Free Radical Biology & Medicine, Vol. 33, No. 5, pp. 627–638, 2002

0891-5849/02/$–see front matter

PII S0891-5849(02)00916-4

Original Contribution

EFFECTS OF COENZYME Q_{10} ADMINISTRATION ON ITS TISSUE CONCENTRATIONS, MITOCHONDRIAL OXIDANT GENERATION, AND OXIDATIVE STRESS IN THE RAT

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(*Received* 21 *March* 2002; *Accepted* 9 *May* 2002)

Abstract—Coenzyme Q (CoQ_{10}) is a component of the mitochondrial electron transport chain and also a constituent of various cellular membranes. It acts as an important in vivo antioxidant, but is also a primary source of $O_2^{-\bullet}/H_2O_2$ generation in cells. CoQ has been widely advocated to be a beneficial dietary adjuvant. However, it remains controversial whether oral administration of CoQ can significantly enhance its tissue levels and/or can modulate the level of oxidative stress in vivo. The objective of this study was to determine the effect of dietary CoQ supplementation on its content in various tissues and their mitochondria, and the resultant effect on the in vivo level of oxidative stress. Rats were administered CoQ_{10} (150 mg/kg/d) in their diets for 4 and 13 weeks; thereafter, the amounts of CoQ_{10} and CoQ_9 were determined by HPLC in the plasma, homogenates of the liver, kidney, heart, skeletal muscle, brain, and mitochondria of these tissues. Administration of CoQ_{10} increased plasma and mitochondria levels of CoQ_{10} as well as its predominant homologue CoQ_9. Generally, the magnitude of the increases was greater after 13 weeks than 4 weeks. The level of antioxidative defense enzymes in liver and skeletal muscle homogenates and the rate of hydrogen peroxide generation in heart, brain, and skeletal muscle mitochondria were not affected by CoQ supplementation. However, a reductive shift in plasma aminothiol status and a decrease in skeletal muscle mitochondrial protein carbonyls were apparent after 13 weeks of supplementation. Thus, CoQ supplementation resulted in an elevation of CoQ homologues in tissues and their mitochondria, a selective decrease in protein oxidative damage, and an increase in antioxidative potential in the rat.

Keywords—Coenzyme Q, Antioxidants, Dietary intake, Mitochondria, Protein carbonyls, Free radicals, Oxidative stress

INTRODUCTION

Coenzyme Q (CoQ) or ubiquinone (2,3,-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) is a redox-active, lipophilic substance present in the hydrophobic interior of the phospholipid bilayer of virtually all the cellular membranes. It consists of a quinone head attached to a chain of isoprene units numbering 9 or 10 in the various mammalian species [1]. The quinone head can alternately assume three different redox states: ubiquinone (Q), the fully oxidized form; ubisemiquinone ($^{\bullet}QH$), the partially reduced form, also a free radical; and ubiquinol (QH_2), the fully reduced form [2].

CoQ seems to play multiple functional roles in cells, three of which have been well characterized. First, the most well-known function of CoQ is that its quinone form transfers electrons in the mitochondrial electron transport chain from complexes I and II to complex III, during which protons are extruded to the outer mitochondrial compartment thereby generating a transmembrane electrochemical gradient [3]. Second, it is now unambiguously established that the quinol form of CoQ acts as a potent antioxidant in the inner mitochondrial membrane.

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It inhibits lipid peroxidation by either scavenging free radicals directly or reducing α-tocopheroxyl radical to α-tocopherol [3–6]. The third established function is that autoxidation of its semiquinone form is the major intracellular source of superoxide and hydrogen peroxide generation [7,8].

The amounts of CoQ in different tissues vary greatly [1]. It is presently little understood how the characteristic tissue concentrations of CoQ are maintained and what is the relative contribution of endogenous biosynthesis vs. that from the dietary sources. Furthermore, whether oral administration of CoQ can enhance tissue amounts of CoQ remains controversial. On the basis of studies involving short-term oral administration of CoQ, it was widely believed that intracellular biosynthesis was the main source of CoQ and that tissues, besides plasma, liver, and spleen, were resistant to an augmentation from exogenous sources [9–12]. More recently, there have been sporadic reports that prolonged CoQ intake may result in elevation of CoQ in the brain [13]. Notwithstanding, a comprehensive study examining CoQ levels in multiple tissues following relatively long-term CoQ intake has not as yet been conducted. Another important, yet currently not well-examined, issue is whether CoQ intake causes a decrease or an increase in oxidative stress/damage in the tissues since CoQ can act both as a pro-oxidant and an antioxidant. Although numerous clinical studies have reported ameliorative effects of CoQ intake on a variety of human diseases, particularly those associated with the nervous system, heart, and skeletal muscles, the validity of such claims and the nature of the mechanisms by which CoQ provides a beneficial effect remain virtually unknown [14–17]. Furthermore, it seems that CoQ is being widely used as a dietary supplement without a clear understanding of its long-term effects.

In the context of the reasons presented above, the purpose of the present study was to determine whether relatively long-term administration of CoQ to rat (i) augments the concentration of CoQ in various tissues and their mitochondrial fraction; and, (ii) affects the redox status of plasma, rates of mitochondrial H_2O_2 generation, activities of antioxidative enzymes, and level of tissue oxidative damage.

MATERIALS AND METHODS

Materials

Q-Gel liquid (provided by Tishcon Corp., Westbury, NY, USA) contained: coenzyme Q_{10} (36.7 mg/g), Span 80 (56.0 mg/g), glycerine (39.1 mg/g), Tween 80 (733.1 mg/g), *d*-α-tocopherol (7.1 mg/g), and medium chain triglycerides (128.0 mg/g). The supplemented diet was NIH-31 modified to contain 9.7% Q-Gel liquid (Purina Mills Test Diet, Richmond, IN, USA). The supplemented NIH-31 was certified to contain 3.24 mg CoQ_{10} and calculated to contain 0.7 IU α-tocopherol/g diet. All solvents used were of HPLC grade (Fisher Scientific, Fair Lawn, NJ, USA). Ubiquinone-9 and ubiquinone-10 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylenedinitrilo-tetraacetic acid disodium salt dihydrate (EDTA) was obtained from Fisher Scientific. Ubiquinol-9 and ubiquinol-10 were prepared by the reduction of corresponding quinones with sodium borohydride (Sigma Chemical Co.), as described by Takada et al. [18].

