Coenzyme Q Intake Elevates the Mitochondrial and Tissue Levels of Coenzyme Q and α-Tocopherol in Young Mice

Sergey Kamzalov, Nathalie Sumien,* Michael J. Forster* and Rajindar S. Sohal2

Department of Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles CA 90089-9121 and *Department of Pharmacology and Neuroscience, University of North Texas Health Science Center at Fort Worth, TX 76107

ABSTRACT The main objective of this study was to resolve the issue of whether the amounts of Coenzyme Q (CoQ), which is endogenously synthesized in cells, can be elevated in tissues and mitochondria of young mice by dietary supplementation with CoQ10. The prevalent view is that the uptake of exogenous CoQ by tissues other than plasma and liver either does not occur or is quite minimal. Mice, 6 mo of age, were fed 0, 148 or 654 mg CoQ10/(kg body - d) in their diets for 11 wk. CoQ10 intake enhanced both CoQ9 and CoQ10 homologues in the plasma, and in homogenates and mitochondria of liver, heart and skeletal muscle. CoQ was elevated in brain mitochondria, but not in the brain homogenate. The uptake of exogenous CoQ was higher in mitochondria of heart and skeletal muscle than those in liver. CoQ10 administration also elevated the α-tocopherol concentration in tissue homogenates and their mitochondria, thereby providing an in vivo indication of the “sparing” effect of CoQ on α-tocopherol. Results of this study demonstrate that, contrary to the historical view, both total and mitochondrial CoQ concentrations in the heart and skeletal muscle and in the mitochondria of brain of young mice can be augmented by dietary supplementation. Furthermore, CoQ intake enhances the antioxidative potential of tissues by elevating the endogenous amounts of α-tocopherol. J. Nutr. 133: 3175-3180, 2003.

KEY WORDS: • coenzyme Q • mitochondria • α-tocopherol • aging • oxidative stress • antioxidants

Coenzyme Q, or ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone), is a redox-active lipoidal substance, present in the hydrophobic middle region of the phospholipid bilayer of cellular membranes, including those of the mitochondria (1). Coenzyme Q (CoQ) consists of a quinone head, which, in mammalian cells, is attached to a chain of 9 or 10 isoprene units (2). CoQ exists in three alternate states of oxidation: ubiquinone (Q), the fully oxidized form; ubisemiquinone (QH), the partially reduced form, also a free radical; and ubiquinol (QH2), the fully reduced form. Endogenous synthesis is thought to be the main source of CoQ supply (3). There are an increasing number of biological functions in which CoQ seems to play a role; however, its most recognized function is the transfer of electrons from NADH-Q oxidoreductase (complex I) and succinate-Q oxidoreductase (complex II) to Q-cytochrome c oxidoreductase (complex III) in the mitochondrial electron transport chain. The transfer of electrons from complex I to CoQ and from CoQ to complex III is linked to the extrusion of protons into the mitochondrial intermembrane space, thereby augmenting the transmembrane proton gradient (4). In addition, CoQ has two other well-characterized, but somewhat incongruous functions, which are related to the modulation of cellular redox state. Ubiquinol is a potent antioxidant (5–7), whereas ubisemiquinone autoxidation is thought to be a major mitochondrial source of superoxide anion radical generation (8,9).

Due to the vital role of CoQ in ATP generation, it has been postulated that an intracellular deficiency of CoQ, occurring during aging and in certain pathological conditions, would have widespread deleterious effects on cellular functions (10,11). Hence, the question has arisen whether endogenous levels of CoQ can be augmented experimentally by dietary supplementation. The prevalent view (12,13), reiterated recently (14), is that although ~6% of the orally administered CoQ permeates the gastrointestinal tract into the blood and is transferred to liver and spleen, its uptake by other tissues such as heart, skeletal muscle and brain is low or completely absent, unless the endogenous levels of CoQ have fallen below a critical physiologic threshold, which occurs during aging.

