Castelli G, Merlo Pich M, Formiggini G, Bovina C, Marchetti M, Lenaz G. Major changes in complex I activity in mitochondria from aged rats may not be detected by direct assay of NADH:coenzyme Q reductase. *Biochemical Journal* 1995;311:105–9.
- [16] Guarnieri C, Muscari C, Calderara CM. Mitochondrial production of oxygen free radicals in the heart muscle during the life span of the rat: peak at middle age. In: Emerit I, Chance B, editors. *Free Radicals and Aging*. Birkhäuser, Basel, 1992. pp. 73–77.
- [17] Hagen TM, Ingersoll RT, Lykkesfeldt J, Liu J, Wehr CM, Vinarsky V, Bartholomew JC, Ames BC. (R)- α -Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB Journal* 1999;13:411–41.
- [18] Joseph JA, Denisova N, Fisher D, Shukitt-Hale B, Bickford P, Prior R, Cao G. Membrane and receptor modifications of oxidative stress vulnerability in aging. *Ann N Y Acad Sci* 1998;854:268–76.
- [19] Joseph JA, Shukitt-Hale B, Denisova NV, Prior RL, Cao G, Martin A. Long term dietary spinach, strawberry or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. *J Neurosci* 1998;18:8047–55.
- [20] Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF. Energy metabolism defects in Huntington's disease and effects of coenzyme Q₁₀. *Annals of Neurology* 1997;41:160–5.
- [21] Kotler M, Rodriguez C, Sainz RM, Anatolin I, Mendenez-Pelaez A. Melatonin increase gene expression for antioxidant enzymes in rat brain. *J Pineal Res* 1998;24:83–9.
- [22] Kwong LK, Sohal RS. Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse. *Archives of Biochemistry and Biophysics* 2000;373:16–22.
- [23] Lass A, Forester MJ, Sohal RS. Effects of coenzyme Q₁₀ and α -tocopherol administration on their tissue levels in the mouse: elevation of mitochondrial α -tocopherol by coenzyme Q. *Free Radical Biology and Medicine* 1999;26:1375–82.
- [24] Lenaz G, Bovina C, Castelluccio C, Fato R, Formiggini G, Genova ML, Marchetti M, Merlo Pich M, Pallotti F, Parenti Castelli G, Biagini G. Mitochondrial complex I defects in aging. *Molecular and Cellular Biochemistry* 1997;174:329–33.
- [25] Lin AMY, Ho LT. Melatonin suppresses iron-induced neurodegeneration in rat brain. *Free Radical Biology and Medicine* 2000;28:904–11.
- [26] Lonnrot K, Holm P, Lagerstedt A, Huhtala H, Alho H. The effects of lifelong ubiquinone Q10 supplementation on the Q9 and Q10 tissue concentrations and life spans of male rats and mice. *Biochem Mol Biol Int* 1998;44:727–37.
- [27] Martin A, Janigian D, Shukitt-Hale B, Prior RL, Joseph JA. Effect of vitamin E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations. *Brain Res* 1999;845:50–9.
- [28] Matthews RT, Yang L, Browne S, Baik M, Beal MF. Coenzyme Q₁₀ administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc Natl Acad Sci USA* 1998;95:8892–7.
- [29] Nishikawa T, Takahashi JA, Fujibayashi Y, Fujisawa H, Zhu B, Nishimura Y, Ohnishi K, Higuchi K, Hashimoto N, Hosokawa M. An early stage mechanism of the age-associated mitochondrial dysfunction in the brain of SAMP8 mice; an age-associated neurodegeneration animal model. *Neuroscience Letters* 1998;254:69–72.
- [30] O'Donnell E, Lynch MA. Dietary anti-oxidant supplementation reverses age-related neuronal changes. *Neurobiol Aging* 1998;19:461–7.
- [31] Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radical Biology and Medicine* 1997;22:359–78.
- [32] Pierpaoli W, Regelson W. Pineal control of aging: effect of melatonin and pineal grafting on aging mice. *Proc Natl Acad Sci USA* 1994;91:787–91.
- [33] Reiter RJ. Oxidative processes and antioxidant defense mechanisms in the aging brain. *FASEB J* 1995;9:526–33.
- [34] Reiter RJ, Craft CM, Johnson JE, King TS, Richardson BA, Vaughn GM, Vaughn MK. Age-associated reduction in nocturnal pineal melatonin levels in female rats. *Endocrinol* 1981;109:1295–7.
- [35] Rieske JS. Preparation and properties of reduced coenzyme Q—cytochrome c reductase (complex III of the respiratory chain). *Meth Enzymol* 1967;10:239–45.
- [36] Sastre J, Pallardó FV, García de la Asunción J, Viña J. Mitochondria, oxidative stress and aging. *Free Rad Res* 2000;32:189–98.
- [37] Sewerynek E, Ortiz G, Reiter R, Pablos M, Melchiorri D, Daniels W. Lipopolysaccharide-induced DNA damage is greatly reduced in rats treated with the pineal hormone melatonin. *Mol Cell Endocrinol* 1996;117:183–8.
- [38] Shults CW, Nasirian F, Ward D, Nakano K, Pay M, Hill L, Haas R. Carbidopa/levodopa and selegiline do not affect platelet mitochondrial function in early parkinsonism. *Neurology* 1995;45:344–8.
- [39] Shults CW, Beal MF, Fontaine D, Nakano K, Haas RH. Absorption, tolerability, and effects on mitochondrial activity of oral coenzyme Q₁₀ in parkinsonian patients. *Neurology* 1998;50:793–5.
- [40] Socci DJ, Crandall BM, Arendash GW. Chronic antioxidant treatment improves cognitive performance of rats. *Brain Res* 1995;693:88–94.
- [41] Sugiyama S, Yamada K, Ozawa T. Preservation of mitochondrial respiratory function by coenzyme Q₁₀ in aged rat skeletal muscle. *Biochemistry and Molecular Biology International* 1995;37:1111–20.
- [42] Trounce IA, Kin YL, Jun AS, Wallace DC. Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmembrane cell lines. *Methods in Enzymology* 1996;264:495–6.
- [43] Urano S, Asai Y, Makebe S, Matsuo M, Izumiyama N, Ohtsubo K. Oxidative injury of synapse and alteration of antioxidant defense systems in rats and its prevention by vitamin E. *Europ J Biochem* 1997;245:64–70.
- [44] Villa RF, Gorini A. Action of L-acetylcarnitine on different cerebral mitochondrial populations from hippocampus and striatum during aging. *Neurochemical Research* 1991;16:1125–32.