Animals

A total of 30 male Sprague Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA). The rats were 14 months old at the time of initiation of the supplementation. Following receipt from the supplier, the rats were housed individually in polycarbonate cages with wire tops (20 $\times$ 24.5 $\times$ 46.5 cm). Food and water were available ad libitum throughout the study, and the colony room was maintained at 23 $\pm$ 1°C, on a 12 h, light-dark cycle beginning at 0600 h. The rats were weighed at weekly intervals for 5 weeks prior to initiation of the CoQ supplementation and thereafter until they were euthanized after 4 or 13 weeks of supplementation.

Coenzyme Q supplementation

After an acclimation period of 5 weeks, 15 rats were randomly assigned to be fed NIH-31 supplemented with CoQ, whereas the remaining 15 rats were fed unsupplemented NIH-31. Seven of the rats in each group were designated for euthanasia after 4 weeks, whereas 8 of the rats in each group were euthanized after 13 weeks of treatment. Based on average body weight through the supplementation period (610 g), a food intake of 33 g/d, and the certified CoQ_{10} content of the diet (3.24 mg/g), the rats received 150 mg/kg/d of CoQ_{10}.

After 4 or 13 weeks on study, the rats were euthanized by carbon dioxide asphyxiation. Approximately 0.5 ml of blood was collected in EDTA tubes by cardiac puncture. Subsequently, the amounts of coenzyme Q homologues were measured in plasma, and homogenates and mitochondria from brain, heart, kidney, liver, and hindlimb skeletal muscle.

Preparation of tissue homogenates and isolation of mitochondria

Tissues were homogenized in 10 volumes (w/v) of the indicated tissue-specific isolation buffer. The homoge-

nate was centrifuged for 5 min at 700 × *g* at 4°C to sediment unbroken cells and cellular debris and an aliquot of the supernatant was removed for the determination of CoQ content. Mitochondria were isolated from the supernatant by differential centrifugation. Mitochondria from liver, kidney, brain, upper hindlimb skeletal muscle, and heart were prepared, respectively, according to Sohal et al. [19], Lash and Sall [20], Sims [21], Trounce et al. [22], and Arcos et al. [23]. Samples for the determination of CoQ homologues were stored at −80°C until analysis.

Measurement of the protein content

Protein content was determined by the BCA protein assay according to the manufacturer's instruction (Pierce, Rockford, IL, USA).

Extraction and quantification of coenzyme Q

Tissue extractions were made according to Takada et al. [18] as described in Lass et al. [24]. Briefly, 10 μl Na_2EDTA (10% w/v) and 750 μl of hexane:ethanol (5:2 v/v) were added to 20–200 μl of the sample and vigorously mixed for 1 min, using a vortex. The mixture was centrifuged for 3 min at 4000 × *g* and 400 μl of the hexane layer was dried under a stream of nitrogen and dissolved in 100 μl of ethanol.

Quantification of CoQ was performed by HPLC according to Katayama et al. [25]. An aliquot of the ethanol extract (5–20 μl) was chromatographed on a reverse-phase C_{18} HPLC column (25.0 cm × 0.46 cm, 5 μm; Supelco, Inc., Bellefonte, PA, USA) using a mobile phase consisting of 0.7% $NaClO_4$ in ethanol:methanol: 70%$HClO_4$ (900:100:1, v/v/v) at a flow rate of 1.2 ml/min. The eluent was monitored with an electrochemical detector (ESA Coulochem II, ESA Inc., Chelmsford, MA, USA). The settings of the electrochemical detector were: guard cell (upstream of the injector), +200 mV; conditioning cell, −550 mV (downstream of the column); analytical cell, +175 mV. The concentrations of ubiquinone-9, ubiquinone-10, ubiquinol-9, and ubiquinol-10 were obtained by comparison of the peak areas with those for the standard solutions of known concentrations. Concentrations of CoQ_9 and CoQ_{10} represent the sum of the respective quinone and quinol values.

Quantification of plasma aminothiols

Plasma levels of glutathione, glutathione disulfide, and other aminothiols were measured by an HPLC method with electrochemical detection according to Lakritz et al. [26].

Protein carbonyls

Protein carbonyl content of tissue homogenates and mitochondria was determined by a modification of the tritiated sodium borohydride reduction method of Lenz et al. [27], as described by Yan and Sohal [28]. The final reaction mixture (1 ml) contained 0.5–1.5 mg protein in 186 mM Tris-HCl, pH 8.8, 2 mM Na_2EDTA, and 40 mM tritiated sodium borohydride in 0.1 M NaOH (specific activity, 1 mCi/mmol). After incubation at 37°C for 30 min, the reaction was terminated and the protein was precipitated by the addition of 1 ml 10% trichloroacetic acid. After standing in ice for 20 min, the mixture was centrifuged for 10 min at 3000 rpm. The pellet was washed three times with 3 ml ethanol:ethyl acetate (1:1 v/v), and then dissolved in 1 ml 25 mM Tris-HCl, pH 6.8, 0.2% SDS. An aliquot of the mixture was added to 4 ml Scintisafe Plus 50% (Fisher Scientific) and radioactivity was quantitated in a Tri-Carb liquid scintillation counter (Parkard BioScience, Meriden, CT, USA).

Biochemical assays

Mitochondrial H_2O_2 production was monitored spectrofluorometrically, with an excitation wavelength of 320 nm and an emission wavelength of 400 nm, by the method of Hyslop and Sklar [29], as modified by Kwong and Sohal [30]. Alpha-glycerophoshate (7 mM) was the substrate for H_2O_2 production in mitochondria from brain and skeletal muscle, while succinate (7 mM) was used in heart mitochondria. Respiratory inhibitors, rotenone (1 μM) and antimycin (0.5 μM), were added to the reaction mixture for brain and skeletal muscle mitochondria.

Antioxidative enzyme activities were measured in the supernatants of tissue homogenates centrifuged at 600 × *g*. Superoxide dismutase activity was measured by the method of Spitz and Oberley [31]. Catalase activity was calculated from the rate of consumption of H_2O_2 as described by Lück [32]. Glutathione peroxidase activity was determined as described by Beutler [33].

Statistical analysis of data

Data for levels of CoQ_{10}, CoQ_9, and total CoQ were considered in separate 1-way analyses of variance for tissue homogenates, plasma, and mitochondria. Planned individual comparisons of each treatment group with the vehicle control group were made using single degree of freedom *F* tests based on the analysis error term.

