

Reduced coenzyme Q10 supplementation decelerates senescence in SAMP1 mice[☆]

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Abstract

The SAMP1 strain is a mouse model for accelerated senescence and severe senile amyloidosis. We determined whether supplementation with coenzyme Q10 (CoQ10) could decelerate aging in SAMP1 mice and its potential role in aging. Plasma concentrations of CoQ10 and CoQ9 decreased with age in SAMP1 but not in SAMR1 mice. Supplementation with reduced CoQ10 (CoQH₂, 250 mg/kg/day) for one week increased plasma CoQ10 concentrations, with an accompanying decrease in plasma CoQ9 concentrations. In two series of experiments, lifelong supplementation with CoQH₂ decreased the senescence grading scores from 10 to 14 months, 7 to 15 months, and at 17 months of age. The body weight of female mice increased from 2 to 10 months of age versus controls in the second series of experiments. Lifelong CoQH₂ supplementation did not prolong or shorten the lifespan, nor did it alter the murine senile amyloid (AApoAII) deposition rate or cancer incidence. In the second series of experiments, urinary levels of 8-hydroxydeoxyguanosine did not change with age or long-term supplementation with CoQH₂. Urinary levels of acrolein (ACR)-lysine adduct increased significantly with age in SAMP1 mice; however, CoQH₂ had no effect. Thus, lifelong dietary supplementation with CoQH₂ decreased the degree of senescence in middle-aged SAMP1 mice.

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1. Introduction

Coenzyme Q (CoQ), or ubiquinone (2, 3-dimethoxy-5-methyl-6-polyprenyl-1, 4-benzoquinone), has been identified in microorganisms, plants, and animals. It consists of a redox active quinoid moiety and a hydrophobic side chain comprised of 6 to 10 isoprenoid units, depending on the species. Coenzyme Q10 (CoQ10), which contains 10 isoprenoid units in its side chain, is the most predominant form in most animals, including humans. However, CoQ9 is the primary form found in rats and mice. CoQ6, CoQ7, and CoQ8 are found in yeast and bacteria (Overvad et al., 1999). CoQ plays multiple functional roles in cells. First, its most recognized function is

that its quinone form transfers electrons from complexes I and II to complex III in the mitochondrial electron transport chain (Ernster and Dallner, 1995) associated with oxidative phosphorylation, leading to ATP generation. Second, CoQ also has functions in its reduced form (ubiquinol), protecting mitochondrial membrane proteins and DNA from oxidative damage accompanying lipid peroxidation, either by scavenging free radicals directly or by reducing the α -tocopheroxyl radical to α -tocopherol (Forsmark-Andree et al., 1994, 1995, 1997; Ernster and Dallner, 1995; Lass and Sohal, 1998).

Endogenous synthesis is thought to be the main source of the CoQ supply (Dallner and Sindelar, 2000). Increasing intracellular deficiency of CoQ during aging and in certain pathologic conditions induces cellular dysfunction (Littarru et al., 1996; Rotig et al., 2000). Thus, exogenously supplied CoQ10 may prevent the progression of aging to some extent. Whether dietary supplementation with CoQ10 can elevate tissue levels of CoQ10 remains controversial. In studies of short-term dietary CoQ10 supplementation, it was widely believed that tissues besides plasma, liver, and spleen, were resistant to augmentation from an exogenous source (Reahal

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and Wrigglesworth, 1992; Zhang et al., 1995, 1996; Ibrahim et al., 2000). However, recent studies found that relatively long-term CoQ10 intake elevated the mitochondrial and tissue levels of CoQ10 in both the rat and mouse (Kwong et al., 2002; Kamzalov et al., 2003). Furthermore, one report showed that prolonged CoQ10 intake may result in the elevation of CoQ10 in brain (Matthews et al., 1998). Although numerous clinical studies have reported ameliorative effects of CoQ10 intake on a variety of human diseases, particularly those associated with the nervous system, skeletal muscles, and heart, the validity of such claims and the nature of the mechanisms by which CoQ10 provides a beneficial effect remain virtually unknown (Ihara et al., 1989; Abe et al., 1991).

Aging in higher organisms is a complex process that is likely controlled by a combination of many different factors: genetic, environmental, nutritional, and pathologic. The senescence-accelerated mouse (SAM) provides a unique model system for the study of the aging process in higher organisms (Takeda et al., 1991, 1994, 1997; Kuro-o, 2001). The SAM strains include the accelerated senescence-prone SAMP series and the accelerated senescence-resistant SAMR series (which serve as controls for the SAMP strains). The SAMP strains grow normally, but they show early signs of aging, including greatly reduced physical activity and loss of hair glossiness, coarseness of the skin, hair loss, periophthalmic lesions, and increased lordokyphosis of the spine. Their life spans are markedly shorter than normal. Analysis of aging dynamics, based on survival curves, scores of senescence, and growth rate, suggests that the aging pattern in the SAMP strains is one of accelerated senescence after normal development (Takeda et al., 1981; Hosokawa et al., 1984). Molecular genetic characterization of the SAM strains revealed that they might be a group of recombinant inbred strains involving the AKR/J strain and an unknown strain developed by accidental outbreeding (Kitado et al., 1994). SAMP1, a strain in the SAMP series, manifests a variety of senescence-associated pathologic phenotypes, such as senile amyloidosis (AApoAII), contracted kidneys, impaired immune responses, hyperinflation of the lungs, hearing impairment, and hypertensive vascular disease. Among these phenotypes, senile amyloidosis is one of the most characteristic age-associated disorders. Furthermore, accumulating evidence accounts for higher oxidative status and mitochondrial dysfunction in various organs such as the skin, brain, liver, heart, eye, and artery (Hosokawa, 2002) in SAMP1 mice. The hyperoxidative status mentioned above may incur damage to the DNA, proteins, and lipids of the cell, and may impair cell proliferation, cell survival, and various cellular functions.

CoQ10 is the only lipophilic antioxidant that has been biosynthesized. Exogenous CoQ10 may protect cells from oxidative stress after conversion to its reduced antioxidant form by cellular plasma membrane oxidoreductases and DT-diaphorase (Lenaz et al., 2002; Genova et al., 2003, 2004). In the present study, we examined whether dietary supplementation with coenzyme Q10 could exert beneficial effects against accelerated senescence in SAMP1 mice.

2. Materials and methods

2.1. Animals

SAMP1 and SAMR1 mice were raised in the Division of Laboratory Animal Research, Research Center for Human and Environmental Sciences, Shinshu University, under specific pathogen-free (SPF) conditions at 24 ± 2 °C with a light-controlled regimen (12-h light/dark cycle). Tap water and food were available ad libitum. At the start of the longitudinal study, 4-week-old SAMP1 mice of the same gender were selected and housed 3 to 6 per cage (20 cm wide \times 30 cm long \times 10 cm deep). Housing groups remained the same, with no regrouping throughout their life spans. Every month, all mice were weighed, food intake was calculated, and the degree of senescence was evaluated. Mice were inspected at least twice daily, and mice that died spontaneously were necropsied immediately. The liver, spleen, heart, kidney, lungs, and neoplasms were weighed, and tissues of the whole body were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 4- μ m sections that were stained with hematoxylin and eosin or used for histopathologic demonstration of amyloid deposition. All experiments were performed with the consent of the Animal Care and Use Committee of Shinshu University School of Medicine.

2.2. Preparation of mouse diet

CoQ10 or its reduced form (CoQH₂) was mixed into standard laboratory mouse diet (powdered CE-2, CLEA Japan) using corn oil in equal proportions to 1% (v/w) of the diet as a vehicle, to achieve a final concentration of CoQ10 or CoQH₂ (0.2%, w/w), respectively. The mixture was processed into pellet-type chow by adding ethanol (30% in water), pressure shaping, and drying. The control diet was prepared using corn oil only. The diet was preserved at 4.0 °C (control and CoQ10) and -20 °C (CoQH₂) for up to two weeks before administration.