Whether CoQ levels can be elevated in tissues other than plasma and liver in young healthy animals by dietary supplementation is an issue of particular importance because 1) it would provide a strategy for the modification of mitochondrial content of CoQ; and 2) a large number of humans consume CoQ as a dietary supplement without any clear understanding of the long-term effects of such a regimen. Augmentation of CoQ in cells can be potentially deleterious because autoxidation of ubisemiquinone and the resultant formation of O2−/H2O2 may have a prooxidizing effect. Furthermore, CoQ can enhance the antioxidative potential of tissues because ubiquinol is a potent antioxidant, and CoQ can also elevate mitochondrial levels of α-tocopherol (5,7). In vitro studies in this

1 Supported by grant R01 AG17526 from National Institutes of Health-National Institute on Aging. Q-gel liquid was donated by Tischcon, Westbury, NY.
2 To whom correspondence should be addressed. E-mail: sohal@usc.edu.
and other laboratories have indicated that in respiring mito-
chondria, CoQ (ubiquinol) reacts with tocopherol radicals,
thereby regenerating α-tocopherol. Whether a similar me-
chanism exists in vivo is presently unclear.

Thus, in the above context, the purpose of this study was to
determine whether supplementation of the diet with CoQ
augments the tissue and mitochondrial levels of CoQ and
α-tocopherol in young mice.

MATERIALS AND METHODS

Materials. Q-gel liquid (provided by Tishcon, Westbury, NY)
was added to Purina diet 5001 to yield two concentrations of CoQ10:
0.72 mg/g (low dose) or 2.81 mg/g (high dose). The Q-gel liquid
contained coenzyme Q10 (36.7 mg/g), Span 80 (56 mg/g), glycerine
(39.1 mg/g), Tween 80 (733.1 mg/g), RRR-α-tocopherol (7.1 mg/g)
and medium-chain triglycerides (128 mg/g). The Q-gel liquid vehicle
(containing all ingredients except coenzyme Q10) was added to the
control and low CoQ10 diets such that all three diets differed only in
the concentration of CoQ10. (catalog # 46002, 46003, 46004, respect-
ively; Purina Mills Test Diet, Richmond, IN). All solvents used were
HPLC grade (Fisher Scientific, Fair Lawn, NJ). Ubiquinone-9,
ubiquinone-10, (+)-α-tocopherol and (+)-α-tocopherol acetate were
purchased from Sigma Chemical (St. Louis, MO). EDTA was
obtained from Fisher Scientific. Ubiquinol-9 and ubiquinol-10 were
prepared by the reduction of corresponding quinones with sodium
borohydride (Sigma Chemical), as described by Takada et al. (15).

Animals. Male mice (n = 36; 3 mo old) were obtained from the
National Institute on Aging (NIA) and maintained subsequently in
the University of North Texas Health Science Center vivarium.
The mice were C57BL/6 characterized by the NIA as “at risk” for genetic
contamination on May 3, 2002. At the time of notification, no tissues
from these mice were available for genotyping. However, genotyping of
C57BL/6 populations obtained at the same time as those in the
current studies revealed that >90% of mice had one or more non-
C57BL/6 markers. The mice were housed individually in clean poly-
carbonate cages modified into two separate compartments with a
stainless steel divider, at 23 ± 1°C, under a 12-h light:dark cycle
starting at 0600 h; food was consumed ad libitum.

Coenzyme Q supplementation. After 1 mo of acclimation, mice
were randomly assigned to one of 3 experimental groups and subse-
quently fed either the control diet or low or high dose CoQ10 diet for
11 wk. The mice were weighed at weekly intervals and food intake
was determined during wk 1 and 6 of the study. Neither body weight
nor food intake was affected by CoQ10 supplementation. Based on
mean body weights and mean food intakes, the three groups of mice
received 0, 148 or 654 mg CoQ10/(kg body wt). Intake of vitamin E
by the three groups was 189 IU/(kg body wt) and 179 IU from the Q-gel liquid vehicle). Mice were killed by carbon
dioxide asphyxiation. Blood (−0.5 mL) was collected in EDTA-
coated tubes by cardiac puncture. Subsequently, the amounts of CoQ
homologues (CoQ9, CoQ10) and α-tocopherol were measured in
plasma, and homogenates and mitochondria from brain, heart, liver
and skeletal muscles were isolated by differential

Preparation of tissue homogenates and isolation of mitochondria.
Tissues were homogenized in 10 volumes (wt/vol) of the indicated
tissue-specific isolation buffer. The homogenate was centrifuged for 5
min at 700 × g at 4°C to sediment unbroken cells and cellular debris;
an aliquot of the supernatant was removed for the determination of
coenzyme Q and α-tocopherol concentration. Mitochondria from
brain, heart, liver and skeletal muscles were isolated by differential
centrifugation, according to Sims (16), Arcos et al. (17), Sohal et al.
(18) and Trounce et al. (19), respectively. Samples were stored at
−80°C until analysis. Protein concentration was determined by
bicinchoninic acid protein assay, according to the manufacturer’s
instructions (Pierce, Rockford, IL).