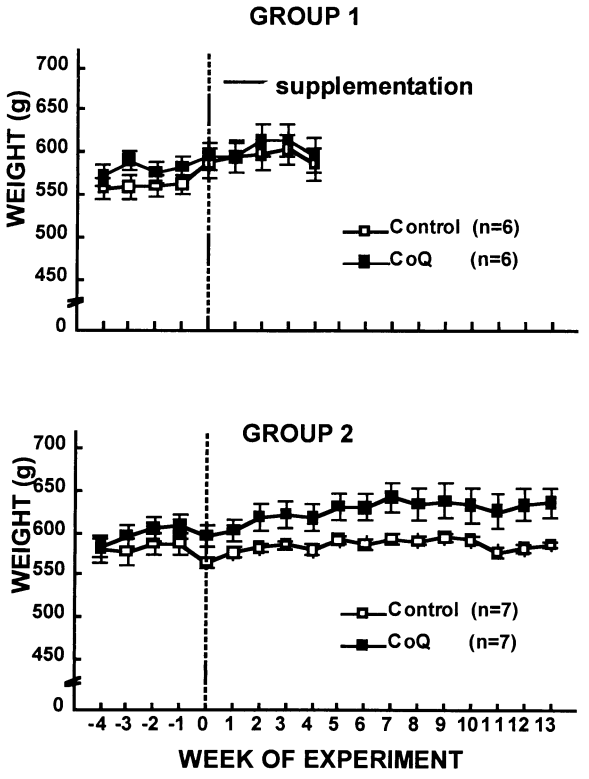

Fig. 1. Effect of coenzyme Q_{10} on mean body weight (g ± SEM) of rats after 4 (Group 1, upper panel) or 13 weeks of dietary supplementation (Group 2, lower panel). Separate groups of rats were placed on control or CoQ-supplemented diets after a 5 week adaptation period (−4 to 0), and euthanized 4 or 13 weeks later. Separate three-way analyses of variance (diet by group with repeated measures on weeks) for the preatreatment period (−4 to 0) and for the first four weeks (0 to 4) failed to indicate significant main effects or interactions with diet (all $p > .128$). A separate ANOVA for Group 2 (0 to 13 weeks) indicated a significant interaction of diet with weeks ($p = .005$).

RESULTS

Body weight and survival

Two rats (1 control and 1 CoQ supplemented) died during the supplementation period and a third (CoQ supplemented) was euthanized because of a large tumor. A control rat, removed after three weeks of study, was negative on a standard serology test panel. Body weights (Fig. 1) showed an overall increase during the 18 weeks of study. For the group of rats euthanized 4 weeks after the initiation of the study, no significant effect of the CoQ diet was evident. On the other hand, CoQ-supplemented rats in the 13 week group weighed significantly more than unsupplemented rats and showed greater weight gain from 0 to 13 weeks. It should be noted that a nonsignificant trend in this direction existed on the day these rats began supplementation (week 0).

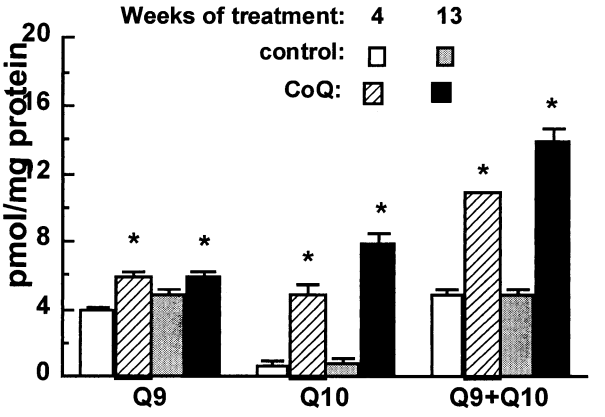

Fig. 2. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in the plasma of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; $*p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

Plasma concentrations of CoQ homologues

The concentration of CoQ_9 in the plasma of the control (nonsupplemented) rats at 4 or 13 weeks was approximately 5-fold higher than that of CoQ_{10} (Fig. 2), in accordance with previous findings that the predominant CoQ homologue in rodents is CoQ_9 [34,35]. Administration of CoQ resulted in a more than 6-fold increase in the CoQ_{10} plasma concentration by 4 weeks, and there was a further increase of greater than 9-fold ($p < .001$) after 13 weeks of treatment. The concentration of CoQ_9 increased by 40% after 4 weeks of CoQ_{10}, but no further increase occurred thereafter ($p < .001$). These results indicated that dietary supplementation with CoQ_{10} resulted in an increase in plasma CoQ_9, in addition to a marked increase in concentration of CoQ_{10}.

CoQ_9, CoQ_{10}, and total CoQ concentration in different tissue fractions of control rats

In homogenates from all tissues (heart, skeletal muscle, liver, kidney, and brain) of control rats at 4 and 13 weeks, concentrations of CoQ_9 were 3- to 11-fold higher than those of CoQ_{10}. A similar differential composition of CoQ_9 and CoQ_{10} was evident in mitochondria prepared from these tissues. The concentrations of CoQ_9, CoQ_{10}, and total CoQ were consistently 1.5 to 5 times higher in mitochondria than in homogenates from all tissues, except the skeletal muscle where the concentrations in mitochondria were 20 times higher. The concentration of CoQ_9 and total CoQ varied greatly with tissue, one order of magnitude greater in the kidney homogenate

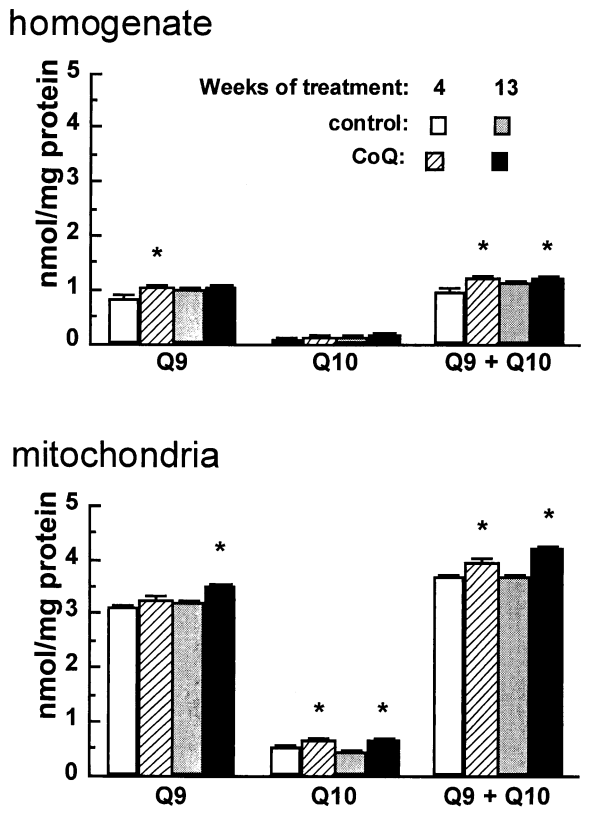

Fig. 3. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in homogenates (upper) and mitochondria (lower) from heart of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; $*p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