2.3. CoQ10 supplementation

In the first series of experiments, SAMP1 mice were randomly assigned to six groups: control males ($n=15$), control females ($n=12$); CoQ10 males ($n=9$), CoQ10 females ($n=11$); CoQH₂ males ($n=9$), and CoQH₂ females ($n=10$). In the second series of experiments, owing to fighting among the male mice, only female mice were used in the control ($n=14$) and CoQH₂ ($n=14$) groups.

2.4. Evaluation of degree of senescence

The degree of senescence was evaluated by a grading system (Hosokawa et al., 1984). Eleven categories of behavioral activity and gross appearances of the skin, eyes, and spine were considered to be associated with the aging process: reactivity, passivity (general); loss of hair and of glossiness, skin coarseness, skin ulcers; periophthalmic

lesions, cataracts, corneal ulcers, corneal opacity, and lordokyphosis of the spine. Each category was graded 0 to 4 according to the degree of change, and the grading score for each mouse was the sum of the grades of each category. Since grading scores increased irreversibly and universally with advancing age in all strains of mice tested, and there was a statistically significant inverse correlation between the remaining life span and the total grading score, this grading score appears valid for the evaluation of the degree of senescence (Hosokawa et al., 1984, 1994). Generally, the grading was done at a fixed time (from 2 pm to 4 pm) by an observer who was blinded to the treatment of the mice examined.

2.5. Extraction and quantification of CoQ homologues

Female SAMP1 and SAMR1 mice aged 2, 10, and 16 months without CoQ supplementation were anesthetized with diethyl ether, and blood was collected in a heparin-coated tube by cardiac puncture and centrifuged at $6000\times g$ at room temperature for 15 min. Plasma was used to determine the variation of CoQ concentrations with age. For the short-term CoQ10 or CoQH₂ supplementation study, 2-month-old female SAMP1 mice were supplemented with a control, 0.2% CoQ10, or 0.2% CoQH₂ diet for one week, and then treated as described above.

Total plasma concentrations of CoQ9 or CoQ10 (sum of the concentrations of oxidized and reduced forms of CoQ9 or CoQ10) were determined by HPLC, as described by Yamashita and colleagues (1997), with a minor modification. Briefly, 20 μ l internal standard (Coenzyme Q7, 5 μ g/ml in ethanol), 500 μ l distilled water, and 10 μ l 1% FeCl₃ were added to 100 μ l plasma, vortexed for 5 s, and then 2.0 ml methanol and 3.0 ml hexane were added. After vigorous mixing for 5 min and centrifugation at $1870\times g$ for 10 min, the hexane layer was collected. The remaining aqueous layer was further extracted with 3.0 ml of hexane in a similar process. The two hexane layers were mixed and evaporated, reconstituted in 200 μ l of ethanol, and 50 μ l of the solution was injected onto an HPLC system (Shimadzu LC-10A system, Shimadzu, Kyoto, Japan) with an analytical column (25.0 cm \times 0.46 cm, 5- μ m; YMC-Pack ODS-A303, YMC, Kyoto, Japan), a reduction column (15.0 mm \times 4 mm; RC-10, Shiseido, Tokyo, Japan), and an amperometric electrochemical detector (ECD, SI-2, Shiseido, Tokyo, Japan). The mobile phase consisted of 0.05 M NaClO₄ monohydrate in methanol/hexane (88:12) at a flow rate of 1.0 ml/min. The oxidation potential for the ECD was 600 mV. The concentrations of total CoQ9 and total CoQ10 were obtained by comparison of the peak area ratios of CoQ9 or CoQ10 to the internal standard and those for the standard solutions of known concentrations.

2.6. Determination of urinary oxidative stress markers

In the second series of experiments, each of the SAMP1 mice in the CoQH₂ lifelong supplementation group and the control group was put in one metabolic cage for 12 h (from 9:00 pm to 9:00 am). The urine of each mouse was collected at

2-month intervals from 10 to 16 months of age, centrifuged at $3000\times g$, 4.0 °C for 5 min to pellet debris and preserved at -70 °C for later analyses.

Urinary acrolein-lysine adduct and 8-OHdG were determined using an enzyme-linked immunosorbent assay kit (ACR-Lysine Adduct ELISA System N213300, Nihon Yushi, Tokyo, Japan) and New 8-OHdG Check kit (Japan Institute for the Control of Aging, Shizuoka, Japan), respectively. The values were then corrected to the urinary concentration of creatinine that was determined using the Creatinine Test Wako (Wako Pure Chemical, Osaka, Japan); the urinary acrolein-lysine adduct/creatinine and 8-OHdG/creatinine levels were used in subsequent analyses.

2.7. Detection of amyloid deposition

Amyloid was identified by green birefringence in Congo-red stained sections under polarizing microscopy (Puchtler et al., 1962). The intensity of AApoAII amyloid deposition was determined using the amyloid index (AI) as a parameter. AI was the average of grades 0 to 4 in the following seven organs: liver, spleen, skin, heart, stomach (Xing et al., 2002), small intestine, and tongue (Higuchi et al., 1998). Two observers who had no information about the tissue examined graded amyloid deposition in each tissue and averaged the values to obtain the AI for each mouse.

2.8. Statistical analysis

Concentrations of CoQ homologues at various ages, urinary levels of acrolein-lysine adduct and 8-OHdG at various ages, and the ratio of CoQ10 to CoQ9 in the two strains (SAMP1, SAMR1) at various ages were compared by two-way analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) test. The senescence grading scores and the degree of AApoAII deposition (AI) in SAMP1 mice supplemented with control, 0.2% CoQ10, or 0.2% CoQH₂ were compared with the Mann-Whitney-*U* test. Body weights were compared by unpaired Student's *t* test. The incidence of each pathologic finding was compared with the Fisher's exact test. Survival data were analyzed using the Log-rank (Mantel-Cox) test and Kaplan-Meier survival curves.

3. Results

3.1. Plasma concentrations of CoQ homologues

Female SAMP1 and SAMR1 mice showed different age-related changes in plasma concentrations of CoQ10 or CoQ9 from 2 to 16 months of age. Two-way ANOVA of age and strain showed that the age effect was significant for CoQ10 ($p=0.0098$) and CoQ9 ($p=0.0008$) in SAMP1 mice. CoQ9 in SAMP1 mice increased between 2 and 10 months of age, and then decreased between 10 and 16 months of age. However, the age effect was not significant for CoQ10 or CoQ9 in SAMR1 mice. The strain effect and the interaction of age and strain were not significant. (Fig. 1A and B). Two-way ANOVA of