Extraction and quantification of coenzyme Q. Extractions of CoQ
were made according to the method of Takada et al. (15), as
described in Lass et al. (20). Briefly, 10 μL NaCl, EDTA (10% wt/v)
and 750 μL hexane/ethanol (5:2 v/v) were added to 20−200 μL of the
sample and mixed vigorously 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,
following the procedure of Katayama et al. (21). An aliquot of the
ethanol extract (5−20 μL) was chromatographed on a reversed-phase
C18 HPLC column (25.0 cm × 0.46 cm, 5 μm; Supelco, Bellefon-
te, PA), using a mobile phase consisting of 0.7% NaClO4, in ethanol/
water/methanol/HClO4 (900:100:1:70 v/v/v) at a flow rate of 1.2 mL/
min. The eluent was monitored with an electrochemical detector
(ESA Coulochem II, ESA, Chelmsford, MA). The settings of the
electrochemical detector were as follows: guard cell (upstream from
the injector), +200 mV; conditioning cell (downstream of the column), −550 mV; analytical cell, +175 mV. The concentrations of
ubiquinone-9, ubiquinone-10, ubiquinol-9, ubiquinol-10 and (+)-α-
tocopherol were obtained by comparison of the peak areas with those
for standard solutions of known concentrations. Concentrations of
CoQ9, CoQ10 and α-tocopherol, the sum of the respective quinone and
quinol values. Each sample was injected at least twice, using 5 and 20
μL injection volumes. This was necessary because concentrations of
α-tocopherol and coenzyme Q often differed by an order of magni-
tude. Samples in which concentrations of α-tocopherol and coen-
zyme Q differed by 2 orders of magnitude were diluted 1:10 before the
injections.

Statistical analysis. Differences in the concentrations of CoQ9,
CoQ10, total CoQ, and α-tocopherol in each tissue were evaluated by
one-way ANOVA. Planned individual comparisons of each treat-
ment group with the control group were made using single df F-tests
based on the analysis error term. An α of 0.05 was set for all analyses.

RESULTS

Plasma concentrations of CoQ homologues and α-tocopherol.
CoQ9 and CoQ10 constituted 60 and 40%, respectively, of CoQ present in the plasma of control mice (Fig. 1). Admin-
istration of both low and high doses of CoQ10 for 11 wk dose dependently increased plasma CoQ9 and CoQ10 concentra-
tions (P < 0.001). In the plasma of the controls, the molar amount of α-tocopherol was 18-fold greater than that of CoQ.
Notably, intake of low and high doses of CoQ10 increased the amounts of α-tocopherol in the plasma to 200 and 260%,
respectively, of the controls (P < 0.001).

CoQ homologues and α-tocopherol in tissue homogenates.
Concentrations of CoQ (CoQ9, CoQ10) in the control mice varied greatly in different tissues (Figs. 2−5; Table 1). For
instance, compared with plasma, the CoQ concentration in

FIGURE 1 Effect of coenzyme Q10 supplementation on concen-
trations of CoQ9, CoQ10 and α-tocopherol in the plasma of mice. Values
are means ± SEM, n = 6. *Different from control, P < 0.05. In some
instances, SEM bars are too small to be discernible.
homogenates was 25-fold higher in the liver, 151-fold higher in the heart, 45-fold higher in the brain and only 1.6-fold greater in the skeletal muscle. CoQ9 was the most abundant CoQ homologue, constituting 92, 92, 92 and 67% of the total CoQ concentration in the homogenates of liver, heart, skeletal muscle and brain, respectively. As in plasma, CoQ intake dose dependently enhanced both CoQ9 and CoQ10 in all the tissue homogenates except the brain (P < 0.022). The absolute increases in the amounts of CoQ (CoQ9 + CoQ10) in tissue homogenates in response to supplementation had the following rank order: liver > heart > brain > skeletal muscle (Fig. 6).