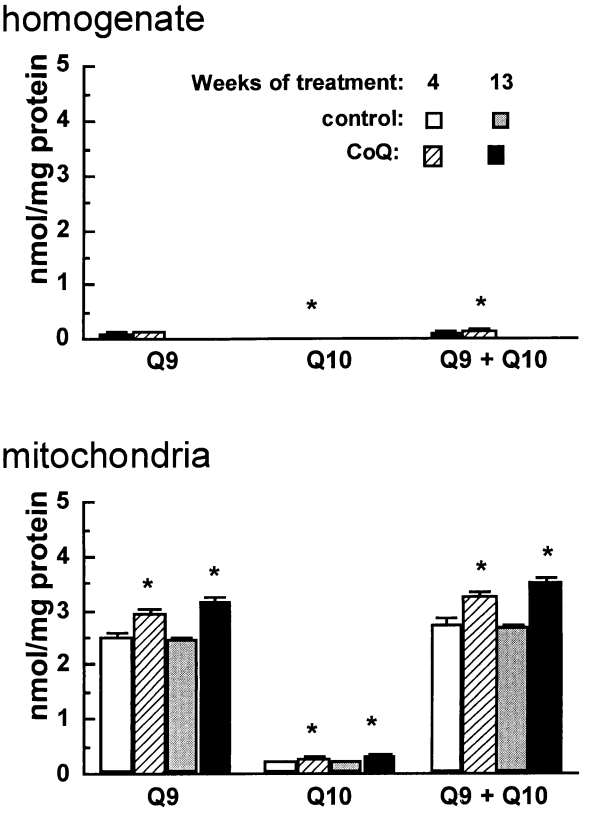

Fig. 4. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in homogenates (upper) and mitochondria (lower) from hindlimb skeletal muscle tissue of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; $*p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

than in the skeletal muscle, with the rank order as follows: kidney > heart > brain > liver > skeletal muscle. However, the variations in mitochondrial CoQ_9 and total CoQ concentration among the tissues were not as great, 2.5-fold higher in kidney than in the liver, with rank order: kidney > heart > skeletal muscle > brain > liver (see Figs. 3–7).

Effect of CoQ supplementation on levels of CoQ homologues in heart and skeletal muscle

CoQ supplementation resulted in an increase in total CoQ content in the heart and the skeletal muscle homogenates. After 4 weeks of supplementation, total CoQ content showed a significant increase in homogenates of heart and skeletal muscle (~23% and ~45%, respectively; $p < .05$). The increases in the individual CoQ homologues were statistically significant for CoQ_9 in the heart homogenates and CoQ_{10} in the skeletal muscle homogenate. No further increase was noted in the heart homogenates at 13 weeks. The contents of CoQ homologues in skeletal muscle homogenates were extremely small, ~9-fold less than those in the heart (see Figs. 3 and 4).

In the heart mitochondria, CoQ_{10} was increased by ~20% after 4 and 13 weeks of supplementation ($p < .02$), whereas increases in CoQ_9 were not evident until after 13 weeks of supplementation (~9%; $p < .0001$). In the skeletal muscle mitochondria, there were also time-dependent increases of both CoQ_9 and CoQ_{10}, ~10% after 4 weeks ($p < .005$) and ~30% after 13 weeks ($p < .005$). Total CoQ contents in both tissue mitochondria were significantly elevated after 4 and 13 weeks. Unlike the homogenates, the relative differences between the levels of CoQ homologues in heart and skeletal muscle mitochondria were not immense, ~15% higher for CoQ_9 and ~12% for CoQ_{10}.

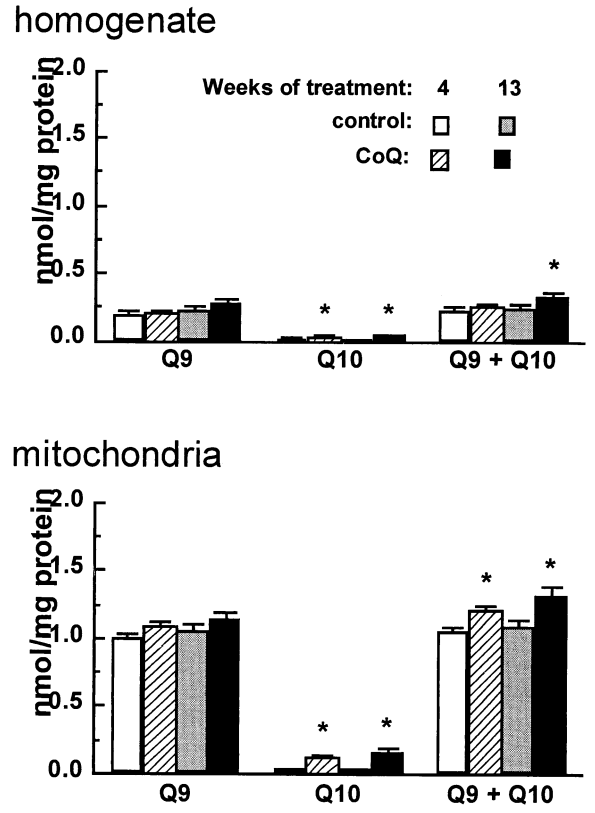

Fig. 5. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in homogenates (upper) and mitochondria (lower) from liver of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of α-tocopherol and CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; $*p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