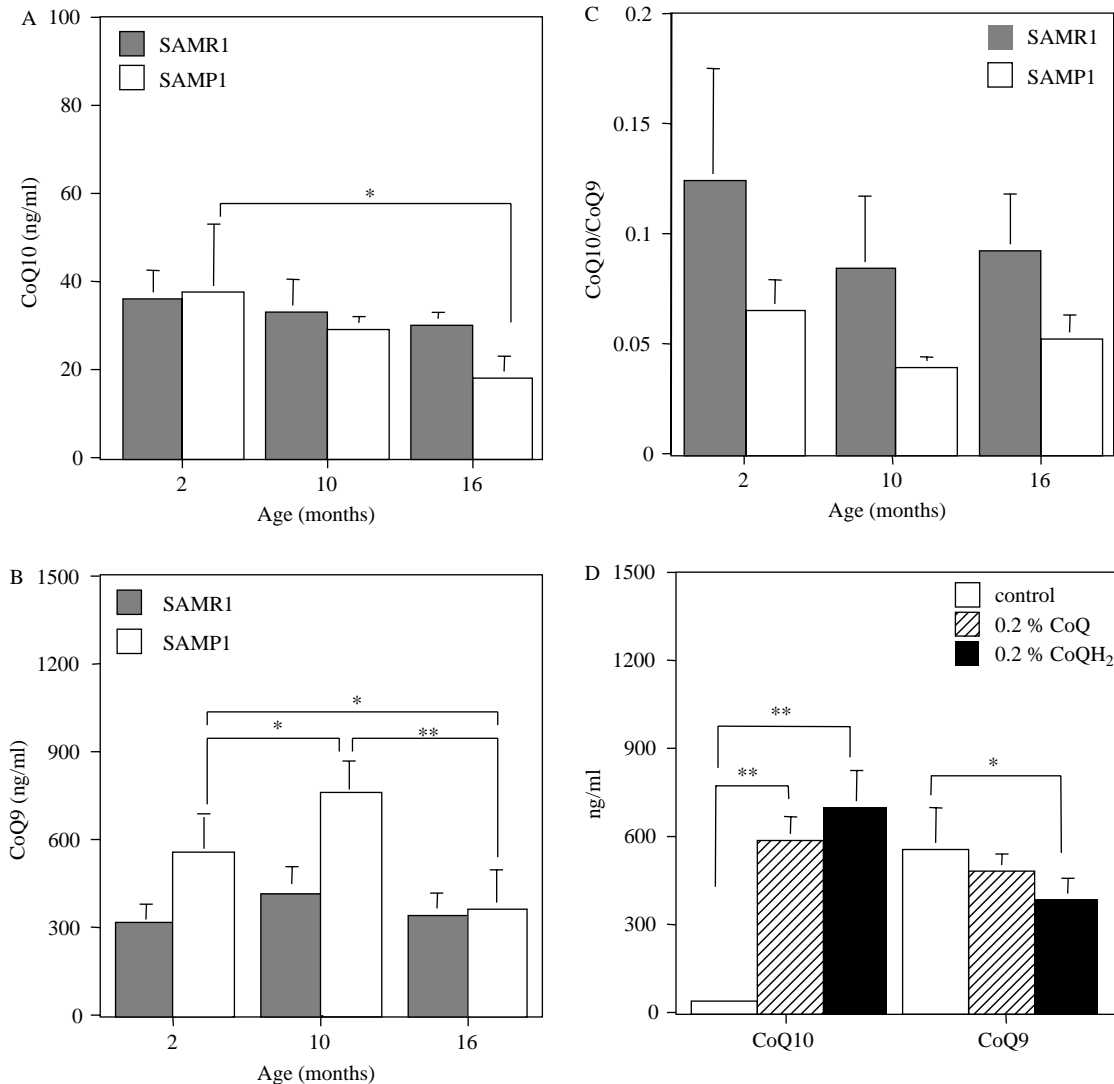


Fig. 1. Concentrations of plasma CoQ homologues. Data are mean \pm SD. (A) Age-related changes in the plasma concentration of CoQ10 in SAMR1 ($n=4, 4, 5$) and SAMP1 mice ($n=9, 4, 7$). *: $p < 0.01$. (B) Age-related changes in the plasma concentration of CoQ9 in SAMR1 and SAMP1 mice. *: $p < 0.05$, **: $p < 0.001$. (C) Age-related changes in the ratio of CoQ10 levels to CoQ9 levels in SAMR1 and SAMP1 mice. (D) Variations in the concentration of plasma CoQ10 and CoQ9 after dietary supplementation with control ($n=9$), CoQ10 ($n=4$), or CoQH₂ ($n=6$) for one week in 2-month-old SAMP1 female mice. *: $p < 0.05$, **: $p < 0.0001$.

strain and age showed significance for the strain effect ($p < 0.0001$) and the age effect ($p = 0.0186$) for the ratio of CoQ10 levels to CoQ9 levels. The interaction of age and strain was not significant. The ratio of CoQ10 levels to CoQ9 levels in SAMR1 mice was about twofold that in SAMP1 mice at any age point (Fig. 1C). Supplementation with CoQ10 or CoQH₂ for one week significantly elevated plasma concentrations of CoQ10 15.7 and 18.7 fold, respectively. However, concentrations of CoQ9 decreased significantly in SAMP1 mice supplemented with CoQH₂ (Fig. 1D).

3.2. Effect of CoQH₂ dietary supplementation on food intake and body weight

Food intake decreased slowly with increasing age in both sexes and in both series of experiments. Food intake was calculated monthly; no difference in food consumption was apparent among the three groups at each monthly time point,

Based on mean food intake (3.5 g/day) and mean body weight (28 g), the mice consumed 250 mg/kg/day CoQ10 (CoQ10 group) and 250 mg/kg/day CoQH₂ (CoQH₂ group), respectively. In both series of experiments, body weight increased steeply up to 4 months of age in males and up to 5 months of age in females, then it stabilized until 10 months of age, after which it tended to decrease. In the first series of experiments, the CoQ10 and CoQH₂ male groups tended to be heavier than the control male group, however, there was no significant difference in body weight between CoQ10 and control groups (data not shown). In females, age-related changes in body weight of the CoQ10 and control groups were the same. The body weight of the CoQH₂ group tended to be greater than that of the control group from 5 to 18 months, except at 14 months of age; however, there was no statistical difference between the CoQH₂ and control groups by age-matched analysis using an unpaired Student's *t* test (Fig. 2A). In the second series of experiments, the body weights of the CoQH₂ group were

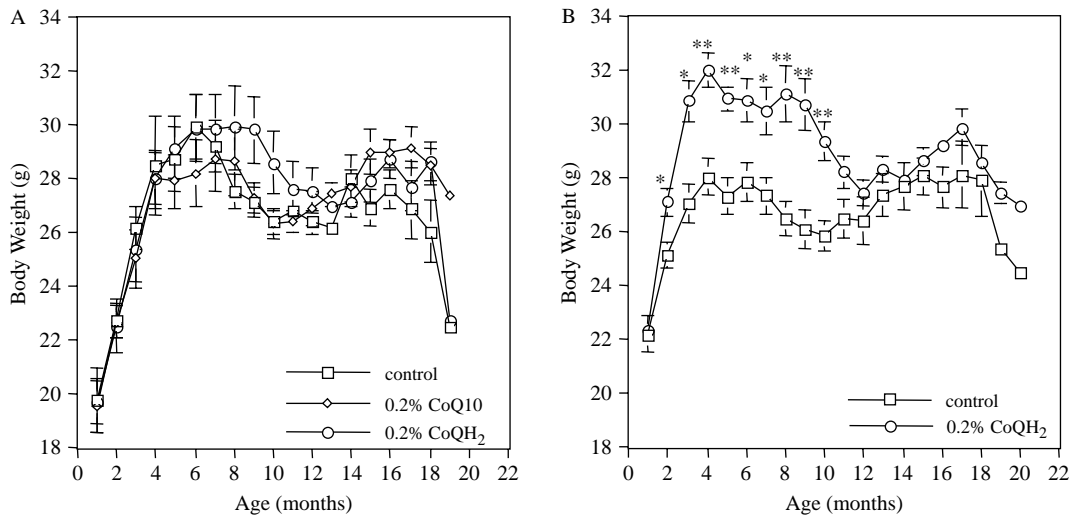


Fig. 2. Age-related changes in body weight in SAMP1 mice supplemented with control, CoQ10, or CoQH₂ in the first series of experiments (A) and supplemented with control or CoQH₂ in the second series of experiments (B). *: $p < 0.01$, **: $p < 0.001$. Data are mean \pm SEM.

greater than those of the control group, and there was a significant difference between the CoQH₂ and the control group from 2 to 10 months of age by age-matched analysis using an unpaired Student's *t* test (Fig. 2B).