In contrast to CoQ, the amounts of α-tocopherol present in the tissue homogenates were, in general, lower than those in the plasma, i.e., 13% in the liver, 19% in the heart, 25% in the skeletal muscle and 61% in the brain. Intake of CoQ10, particularly at the high doses, increased the amounts of α-tocopherol in the tissue homogenates (P < 0.03).

Mitochondrial concentrations of CoQ and α-tocopherol. As in the tissue homogenates, CoQ9 was also the predominant CoQ homologue in mitochondria (Figs. 2–5). Compared with the tissue homogenate, CoQ concentration (Q9 + Q10) of mitochondria in controls was 4.5-fold higher in the liver, 2.5-fold in the heart, 161-fold in the skeletal muscle and 3.1-fold in the brain. Experimental intake of both low and high doses of CoQ10 increased the level of CoQ (Q9 + Q10) in mitochondria of heart and skeletal muscle (P < 0.012). In contrast to the other tissues, CoQ levels in the liver and brain mitochondria were significantly increased only in mice fed the high dose of CoQ10 (P < 0.028). A noteworthy finding was that on a per mg protein basis, relatively greater increases in

FIGURE 2 Effect of coenzyme Q10 supplementation on concentrations of CoQ9, CoQ10 and α-tocopherol in the liver homogenate (A) and mitochondria (B) of mice. Values are means ± SEM, n = 4 (the pool of two mice). *Different from control, P < 0.05.

FIGURE 3 Effect of coenzyme Q10 supplementation on concentrations of CoQ9, CoQ10 and α-tocopherol in the heart homogenate (A) and mitochondria (B) of mice. Values are means ± SEM, n = 5–6. *Different from control, P < 0.05.
the amounts of CoQ occurred in the mitochondria of the heart and skeletal muscle than in the liver \((P < 0.001)\). The mitochondrial response to supplementation had the following rank order: heart > skeletal muscle > liver > brain (Fig. 6). This is in contrast to the pattern of CoQ elevation in the homogenates, in which the largest increase was in the liver.

Compared with the homogenates, the amounts of \(\alpha\)-tocopherol in mitochondria of controls were 2.2-fold higher in the liver and 2.1-fold greater in the heart. There were no differences in the skeletal muscle or the brain. Administration of CoQ\(_{10}\), particularly at the high dose, increased \(\alpha\)-tocopherol in the mitochondria of various tissues \((P < 0.024)\).

**DISCUSSION**

Results of this study indicate that intake of CoQ\(_{10}\) enhances the levels of CoQ homologues and \(\alpha\)-tocopherol in

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Low CoQ intake</th>
<th>High CoQ intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>20.6 ± 1.4</td>
<td>34.8 ± 1.0</td>
<td>67.9 ± 7.2</td>
</tr>
<tr>
<td>Heart</td>
<td>14.2 ± 2.2</td>
<td>20.9 ± 1.5</td>
<td>23.1 ± 1.3</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.64 ± 0.16</td>
<td>1.13 ± 0.18</td>
<td>1.24 ± 0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>6.3 ± 2.2</td>
<td>9.1 ± 0.4</td>
<td>13.0 ± 1.0</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM, \(n = 4\).
plasma and homogenates and in mitochondria of liver, heart and skeletal muscle. In brain, the CoQ augmentation occurred in mitochondria but not in the homogenate.

The finding that amounts of CoQ, especially in mitochondria from skeletal muscle, heart and brain, can be elevated in young mice by dietary supplementation of CoQ argues against the classical view that uptake of exogenous CoQ by tissues other than plasma and liver is very low or absent (12–14). In contrast, the present results clearly indicate that CoQ levels were not only elevated in heart and skeletal muscle mitochondria in response to dietary supplementation, but such increases were greater than those occurring in the liver. It appears that procedural differences may be responsible for the discrepant results of the respective studies. It is worth noting that the CoQ concentration is 4–250 times higher in mitochondria than in the tissue homogenate. Such selective sequestration of CoQ in a subcellular component, i.e., mitochondria, can appear obscure and insignificant when the quantification of CoQ is made in reference to the entire organ, which contains not only large amounts of nonmembranous material but also the extracellular connective tissue. This reasoning is supported by the previous finding in the brain, and previous findings in skeletal muscle and heart (20) that CoQ augmentation in response to experimental administration is detectable in the mitochondria, but not in the tissue homogenates.