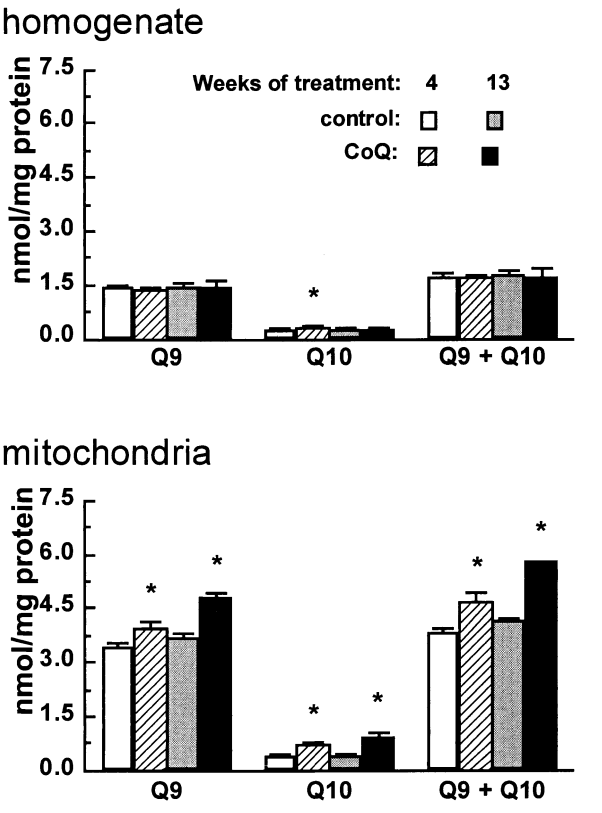

Fig. 6. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in homogenates (upper) and mitochondria (lower) from kidney of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; $*p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

In summary, experimental supplementation of CoQ in the diet for 13 weeks increased the CoQ homologue contents in the homogenates and mitochondria of heart and skeletal muscle.

Effect of CoQ supplementation on levels of CoQ homologues in liver and kidney

The concentration of CoQ_{10}, but not CoQ_9 increased with supplementation in liver and kidney homogenates. After 4 weeks, the concentration of CoQ_{10} in liver homogenates increased by ~75%, which further increased to ~150% after 13 weeks. However, in the kidney homogenates only a small increase in CoQ_{10} content was evident after 4 weeks, and no appreciative increase was evident after 13 weeks. Significant increase in total CoQ content was only evident in liver homogenates after 13 weeks. The CoQ_9 and CoQ_{10} content in the kidney homogenates of control rats were ~6-fold and ~12-fold higher, respectively, than those in the liver homogenates (compare Figs. 5 and 6). However, in the supplemented groups, the differences were appreciatively less, only ~4-fold higher in kidney after 13 weeks.

Both liver and kidney mitochondria showed an increase in CoQ_{10} content after 4 and 13 weeks, whereas an increase in CoQ_9 content occurred only in kidney mitochondria. The magnitude of the increase in CoQ_{10} concentration was ~3-fold greater in liver homogenates than in kidney. However, the absolute levels of CoQ_9 and CoQ_{10} were higher in the kidney mitochondria, ~2.5-fold higher in control rats and ~4-fold in supplemented rats (compare Figs. 5 and 6). In addition, there was a time-dependent, statistically significant increase in total CoQ content in mitochondria of both tissues ($p < .05$ after 4 weeks and $p < .001$ after 13 weeks).

In summary, 13 weeks of CoQ supplementation in the diet resulted in an increase of total CoQ and CoQ_{10} content in homogenates and mitochondria of liver and

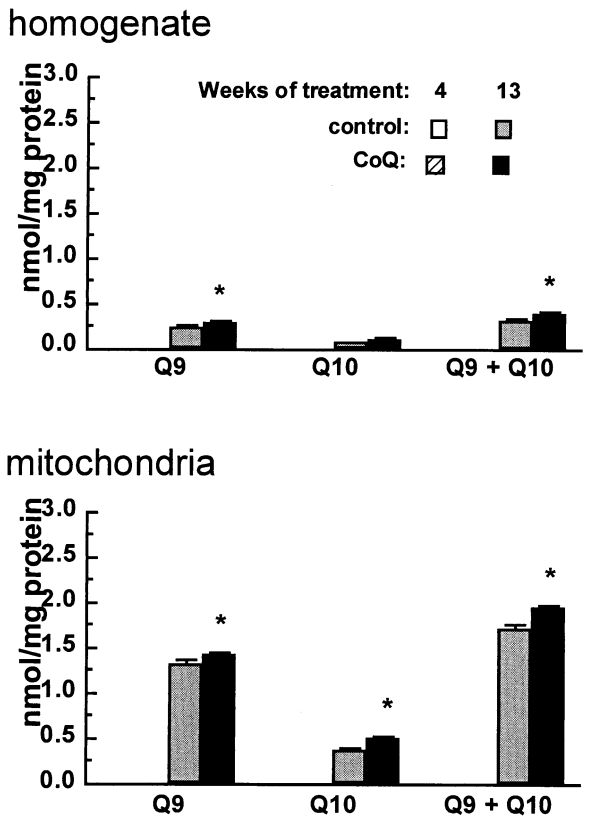

Fig. 7. Effect of CoQ_{10} supplementation on concentrations of CoQ_9 and CoQ_{10} in homogenates (upper) and mitochondria (lower) from whole brain of rats. Separate groups of rats were placed on control or CoQ-supplemented diets and euthanized 4 or 13 weeks later. Levels of CoQ homologues were measured by HPLC. All values represent the mean ± SEM (nmol/mg protein) of six to seven samples, each obtained from a different rat; *$p < .05$ when compared with the duration-matched control group (planned individual comparison within one-way ANOVA).

mitochondria of kidney. However, CoQ_9 content was elevated only in the kidney mitochondria.

Effect of CoQ supplementation on levels of CoQ homologues in the brain

Homogenates and mitochondria were prepared from whole brains of rats after 13 weeks of supplementation. The magnitude of increase in CoQ homologues was similar in both cellular fractions, ~10% for CoQ_9, ~30% for CoQ_{10}, and ~15% for total CoQ (Fig. 7). Although the increase in CoQ_{10} level in the homogenates was not statistically significant, the other increases were significant (CoQ_9: $p < .05$ for homogenates and $p < .005$ for mitochondria; CoQ_{10}: $p < .001$ for mitochondria; total CoQ: $p < .01$ for homogenates and $p < .001$ for mitochondria). In summary, CoQ supplementation increased the concentration of CoQ homologues in brain homogenate and mitochondria.