3.3. Effect of CoQH₂ dietary supplementation on senescence

Senescence grading scores increased from 4 or 5 months of age in SAMP1 mice of both sexes and in both series of experiments. In the first series, in the male groups, from 7 months of age, senescence grading scores in the control group were higher than those in the CoQ10 and CoQH₂ groups. There was a significant difference between the control and the CoQ10 or CoQH₂ groups at 8, 10, and 11 months of age (data not shown). In the female groups, the grading scores in the control group were higher than those of the CoQH₂ group from 7 months of age throughout the rest of life, there was a significant difference between the CoQH₂ and control groups from 10 to 14 months (Fig. 3A). There was no significant difference between the CoQ10 group and the control group (Fig. 3A).

To determine whether the results of the first series of experiments were reproducible, the second series of experiments was conducted. Senescence grading scores in both the control and CoQH₂ groups increased from 5 months of age, grading scores in the control group were higher than those of the CoQH₂ group throughout the rest of the lifespan. There was a significant difference in grading scores between the control and CoQH₂ groups from 7 months of age to 15 months of age, as well as at 17 months of age (Fig. 3B). At 11 months of age, the 11 categories of grading scores were divided into four groups: skin, general, eye, and spine. In all 4 groups, the grading scores in the CoQH₂ group were significantly lower than those in the control group (Fig. 3C).

3.4. Effect of CoQH₂ dietary supplementation on survival

In the first series of experiments, the mean (\pm SD) lifespans of the control groups were 544.07 ± 64.76 (males) and

494.67 ± 95.66 (females) days; in the CoQ10-supplemented groups, they were 509.78 ± 92.31 (CoQ10 males), 512.91 ± 77.26 (CoQ10 females), 517.89 ± 82.39 (CoQH₂ males), 471.70 ± 98.84 (CoQH₂ females) days, respectively. There were no significant differences in lifespan between the control and the CoQ10- or CoQH₂-supplemented groups in both females (Fig. 4A) and males (data not shown). In the second series of experiments, the mean lifespans of the control and CoQH₂-supplemented groups were 489.86 ± 91.33 and 519.64 ± 79.53 days, respectively. There was no significant difference in lifespan between the CoQH₂ and control groups when analyzed using the Log-rank (Mantel–Cox) test (Fig. 4B).

3.5. Effect of CoQH₂ dietary supplementation on oxidative stress

SAMP1 mice supplemented without or with CoQH₂ showed similar age-related changes in the concentration of acrolein–lysine adduct from 10 to 16 months of age (Fig. 5A), two-way ANOVA of age and CoQH₂ showed that the age effect was significant ($p < 0.0001$), but the CoQH₂ effect was not significant ($p = 0.3341$), interaction of age and CoQH₂ were not significant. Two-way ANOVA of age and CoQH₂ showed that the effect of age and CoQH₂, interaction of age and CoQH₂ were not significant in 8-OHdG in SAMP1 mice supplemented without or with CoQH₂ (Fig. 5B).

3.6. Effect of CoQ10 dietary supplementation on amyloidosis and general pathology

Fifty-six mice in the first series of experiments (control males, 14; females, 10; CoQ10 males, 8; females, 9; CoQH₂ males, 7; females, 8) and 28 mice in the second series of experiments (control females: 14; CoQH₂ females: 14) without severe autolysis were selected for pathologic examination. The primary pathologic findings were amyloidosis, inflammatory

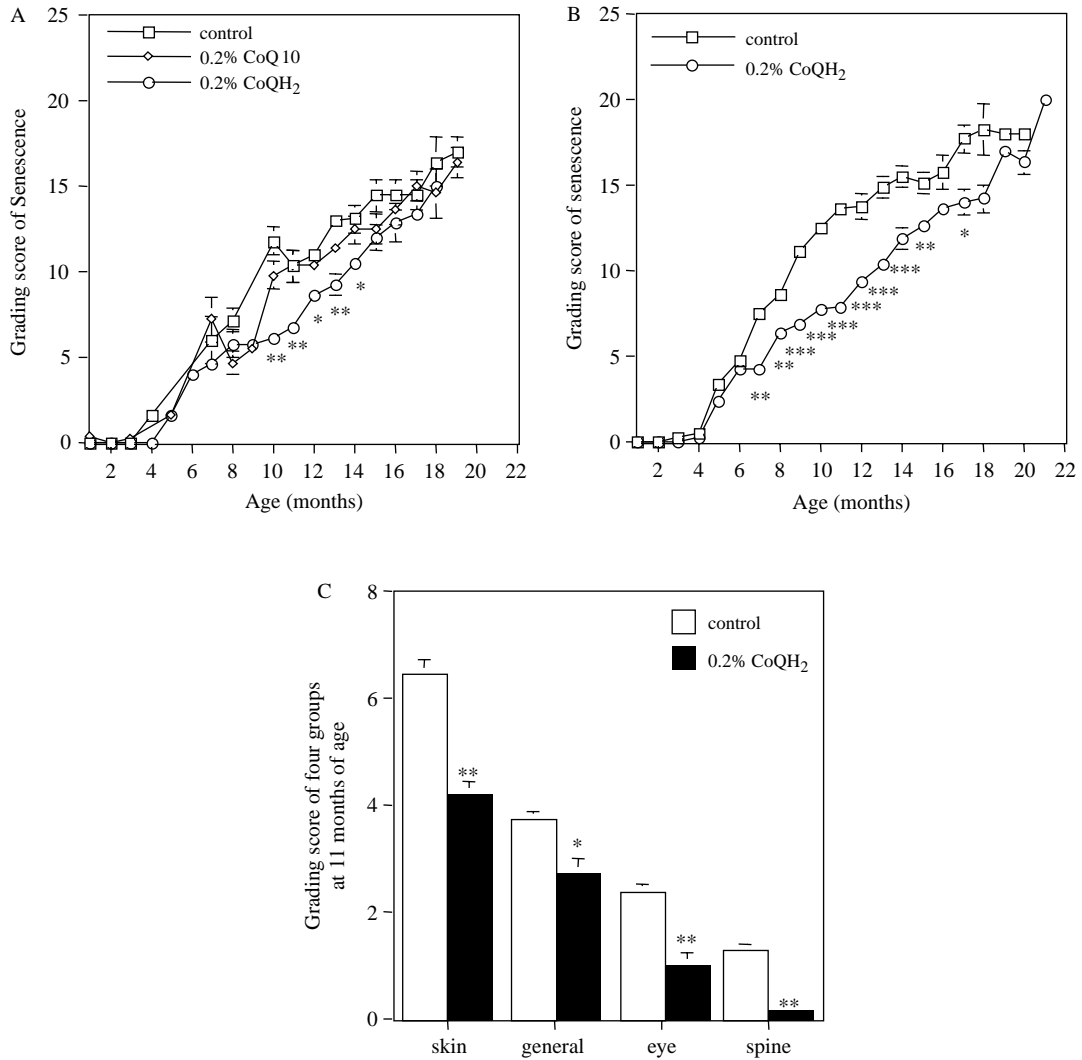


Fig. 3. Age-related changes in senescence grading scores in SAMP1 mice supplemented with control, CoQ10 or CoQH₂. The values are means \pm SEM of mice in each age group. (A) In the first series of experiments, there was a significant difference between the control and CoQH₂ groups from 10 to 14 months of age. *: $p < 0.05$, **: $p < 0.01$. (B) In the second series of experiments, there was a significant difference between the control and CoQH₂ groups from 7 to 17 months of age, except at 16 months of age. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. (C) In the second series of experiments, there was a significant difference between the control and CoQH₂ groups in four groups of grading scores of senescence at 11 months of age. *: $p < 0.05$, **: $p < 0.01$.