Other factors that may limit augmentation of CoQ in tissues in response to its experimental intake include the dietary dosage and duration. The prevalent view, based mainly on studies by Dallner and his associates (12–14), derives from studies in which 20 mg CoQ10 was administered to 1- to 3-month-old rats each day for 3 wk. In a previous study, 1- to 3-month-old rats were administered CoQ10 for 13 wk, which resulted in widespread uptake of CoQ by mitochondria in the liver, kidney, skeletal muscle and brain (22). In a similar study (23), 200 mg CoQ/kg · d was administered to 1- to 3-month-old rats for 2 mo, which increased CoQ in the brain mitochondria. In the present study, 6- to 8-month-old mice were administered 148 and 654 mg CoQ/(kg · d) for 11 wk, which, as reported here, significantly elevated CoQ concentrations of mitochondria and tissue homogenates. An analysis of the dosage and duration of CoQ administration, employed in various studies, suggests that a relatively prolonged period of CoQ intake at fairly high dosages is required to increase CoQ in tissues. Furthermore, the capacity for CoQ augmentation varies in different tissues.

A noteworthy finding of this study is that administration of CoQ10 enhances CoQ9, the predominant CoQ homologue, as well as CoQ10. In all tissues, except skeletal muscle, the ratio of Q9/Q10 appears to be unaffected by a supplement consisting entirely of CoQ10. It seems that the various tissues possess the ability to trim an isoprenoid unit from the CoQ10 homologue to form the CoQ9 homologue. The possibility that exogenous CoQ stimulates the synthesis of endogenous CoQ has been ruled out by previous studies (3,14). Furthermore, both the endogenously synthesized as well as exogenously supplied CoQ are more highly concentrated in mitochondria than in the remaining tissue. Whether experimental augmentation of mitochondria with CoQ enhances or adversely affects mitochondrial functions is presently unknown. Nevertheless, the finding that endogenous levels of CoQ can be increased by dietary supplementation of CoQ seems to be relevant to the attenuation of both CoQ9 and CoQ10 deficiencies, which block the mevalonate pathway and are widely used as antihypercholesterolemic agents. Statins inhibit the activity of 3-hydroxy-3-methylglutaryl-CoA reductase, which depresses the synthesis of not only cholesterol but also of CoQ10 because they share a common biosynthetic pathway. Dietary intake of CoQ may thus ameliorate the statin-induced decrease in endogenous CoQ, as observed in clinical trials (24).

Experimental administration of CoQ10 also resulted in the elevation of the tissue and mitochondrial concentrations of α-tocopherol. A previous study on the in vitro antioxidation of rat heart mitochondria indicated that in the presence of succinate as a respiratory substrate, mitochondrial CoQ was reduced to the ubiquinol form, whereas the α-tocopherol concentration was depleted (7). The total CoQ (ubiquinone + ubiquinol) concentration of mitochondria remained unaltered irrespective of the presence or absence of succinate. A separate experiment on bovine heart mitochondria (7) that had been experimentally enriched with different amounts of α-tocopherol, indicated that oxidative damage to mitochondrial proteins and lipids, in the absence of succinate, was inversely related to α-tocopherol concentration. However, such damage was greatly attenuated in the presence of ubiquinol. Results of such studies (2,20,25) suggest that the antioxidant role of CoQ (ubiquinol) is dependent upon α-tocopherol, which reacts directly with the peroxyl radical to form the tocopheroxyl (phenoxyl) radical and is subsequently reduced to α-tocopherol by ubiquinol. Thus, the present finding, that CoQ intake elevates the level of mitochondrial α-tocopherol in vivo is consistent with the hypothesis that in respiring mitochondria, CoQ has a "sparing" effect on α-tocopherol. CoQ intake therefore elevates the mitochondrial concentration of both CoQ and α-tocopherol.

In conclusion, results of this study clearly demonstrate that, in contrast to the prevailing view, endogenous levels of CoQ in mitochondria of brain, skeletal muscle and heart can be augmented in young mice by the administration of exogenous CoQ. Furthermore, CoQ intake enhances the α-tocopherol concentration of tissues and mitochondria. The long-term physiologic effects of such modifications on mitochondrial functions remain to be investigated.

LITERATURE CITED