Effect of CoQ supplementation on protein oxidative damage in homogenates and mitochondria of skeletal muscle and liver

It has been postulated that CoQ is a potent cellular antioxidant as well as an oxidant generator. Thus, dietary CoQ supplementation may either attenuate or exacerbate in vivo oxidative damage. Protein oxidative damage, measured by [^{3}H]-borohydride reduction of carbonyl modifications, was determined in the homogenates and mitochondria isolated from upper hindlimb skeletal muscles and liver of control and supplemented rats after 13 weeks. Carbonyl content was 100% higher in liver homogenates than in skeletal muscle homogenates. Although a trend of lower carbonyl content in the skeletal muscle homogenates of supplemented rats was observed, there was no statistically significant difference between the two groups of rats for both tissues (Fig. 8). However, there was a statistically significant decrease of ~40% in skeletal muscle mitochondrial carbonyl content in supplemented rats ($p < .05$). The 30% decrease in carbonyl content in liver mitochondria was not statistically significant. Thus, CoQ supplementation has a protective effect against oxidative damage to protein in skeletal muscle mitochondria.

Effect of CoQ supplementation on mitochondrial oxidant generation in brain, heart, and skeletal muscle

It is well established that the autoxidation of semiquinone intermediate in the electron respiratory chain is the major source of mitochondrial oxidants. To determine if CoQ supplementation has an effect on mitochondrial oxidant generation, the rate of H_2O_2 production in brain, heart, and skeletal muscle mitochondria were measured in control and CoQ-supplemented rats after 13 weeks (Fig. 9). In the absence of respiratory inhibitors and with succinate as a substrate, there was no difference in the rate of H_2O_2 production in heart mitochondria isolated from the control and supplemented rats, 1270 ± 240 and 1280 ± 230 pmol H_2O_2/mg protein/min, respectively. Similarly, in the presence of respiratory inhibitors (antimycin and rotenone), CoQ supplementation had no effect on α-glycerophosphate-supported rate of H_2O_2 production in brain and skeletal muscle mitochondria. Therefore, the data indicate that CoQ supplementation in rats does not appear to have an effect on the rate of mitochondrial H_2O_2 generation.

Effect of CoQ supplementation on antioxidative enzymatic defenses

To determine if CoQ supplementation resulted in a compensatory change in the antioxidative enzymatic system, the activity levels of catalase, superoxide dismutase, and glu-

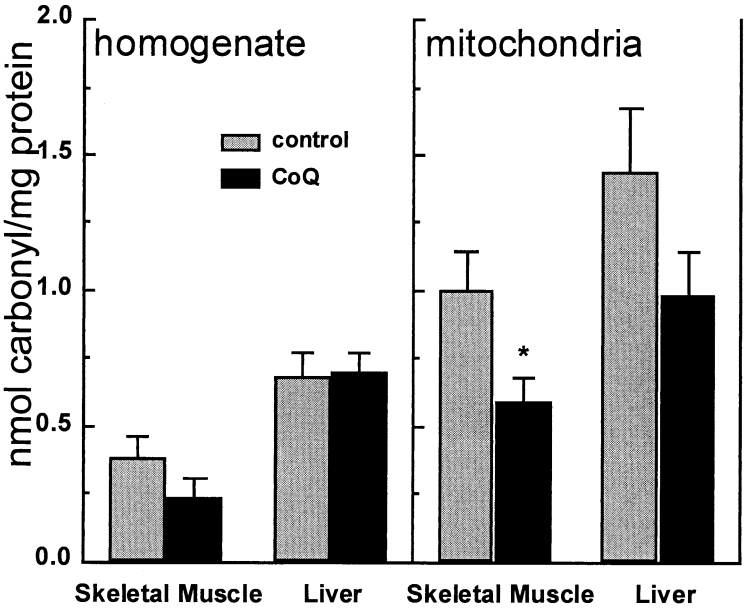

Fig. 8. Comparison of protein carbonyl content in the homogenates and mitochondria of hindlimb skeletal muscle and liver between control and CoQ-supplemented rats at 13 weeks of study. Carbonyl content was measured by the [^{3}H]-borohydride reduction method of Lenz et al. [27], as modified by Yan and Sohal [28]. All values represent the mean ± SEM of four to six samples, each obtained from a different rat; *$p < .05$ based on two-tailed, two-sample *t*-test.

tathione peroxidase were measured in the homogenates of skeletal muscle and liver of control and supplemented rats after 13 weeks. As shown in Fig. 10, there were no statistically significant differences between control and supplemented rats in the activities of these three enzymes in muscle and liver homogenates. Hence, CoQ supplementation did not result in a decrease of antioxidative enzymes in the skeletal muscle and liver homogenates.

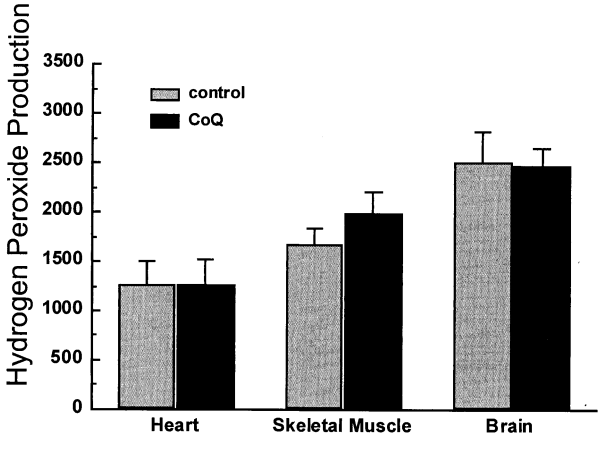

Fig. 9. Rates of H_2O_2 production by mitochondria from heart, skeletal muscle, and brain of control and CoQ-supplemented rats at 13 weeks of study. Hydrogen peroxide release was measured using 7 mM succinate (heart) or 7 mM α-glycerophosphate (skeletal muscle and brain) as substrate in the presence of 0.5 μM antimycin and 1 μM rotenone (skeletal muscle and brain only) by the method of Kwong and Sohal [30]. All values represent the mean ± SEM of five to seven samples, each obtained from a different rat, and represented four to six determinations.