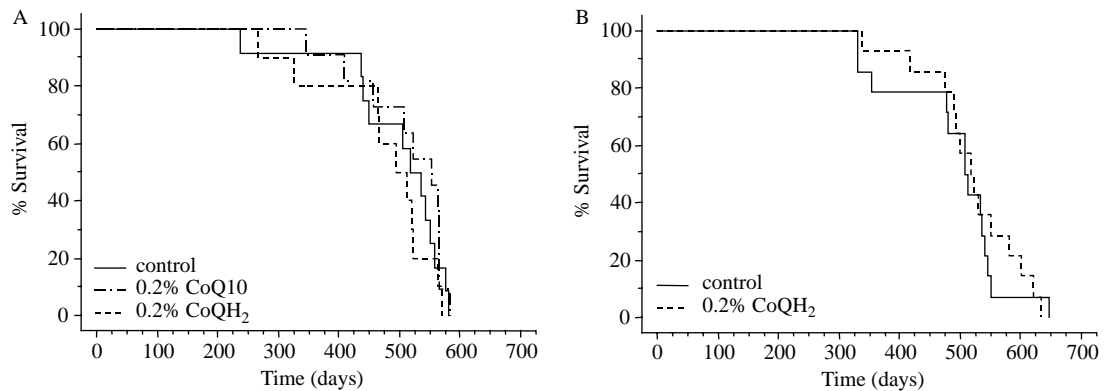


Fig. 4. Survival curves of female SAMP1 mice supplemented with control, CoQ10 or CoQH₂ in the first series of experiments (A) and supplemented with control or CoQH₂ in the second series of experiments (B).

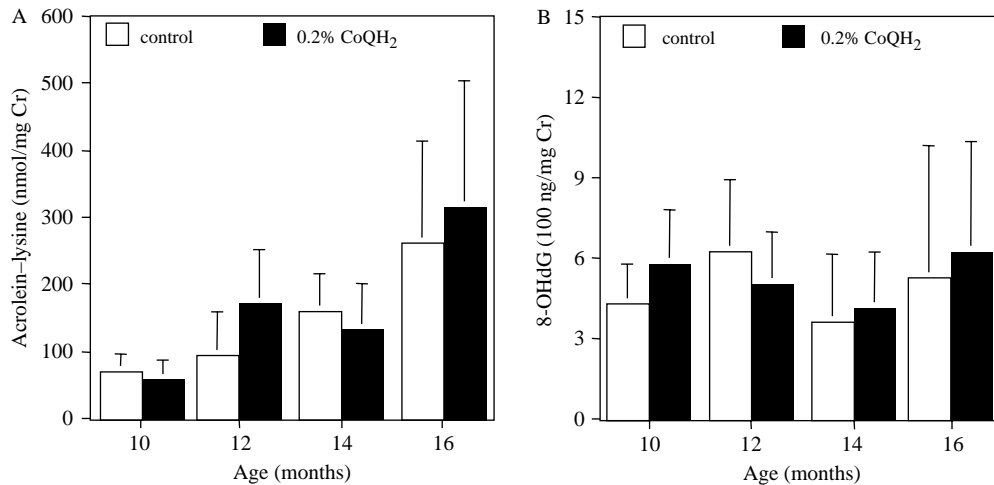


Fig. 5. Age-related changes in urinary oxidative stress markers in SAMP1 mice supplemented with control or CoQH₂. Open squares: $n=8, 8, 8, 7$ at 10, 12, 14, 16 months of age, respectively; closed squares: $n=11, 11, 12, 8$ at 10, 12, 14, 16 months of age, respectively. Data are mean \pm SD. (A) Two-way ANOVA of age and CoQH₂ showed that the age effect was significant ($p<0.0001$) in urinary concentration of acrolein-lysine adducts, but the CoQH₂ effect was not significant ($p=0.3341$). (B) Two-way ANOVA of age and CoQH₂ showed that neither the age nor the CoQH₂ effect was significant in urinary concentration of 8-hydroxydeoxyguanosine (8-OHdG).

changes (mainly abscess), neoplasm (mainly malignant lymphoma and histiocytic sarcoma), contracted kidney, and others (mainly skin ulcer, interstitial pneumonia, chronic cervical lymphadenitis, chronic splenitis, pulmonary embolism, ovarian cyst, etc.) (Table 1). The incidence of each pathological finding was not significantly different between the control and the CoQ or CoQH₂ groups. The incidence of AApoAII amyloidosis in each group was 100%, there was no significant difference in AI between the control and CoQH₂supplemented groups (Fig. 6).

4. Discussion

Previous studies have shown that CoQ10 administration exerts beneficial effects in humans and experimental animals. Some patients with mitochondrial disorders treated with CoQ10 have shown clinical and biochemical improvements (Ihara et al., 1989; Abe et al., 1991). Oral administration of coenzyme Q10 significantly extended survival and delayed the

development of motor deficits, weight loss, cerebral atrophy, and neuronal intranuclear inclusions in the R6/2 transgenic mouse model of Huntington's disease (Ferrante et al., 2002). Coenzyme Q10 extended the lifespan of wild-type and *mev-1* (which encodes cytochrome *b*) mutant *C. elegans* by reducing superoxide anion levels (Ishii et al., 2004). Long-term supplementation with coenzyme Q10 extended the lifespan of rats by attenuating oxidative DNA damage owing to a polyunsaturated fatty acid (PUFA)-based diet (Quiles et al., 2004).

In the present study, we examined whether dietary supplementation of CoQH₂ could exert beneficial effects in SAMP1 mice, which are a mouse model of accelerated senescence. We found that dietary supplementation with CoQH₂ produces a significant decrease in the senescence grading score and a significant increase of body weight in SAMP1 mice. The grading system was developed for evaluation of the degree of senescence of a mouse and maintenance of the phenotype of accelerated senescence in

Table 1
Pathologic findings in SAMP1 mice supplemented with control, CoQ or CoQH₂

		Amyloidosis	Neoplasm					Contracted kidney	Abscess	Others
			Lymphoma	Histiocytic sarcoma	Breast cancer	Fibrosarcoma	Malignant neurinoma			
A										
Control	M	14/14	0/14	2/14	0/14	0/14	1/14	12/14	2/14	10/14
CoQ	M	8/8	4/8	0/8	0/8	0/8	0/8	8/8	1/8	3/8
CoQH ₂	M	7/7	0/7	0/7	0/7	0/7	0/7	6/7	4/7	6/7
Control	F	10/10	2/10	0/10	0/10	0/10	0/10	8/10	2/10	7/10
CoQ	F	9/9	0/9	1/9	0/9	0/9	0/9	6/9	1/9	3/9
CoQH ₂	F	8/8	1/8	1/8	0/8	0/8	0/8	8/8	3/8	5/8
B										
Control	F	14/14	2/14	1/14	1/14	0/14	0/14	9/14	7/14	2/14
CoQH ₂	F	14/14	1/14	1/14	1/14	1/14	0/14	6/14	3/14	5/14

Note: A, first series of experiments; B, second series of experiments; M, male; F, female.

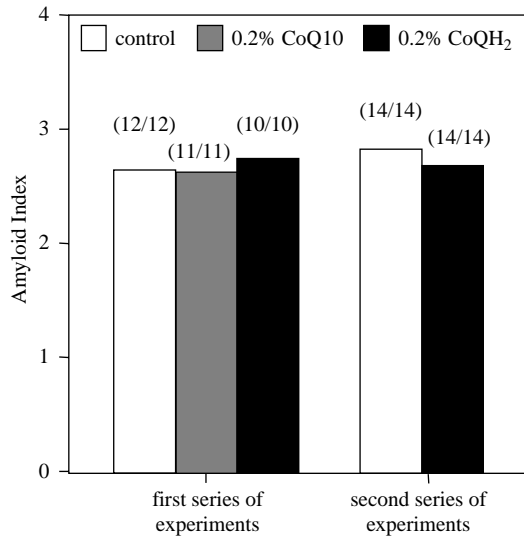


Fig. 6. AApoAII systemic senile amyloid deposits in SAMP1 mice supplemented with control, CoQ10 or CoQH₂ in the first series of experiments and supplemented with control, or CoQH₂ in the second series of experiments. In two series of experiments, there was no significant difference in AI between the control and CoQH₂ groups. Numbers in parentheses indicate the number of mice having AApoAII deposits and the number of mice examined.

SAMP strains. This system was designed to satisfy the following criteria: (1) appropriate for longitudinal studies of individual mice, (2) easy to apply, (3) results expressed numerically and analyzed statistically, and (4) objective and reproducible grading scores (Hosokawa, 1994). Mice are graded according to clearly defined criteria (Takeda et al., 1981). This system has been used as a method to evaluate the effect of manipulations that modify lifespan and senescence in many laboratories (Naiki et al., 1993; Umezawa et al., 1993). The grading system consists of 11 items classified into four groups: behavioral, gross appearance of the skin, ophthalmic lesions, and lordokyphosis of the spine. Lower scores in SAMP1 mice supplemented with CoQH₂ suggest that CoQH₂ might delay senescence. The body weight of SAMP1 mice is greater than that of SAMR1 mice (Yanagidaira et al., 2003). Though food intake was the same among the three groups, CoQH₂ supplementation increased the body weight of SAMP1 mice at adolescence. The effect of CoQH₂ on body weight may reflect an increased level of activity in SAMP1 mice. However, further study is required to elucidate the mechanism of increased body weight brought about by CoQH₂ supplementation.

The onset of amyloidosis, characterized by the extracellular accumulation of fine amyloid fibrils, is usually in the middle and late stages of life. Senile amyloidosis occurs in SAMP1 mice. Severe AApoAII deposition replaces and destroys cells throughout the body and might accelerate the functional loss of each tissue. This accelerated functional loss might increase the senescence grading score in SAMP1 mice. AApoAII amyloid deposition was not altered by CoQ10 and CoQH₂ supplementation, and CoQH₂ could not block the initiation or inhibit the propagation of AApoAII senile amyloidosis. Although there is

a significant decrease in the grading of senescence, there does not appear to be an effect on amyloidosis progression.

To evaluate the mechanism of deceleration of the grade of senescence by CoQ10 supplementation, we tried to determine the oxidative stress in SAMP1 mice. Acrolein (CH₂=CH-CHO) is one of the major lipid peroxidation products with cytotoxic and mutagenic activities. Among α,β -unsaturated aldehydes, acrolein is by far the strongest electrophile and reacts with nucleophiles, such as the sulfhydryl group of cysteine, the imidazole group of histidine, and the amino group of lysine. Acrolein-lysine adducts can be used as biological markers of lipid peroxidation (Uchida et al., 1998a, 1998b; Tsukahara et al., 2003, 2004). 8-OHdG is used as a sensitive marker for oxidative DNA damage. When reactive oxygen intermediates (including superoxide, hydrogen peroxide, and hydroxyl radical) attack DNA, guanine is changed to water-soluble 8-oxo-guanine. This DNA damage is usually extracted by repair enzymes as 8-OHdG and is finally excreted in the urine without being further metabolized (Toyokuni, 1999). Quantification of these two markers in the urine may serve as a noninvasive determination of oxidative stress in vivo and enable repeated monitoring in our longitudinal study. We found that SAMP1 mice suffered age-related lipid peroxidation damage ($p < 0.0001$) that was not alleviated by lifelong dietary CoQH₂ supplementation. In addition, urinary 8-OHdG levels did not correlate with aging and were not affected by CoQH₂ supplementation. Thus, decrease in the senescence grading score may not be the result of an effect on oxidative DNA damage or lipid peroxidation damage.

How does CoQH₂ exert its beneficial effects? For CoQ10 supplementation to have a treatment effect, it must be absorbed to a significant degree and incorporated into the mitochondrial membrane. Animal studies have demonstrated that exogenous CoQ10 can elevate plasma concentrations of CoQ10 several-fold (Reahal and Wrigglesworth, 1992; Zhang et al., 1995, 1996; Matthews et al., 1998; Ibrahim et al., 2000; Kwong et al., 2002; Kamzalov et al., 2003) and CoQ10 levels in homogenates of the liver, kidney, heart, skeletal muscle, and brain, as well as the mitochondria of these tissues (Kwong et al., 2002; Kamzalov et al., 2003). The CoQ10 or CoQH₂ from the supplements in our study was absorbed, as plasma CoQ10 levels increased about 20 fold after 1 week of oral administration, although the degree of mitochondrial incorporation is still unclear. Studies on oxidative phosphorylation in the livers of SAMP8 and SAMR1 mice showed that the respiratory control ratio decreased during aging in SAMP8 mice, and it was estimated that by 18 months of age, it might be inefficient to provide the ATP synthesis levels necessary for normal cell metabolism (Nakahara et al., 1998). A defect in oxidative phosphorylation may result in increased consumption of CoQ10 and therefore contribute to lower CoQ10 levels (Littarru and Battino, 2001). This may be the case for the SAMP1 mice in our study, as the plasma concentration of CoQ10 was reduced significantly with age ($p = 0.0098$), and the ratio of CoQ10 levels to CoQ9 levels was significantly lower compared with SAMR1 mice ($p < 0.0001$). We suggest

that the accelerated decrease in CoQ10 concentration may contribute to the accelerated senescence of SAMP1 mice. In oxidative phosphorylation disorders, there was a significant increase in ATP synthetic capacity in lymphocytes from patients undergoing CoQ10 treatment (Marriage et al., 2004). Furthermore, in vitro study of CoQ supplementation in control lymphocytes showed that a 49% increase in mitochondrial ATP synthesis can be attributed to supplementation with CoQ10 (Marriage et al., 2004). Thus, we suggest that the decrease in the grading score of senescence might be attributed to the elevated ATP synthesis in SAMP1 mice.

Longevity correlates positively with CoQ10 levels and negatively with CoQ9 levels in different mammalian species, although the exact role played by the CoQ10/CoQ9 ratio has not been determined (Lass et al., 1997). The average life spans of SAMP1 and SAMR1 mice in SPF conditions are 15.4 and 22.0 months, respectively (Takeda, 2004). In the present study, the CoQ10/CoQ9 ratio in SAMR1 mice was about two-fold that in SAMP1 mice at any age point. The lower CoQ10/CoQ9 ratio may correlate with the shorter life span and accelerated senescence in SAMP1 mice. Plasma CoQ9 levels decreased significantly when SAMP1 mice received CoQH₂ for one week. This is contrary to classical views that dietary CoQ10 supplementation did not appear to affect endogenous biosynthesis of CoQ9 (Zhang et al., 1995), and plasma CoQ9 levels remained unchanged (Ibrahim et al., 2000) or elevated (Zhang et al., 1995; Lonrot et al., 1998; Matthews et al., 1998). We suggest that CoQ9 may redistribute between plasma and tissues with an overwhelming CoQ10 increase in plasma, we also do not exclude the possibility that the endogenous biosynthesis of CoQ9 might be inhibited. The significantly decreased CoQ9 level brought about by CoQH₂ supplementation versus CoQ10 supplementation may underlie the discrepancy in the variance of senescence degree; CoQH₂ supplementation significantly decreased senescence in middle-age, while the effect of CoQ10 supplementation was diminished.