Effect of CoQ supplementation on the levels of plasma aminothiols

Results from numerous studies have suggested that plasma homocysteine content and GSH/GSSG ratio are appropriate indices of in vivo oxidative stress. To determine the efficacy of CoQ supplementation in the attenuation of oxidative stress, aminothiol concentrations were measured by HPLC in the plasma of control and supplemented rats. After 13 weeks of supplementation, no change in the level of plasma GSH and methionine was noted (Fig. 11). However, plasma GSSG content was decreased by ~60% ($p < .001$), resulting in an increase of ~2-fold in GSH/GSSG ($p < .001$). Concomitantly, there was also a ~25% decrease in cysteine-glycine ($p < .001$) and a ~45% decrease in homocysteine content ($p < .001$). In summary, CoQ supplementation may attenuate oxidative stress by altering the plasma aminothiol redox balance toward a more reductive and less pro-oxidative environment.

DISCUSSION

The main findings of this study are: (i) experimental administration of CoQ in the diet resulted in an increase

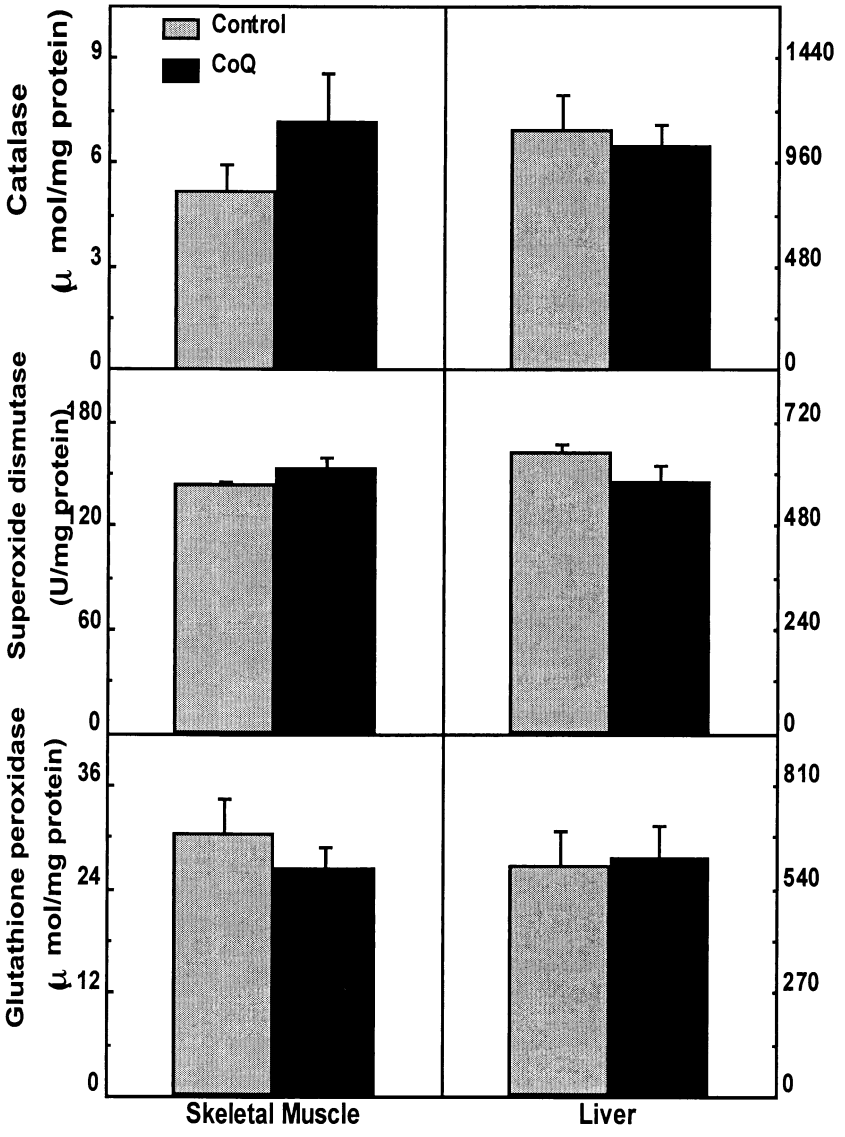

Fig. 10. Activities of antioxidative enzymes in skeletal muscle and liver homogenates of control and CoQ-supplemented rats at 13 weeks of study. All values represent the mean ± SEM of four to six samples, each obtained from a different rat, and represented three determinations. Statistical analysis revealed no significant difference between control and supplemented group for all three enzymes, catalase, superoxide dismutase, and glutathione peroxidase.

in total CoQ level in plasma, homogenates of heart, skeletal muscle, liver, and brain, and mitochondria from all tissue studied (the magnitude of the increase varied with duration of supplementation and in different tissues); (ii) CoQ intake caused an elevation of CoQ_{10} as well as CoQ_9, the predominant rodent homologue, in the plasma and mitochondria of nearly all the tissues (to varying extents in different tissues), and, in the tissue homogenates, increased CoQ_9 content was found only in the brain; and, (iii) CoQ supplementation attenuated oxidative stress in the rats, as indicated by lower protein carbonyls in skeletal muscle mitochondria and improved plasma aminothiol redox status. In addition, no change in mitochondrial pro-oxidant generation and antioxidative defense enzyme system was noted.

This study represents the only reported general uptake of supplemented CoQ into mitochondria of liver, kidney, heart, skeletal muscle, and brain of rat. Results from previous animal studies have been inconsistent as to the uptake of exogenous CoQ by various organs. CoQ supplementation in rats and mice resulted in a restricted uptake of CoQ_{10} into plasma, liver, spleen, and their mitochondria, and not into other tissues such as heart, skeletal muscles, kidney, and brain [10–12,36]. However, others have reported an elevation of CoQ_{10} level in homogenates of heart and skeletal muscles after long-