Portakal and colleagues disclosed that CoQ10 concentrations in breast tumor tissues decreased significantly as compared to the surrounding normal breast tissues (Portakal et al., 2000). Lockwood reported that oral CoQ10 administration regressed metastases of breast cancer in humans (Lockwood et al., 1995). These data imply that CoQ10 supplementation may protect against the development of cancer or metastases. Though there are some publications that demonstrate a positive benefit for CoQ10 as part of a treatment regimen for patients with cancer, it is difficult for cancer studies to be designed as clinical trials with adequate controls and randomization to reveal the true efficacy of CoQ10 supplementation. In this study, SAMP1 mice had spontaneous lymphoma, histiocytic sarcoma, breast cancer, fibrosarcoma, and malignant neurinoma. The cancer incidence in the control and CoQH₂-supplemented groups of the first series of experiments was 20 and 25%, respectively. They were 28.6 and 28.6% in the second series of experiments. Caloric restriction (CR) is the only dietary intervention that has been shown consistently to extend the maximum life span in mammals (Weindruch and Walford, 1988). Though CoQ10

supplementation produced some alterations in gene expression similar to those reported for CR, it failed to affect the life span or tumorigenesis of relatively longer-lived mice. It appears likely that CR retards aging and delays tumorigenesis in mouse tissues, in part by reducing cellular proliferation or increasing apoptosis; however, CoQ10 lacks these effects (Lee et al., 2004). In the present study, the inability of CoQ10 to prevent spontaneous tumorigenesis and amyloidosis may be the major reason why CoQ10 supplementation did not prolong life span. The apparent dissociation of a decrease in the grading score of senescence and no variance of lifespan implies that the grading score of senescence, or some of its components, reflects biological aging processes that may be independent of lifespan.

In conclusion, CoQH₂ supplementation improved the behavior and appearance of SAMP1 mice. The two oxidative stress markers we determined were not affected by CoQH₂ supplementation. But this may not be enough to elucidate the results we found. Many other parameters of oxidative stress such as the GSSG/GSH ratio, malondialdehyde, or antioxidant activities may be involved in the beneficial effects. We should determine other parameters of oxidative stress and mitochondrial ATP generation in our future work.

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References

- Abe, K., Fujimura, H., Nishikawa, Y., Yorifuji, S., Mezaki, T., Hirono, N., Nishitani, N., Kameyama, M., 1991. Marked reduction in CSF lactate and pyruvate levels after CoQ therapy in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). *Acta Neurol. Scand.* 83, 356–359.
- Dallner, G., Sindelar, P., 2000. Regulation of ubiquinone metabolism. *Free Radic. Biol. Med.* 29, 285–294.
- Ernster, L., Dallner, G., 1995. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* 1271, 195–204.
- Ferrante, R.J., Andreassen, O.A., Dedeoglu, A., Ferrante, K.L., Jenkins, B.G., Hersch, S.M., Beal, M.F., 2002. Therapeutic effects of coenzyme Q₁₀ and remacemide in transgenic mouse models of Huntington's disease. *J. Neurosci.* 22, 1592–1599.
- Forsmark-Andree, P., Ernster, L., 1994. Evidence for a protective effect of endogenous ubiquinol against oxidative damage to mitochondrial protein and DNA during lipid peroxidation. *Mol. Aspects Med.* 15 (Suppl), S73–S81.
- Forsmark-Andree, P., Dallner, G., Ernster, L., 1995. Endogenous ubiquinol prevents protein modification accompanying lipid peroxidation in beef heart submitochondrial particles. *Free Radic. Biol. Med.* 19, 749–757.
- Forsmark-Andree, P., Lee, C.P., Dallner, G., Ernster, L., 1997. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radic. Biol. Med.* 22, 391–400.

- Genova, M.L., Pich, M.M., Biondi, A., Bernacchia, A., Falasca, A., Bovina, C., Formiggini, G., Parenti Castelli, G., Lenaz, G., 2003. Mitochondrial production of oxygen radical species and the role of coenzyme Q as an antioxidant. *Exp. Biol. Med.* (Maywood) 228, 506–513.
- Genova, M.L., Pich, M.M., Bernacchia, A., Bianchi, C., Biondi, A., Bovina, C., Falasca, A.I., Formiggini, G., Castelli, G.P., Lenaz, G., 2004. The mitochondrial production of reactive oxygen species in relation to aging and pathology. *Ann. NY Acad. Sci.* 1011, 86–100.
- Higuchi, K., Kogishi, K., Wang, J., Chen, X., Chiba, T., Matsushita, T., Hoshii, Y., Kawano, H., Ishihara, T., Yokota, T., Hosokawa, M., 1998. Fibrilization in mouse senile amyloidosis is fibril conformation-dependent. *Lab. Invest.* 78, 1535–1542.
- Hosokawa, M., 1994. Grading score system: a method of evaluation of the degree of senescence in senescence-accelerated mouse (SAM). In: Takeda, T. (Ed.), *The SAM Model of Senescence*. Excerpta Medica, Amsterdam, pp. 23–28.
- Hosokawa, M., 2002. A higher oxidative status accelerates senescence and aggravates age-dependent disorders in SAMP strains of mice. *Mech. Ageing Dev.* 123, 1553–1561.
- Hosokawa, M., Kasai, R., Higuchi, K., Takeshita, S., Shimizu, K., Hamamoto, H., Honma, A., Irino, M., Toda, K., Matsumura, A., et al., 1984. Grading score system: a method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). *Mech. Ageing Dev.* 26, 91–102.
- Ibrahim, W., Bhagavan, H.N., Chopra, R.K., Chow, C.K., 2000. Dietary coenzyme Q10 and vitamin E alter status of these compounds in rat tissues and mitochondria. *J. Nutr.* 130, 2343–2348.
- Ihara, Y., Namba, R., Kuroda, S., Sato, T., Shirabe, T., 1989. Mitochondrial encephalomyopathy (MELAS): pathological study and successful therapy with coenzyme Q10 and idebenone. *J. Neurol. Sci.* 90, 263–271.
- Ishii, N., Senoo-Matsuda, N., Miyake, K., Yasuda, K., Ishii, T., Hartman, P.S., Furukawa, F., 2004. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mech. Ageing Dev.* 125, 41–46.
- Kamzalov, S., Sumien, N., Forster, M.J., Sohal, R.S., 2003. Coenzyme Q intake elevates the mitochondrial and tissue levels of coenzyme Q and α -tocopherol in young mice. *J. Nutr.* 133, 3175–3180.
- Kitado, H., Higuchi, K., Takeda, T., 1994. Molecular genetic characterization of the senescence-accelerated mouse (SAM) strains. *J. Gerontol. Biol. Sci.* 49, B247–B254.
- Kuro-o, M., 2001. Disease model: human aging. *Trends Mol. Med.* 7, 179–181.
- Kwong, L.K., Kamzalov, S., Rebrin, I., Bayne, A.-C.V., Jana, C.K., Morris, P., Forster, M.J., Sohal, R.S., 2002. Effects of coenzyme Q10 administration on its tissue concentration, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radic. Biol. Med.* 33, 627–638.
- Lass, A., Sohal, R.S., 1998. Electron transport-linked ubiquinone-dependent recycling of α -tocopherol inhibits autooxidation of mitochondrial membranes. *Arch. Biochem. Biophys.* 352, 229–236.
- Lass, A., Agarwal, S., Sohal, R.S., 1997. Mitochondrial ubiquinone homologues, superoxide radical generation, and longevity in different mammalian species. *J. Biol. Chem.* 272, 19199–19204.
- Lee, C.K., Pugh, T.D., Klopp, R.G., Edwards, J., Allison, D.B., Weindruch, R., Prolla, T.A., 2004. The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free Radic. Biol. Med.* 36, 1043–1057.
- Lenaz, G., Bovina, C., D'Aurelio, M., Fato, R., Formiggini, G., Genova, M.L., Giuliano, G., Merlo Pich, M., Paolucci, U., Parenti Castelli, G., Ventura, B., 2002. Role of mitochondria in oxidative stress and aging. *Ann. NY Acad. Sci.* 959, 199–213.
- Littarru, G., Battino, M., 2001. Protection against oxidative stress by chronic administration of coenzyme Q. In: Kagan, V., Quinn, P. (Eds.), *Coenzyme Q: Molecular Mechanisms in Health and Disease*. CRC Press, Boca Raton, FL, pp. 219–226.
- Littarru, G.P., Battino, M., Folkers, K., 1996. Clinical aspects of coenzyme Q: improvement of cellular bioenergetics or antioxidant perfection? In: Cadenas, E., Packer, L. (Eds.), *Handbook of Antioxidants*. Marcel Dekker, New York, NY, pp. 203–239.
- Lockwood, K., Moesgaard, S., Yamamoto, T., Folkers, K., 1995. Progress on therapy of breast cancer with vitamin Q10 and the regression of metastases. *Biochem. Biophys. Res. Commun.* 212, 172–177.
- Lonnrot, K., Holm, P., Lagerstedt, A., Huhtala, H., Alho, H., 1998. The effects of lifelong ubiquinone Q10 supplementation on the Q9 and Q10 tissue concentrations and life span of male rats and mice. *Biochem. Mol. Biol. Int.* 44, 727–737.
- Marriage, B.J., Clandinin, M.T., Macdonald, I.M., Glerum, D.M., 2004. Cofactor treatment improves ATP synthetic capacity in patients with oxidative phosphorylation disorders. *Mol. Genet. Metab.* 81, 263–272.
- Matthews, R.T., Yang, L., Browne, S., Baik, M., Beal, M.F., 1998. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc. Natl Acad. Sci. USA* 95, 8892–8897.
- Naiki, H., Higuchi, K., Shimada, A., Takeda, T., Nakakuki, K., 1993. Genetic analysis of murine senile amyloidosis. *Lab. Invest.* 68, 332–337.
- Nakahara, H., Kanno, T., Inai, Y., Utsumi, K., Hiramatsu, M., Mori, A., Packer, L., 1998. Mitochondrial dysfunction in the senescence accelerated mouse (SAM). *Free Radic. Biol. Med.* 24, 85–92.
- Overvad, K., Diamant, B., Holm, L., Holmer, G., Mortensen, S.A., Stender, S., 1999. Coenzyme Q10 in health and disease. *Eur. J. Clin. Nutr.* 53, 764–770.
- Portakal, O., Ozkaya, O., Erden Inal, M., Bozan, B., Kosan, M., Sayek, I., 2000. Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. *Clin. Biochem.* 33, 279–284.
- Puchtler, H., Sweat, F., Levine, M., 1962. On the binding of Congo red by amyloid. *J. Histochem. Cytochem.* 10, 355–364.
- Quiles, J.L., Ochoa, J.J., Huertas, J.R., Mataix, J., 2004. Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet. *Exp. Gerontol.* 39, 189–194.
- Reahal, S., Wrigglesworth, J., 1992. Tissues concentrations of coenzyme Q10 in the rat following its oral and intraperitoneal administration. *Drug Metab. Dispos.* 20, 423–427.
- Rotig, A., Appelkvist, E.L., Geromel, V., Chretien, D., Kadhom, N., Edery, P., Lebideau, M., Dallner, G., Munnich, A., Ernster, L., Rustin, P., 2000. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. *Lancet* 356, 391–395.
- Takeda, T., 2004. Effects of environment on life span and pathobiological phenotypes in senescence-accelerated mice. In: Nomura, Y. (Ed.), *The Senescence-Accelerated Mouse (SAM): An Animal Model of Senescence*. Elsevier, Amsterdam, pp. 3–12.
- Takeda, T., Hosokawa, M., Takeshita, S., Irino, M., Higuchi, K., Matsushita, T., Tomita, Y., Yasuhira, K., Hamamoto, H., Shimizu, K., Ishii, M., Yamamuro, T., 1981. A new murine model of accelerated senescence. *Mech. Ageing Dev.* 17, 183–194.
- Takeda, T., Hosokawa, M., Higuchi, K., 1991. Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence. *J. Am. Geriatr. Soc.* 39, 911–919.
- Takeda, T., Hosokawa, M., Higuchi, K., 1994. Senescence-accelerated mouse (SAM): a novel murine model of aging. In: Takeda, T. (Ed.), *The SAM Model of Senescence*. Excerpta Medica, Amsterdam, pp. 15–22.
- Takeda, T., Matsushita, T., Kurozumi, M., Takemura, K., Higuchi, K., Hosokawa, M., 1997. Pathobiology of the senescence-accelerated mouse (SAM). *Exp. Gerontol.* 32, 117–127.
- Toyokuni, S., 1999. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol. Int.* 49, 91–102.
- Tsukahara, H., Shibata, R., Ohshima, Y., Todoroki, Y., Sato, S., Ohta, N., Hiraoka, M., Yoshida, A., Nishima, S., Mayumi, M., 2003. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. *Life Sci.* 72, 2509–2516.
- Tsukahara, H., Jiang, M.Z., Ohta, N., Sato, S., Tamura, S., Hiraoka, M., Maeda, M., Mayumi, M., 2004. Oxidative stress in neonates: evaluation using specific biomarkers. *Life Sci.* 75, 933–938.
- Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N., Niki, E., 1998a. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J. Biol. Chem.* 273, 16058–16066.

- Uchida, K., Kanematsu, M., Sakai, K., Matsuda, T., Hattori, N., Mizuno, Y., Suzuki, D., Miyata, T., Noguchi, N., Niki, E., Osawa, T., 1998b. Protein-bound acrolein: potential markers for oxidative stress. *Proc. Natl Acad. Sci. USA* 95, 4882–4887.
- Umezawa, M., Hosokawa, M., Kohno, A., Ishikawa, S., Kitagawa, K., Takeda, T., 1993. Dietary soybean protein compared with casein retards senescence in the senescence accelerated mouse. *J. Nutr.* 123, 1905–1912.
- Weindruch, R., Walford, R.L., 1988. *The Retardation of Aging and Disease by Dietary Restriction*. Charles C. Thomas, Springfield, IL.
- Xing, Y., Nakamura, A., Korenaga, T., Guo, Z., Yao, J., Fu, X., Matsushita, T., Kogishi, K., Hosokawa, M., Kametani, F., Mori, M., Higuchi, K., 2002. Induction of protein conformational change in mouse senile amyloidosis. *J. Biol. Chem.* 277, 33164–33169.
- Yamashita, S., Yamamoto, Y., 1997. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal. Biochem.* 250, 66–73.
- Yanagidaira, Y., Higuchi, K., Nose, H., 2003. Diurnal rhythm disorder of behavioral activity in SAMP1 mice is partially normalized by spontaneous wheel running. *Physiol. Behav.* 80, 195–201.
- Zhang, Y., Aberg, F., Appelkvist, E.L., Dallner, G., Ernster, L., 1995. Intake of dietary coenzyme Q supplement is limited in rats. *J. Nutr.* 125, 446–453.
- Zhang, Y., Turunen, M., Appelkvist, E.L., 1996. Restricted intake of dietary coenzyme Q is in contrast to the unrestricted intake of α -tocopherol into rat organs and cells. *J. Nutr.* 126, 2089–2097.