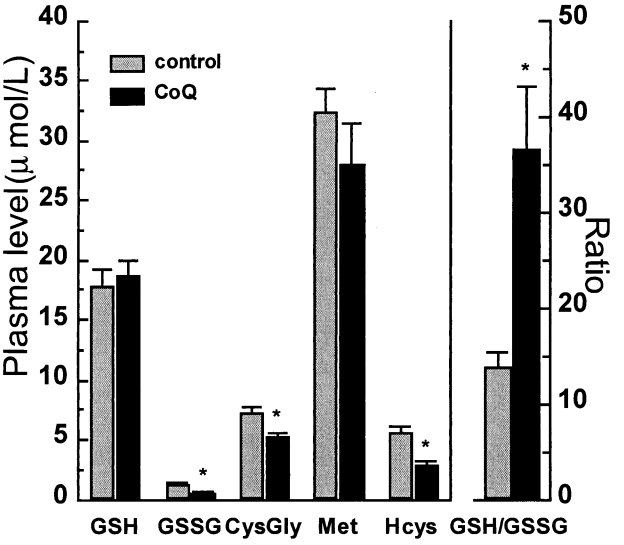

Fig. 11. Effect of CoQ supplementation on plasma levels of aminothiols in rats. Rats were fed control diet or CoQ-supplemented diet for 13 weeks. Plasma was collected in EDTA and analyzed for aminothiols by the HPLC method of Lakritz et al. [26]. All values represent the mean ± SEM (GSH: μmol/l plasma; GSSG: μmol/l plasma; Cys-Gly: μmol/l plasma × 10; Met: μmol/l plasma; and Hcys: μmol/l plasma) of six samples, each obtained from a different rat; $*p < .05$ based on two-tailed, two-sample *t*-test.

term supplementation in rat [37]. Matthews et al. found oral administration of CoQ_{10} to rats resulted in an increase of CoQ_{10} content in cerebral cortex homogenates and mitochondria [13]. As observed in this and our previous study in mice, total CoQ concentration is 1.5–5-fold higher in mitochondria than in tissue homogenates [24], which is in accordance with CoQ being an essential component of the mitochondrial electron respiratory chain. Thus, a more accurate and sensitive assessment of tissue uptake of CoQ is to measure its concentration in the mitochondria. Comparison of CoQ content in tissue homogenates between control and supplemented rodents alone may be unable to validate its uptake into the tissue. This phenomenon is illustrated by the findings in our previous study where CoQ_{10} was administered to old mice for 13 weeks. No increase in CoQ concentration was noted in tissue homogenates, except for the liver; however, significant increase was found in heart, liver, and kidney mitochondria. Furthermore, experimental designs, such as animal species and strains, supplementation protocol, and analytical methodology, may also contribute to the different experimental findings. Thus, our results support the notion that there is a general uptake of supplemented CoQ by all tissues, albeit specific amounts of the uptake may vary in different tissues.

It would seem unreasonable to assume a priori that dietary supplementation of CoQ_{10} in the rodents would affect CoQ_9 level in blood and tissues because the effect may be a corollary increase, a compensatory decrease, or no effect on CoQ_9 levels. Several studies found no change in plasma and tissue level of CoQ_9 in rats when CoQ_{10} levels were elevated manyfold, while other studies, including ours, found an increase in CoQ_9 [10,12, 13,24,36,37]. Interestingly, Ibrahim et al. reported an increase of CoQ_9 content in liver, a decrease in skeletal muscle, and no change in plasma and all other tissues, suggesting that tissue level of CoQ homologues may be independently regulated [12]. Results from the present study support the supposition that administration of CoQ_{10} to rodents can lead to tissue-specific increases in both CoQ_9 and CoQ_{10} concentrations in plasma, tissues, and their mitochondria. Dietary CoQ_{10} supplementation did not appear to have an effect on endogenous biosynthesis of CoQ_9 [10]. Thus, the observed increase in CoQ_9 may be due to the in vivo modification of the isoprene moiety and/or the antioxidative protection of mitochondrial CoQ_9 by the exogenous CoQ_{10} [38].

Data from the present study support the beneficial, antioxidative role of dietary CoQ supplementation. Our results demonstrate that CoQ supplementation causes a definite shift in plasma aminothiol redox status toward a more antioxidative environment (Fig. 11). Low GSH/GSSG, and high concentration of cysteinylglycine and homocysteine in plasma are accepted indicators of oxidative stress. The observed increase in GSH/GSSG and a decrease in cysteinylglycine and homocysteine content in plasma after13 weeks of CoQ supplementation suggest an elevation in extracellular reducing potential. In addition, no compensatory alteration was noted in the measured liver and skeletal muscle antioxidative enzymic defense system (Fig. 10). Thus, CoQ supplementation may be beneficial in lowering in vivo oxidative

stress by tipping the pro-oxidant/antioxidant balance toward the antioxidants.

Our finding of decreased protein carbonyl content in skeletal muscle mitochondria supports the hypothesis that dietary CoQ may reduce oxidative stress in certain loci in the animal. There is a large body of evidence showing increase in tissue and mitochondrial protein carbonyls with age and under oxidative stress [39–41]. Conversely, a decrease in protein carbonyls may be indicative of reduction in oxidative stress. The casual relationship of protein oxidative damage and oxidative stress has been demonstrated [42,43]. It is noteworthy that skeletal muscle has the highest relative enrichment of CoQ in the mitochondria, which may be reflective of its potentially high metabolic demands. Thus, the skeletal muscle may be more receptive to CoQ supplementation. Several clinical studies have demonstrated the efficacy of oral CoQ adjuvant in the amelioration of muscle mitochondrial dysfunctions [44]. In addition, we observed no change in the rate of mitochondrial hydrogen peroxide generation in the heart, skeletal muscle, and brain mitochondria in response to CoQ intake. This is consistent with our previous results that the rate of superoxide anion generation by submitochondrial particles was inversely related to their α-tocopherol content, and not to CoQ content in the liver and kidney, but not in the skeletal muscle [45]. Thus, the decrease in protein carbonyl content in skeletal muscle mitochondria is probably a result of the radical-scavenging activity of CoQ and not to a reduction in free radical generation (Fig. 9).

In conclusion, results of the present study indicate that the concentrations of CoQ homologues in plasma and tissue homogenates and mitochondria can be augmented by dietary CoQ_{10} supplementation, and the magnitude of the increase may be dependent on the duration of supplementation. In addition, CoQ supplementation can modulate the plasma aminothiol redox status towards antioxidants and lower protein oxidative damage in skeletal muscle mitochondria. Notwithstanding, the present results do not imply that CoQ intake can retard the aging process or ameliorate the severity of mitochondrial disorders, because no such effects have as yet been demonstrated.

Acknowledgements — This research was supported by the grant RO1 AG17526 from the National Institutes of Health, National Institute on Aging.

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ABBREVIATIONS

CoQ—coenzyme Q

EDTA—ethylenedinitrilo-tetraacetic acid disodium salt dihydrate

HPLC—high-performance liquid chromatography