

CoQ10 conveys protection from oxidative stress in plasma but not skeletal muscle

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Abstract: Coenzyme Q10 (CoQ10) is commonly consumed as an antiaging supplement at doses of 30–210 mg/day. The aim of the study was to determine if CoQ10 alters markers of antioxidant status, oxidative damage, and gene expression in aging skeletal muscle. Female guinea pigs aged 26 months were supplemented for 6 weeks with CoQ10 at a human equivalent dose of 10 mg/kg/day. Body weight, plasma CoQ10 concentration, and WBC DNA abasic sites were measured at weeks 0, 2, 4, and 6 of the supplementation period. At the end of supplementation, concentrations of skeletal muscle CoQ10, glutathione, malondialdehyde, protein carbonyls, DNA abasic sites, activities of catalase and glutathione peroxidase, and the gene expression of cytochrome *c* oxidase subunits were measured. Dietary supplementation with CoQ10 elevated plasma CoQ10 levels (pre 73 ± 3 nmol/L, post 581 ± 15 nmol/L, $P < 0.05$) and decreased abasic sites in WBC DNA (pre 16.8 ± 0.5 Ap/100000 bp, post 9.7 ± 0.4 Ap/100000 bp, $P < 0.05$). In contrast, all of the measures made in skeletal muscle were not different between groups ($P > 0.05$). These results indicate that dietary supplementation with CoQ10 at a dose of 10 mg/kg/day may be capable of increasing antioxidant protection and reducing oxidative damage in the plasma, but may have no effect in skeletal muscle.

Keywords: coenzyme Q10, aging, skeletal muscle, oxidative damage, gene expression

Background

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a lipid soluble compound endogenously synthesized in cells and located in all cellular membranes.¹ CoQ10 can act as a potent antioxidant, is an important component of the mitochondrial electron transport chain, and has been recognized for its antiaging properties.² A dose of 0.5–1 mg/kg/day of CoQ10 is required to prevent CoQ10 deficiency and to retain elevated CoQ10 serum and plasma levels.^{3,4} Many CoQ10 supplement manufacturers, however, recommend a dose of 1–2 mg/kg/day to alleviate age-associated symptoms such as decreased skeletal muscle function.

Aging can be characterized by a decline in physiological functions arising from progressive oxidative damage to cellular structures such as DNA, proteins, and lipids.⁵ Oxidative damage may be caused by reactive oxygen species (ROS), and continual exposure to ROS is the rationale behind the free radical theory of aging.⁶ Skeletal muscle cells have a complex antioxidant system designed to scavenge and neutralize ROS, however a decreased antioxidant capacity or heightened oxidative stress may overwhelm the system.⁷ In aging skeletal muscle there is a reduction in muscle mass, muscle fibre number, and muscle strength.^{8–10} These aging-induced changes

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in skeletal muscle have been shown to adversely affect the quality of life in elderly populations.¹¹

Given the antioxidant properties of CoQ10, it is not surprising that studies have reported improved antioxidant status and decreased oxidative damage after supplementation. CoQ10 supplementation with doses of 30–60 mg/day elevated plasma concentrations of vitamin E in young mice, and protected human and rat lymphocyte DNA from oxidative damage.^{12–15} In mice aged 24 months CoQ10 supplementation decreased the rate of superoxide anion generation in skeletal muscle and liver.¹³

In addition to acting as an antioxidant, CoQ10 has been shown to alter gene expression in muscle.^{16,17} A study by Linnane et al¹⁷ found that CoQ10 supplementation upregulated and downregulated a number of genes involved in transcription, cell-cycle control, and cell-signalling in the vastus lateralis muscle of aged humans. In a study by Lee et al¹⁶ it was reported that CoQ10 upregulated the expression of genes encoding mitochondrial electron transport chain proteins in the hearts of old mice. Specifically, cytochrome *c* oxidase (complex IV) subunits and ATP synthase (complex V) were upregulated.¹⁶ However, the effect that CoQ10 supplementation has on the gene expression of mitochondrial electron transport chain proteins in aged skeletal muscle has not been examined previously.

As previously mentioned CoQ10 is widely recommended by a range of health professionals as a dietary supplement and therapeutic agent at doses of 30–210 mg/day (0.4–7 mg/kg/day for a 70 kg patient), particularly in older individuals. Doses in this range are known to increase plasma CoQ10 concentrations.^{3,4} It is often assumed that the same dose will increase levels in other tissues such as skeletal muscle, and convey therapeutic effects such as increased antioxidant status and protection against oxidative damage.

Furthermore, CoQ10 is commonly consumed as an anti-aging supplement in the wider community at a dosage of 10 mg/kg/day, with consumers doing so in the belief that it will alleviate age associated symptoms in a range of organs such as skeletal muscle.

Given the scientific evidence presented, and the wide consumer usage of CoQ10 supplements, the aim of the current study was to determine if CoQ10 at a dose equivalent to 10 mg/kg/day in humans alters gene expression, and markers of antioxidant status and oxidative damage in aged skeletal muscle.

A second aim was to determine the effect of this dose on oxidative damage in white blood cells.

Materials and methods

Animals and experimental design

Approval for this project was granted by Victoria University's Animal Experimentation Ethics Committee (project number AEETH 09/03). Ex-breeding female guinea pigs aged 26 months were randomly assigned to either a control (n = 8) or a CoQ10 supplemented group (n = 8), the age of these animals is comparable to 60–70 years in humans, based on the average life span of a guinea pig being 36 months. Guinea pigs were chosen as the model because, like humans, they utilize CoQ10 as the major electron transporter in the mitochondrial electron transport chain, whereas rats use CoQ9.¹⁸

Treatment group animals were supplemented with CoQ10 at a dose of 46 mg/kg/day incorporated into their food for 6 weeks, (Table 1). The diets used contained vitamin C (150 mg/g) and vitamin E (5 IU/g) and were identical except for the addition of CoQ10 in the supplemented diet. The dose of 46 mg/kg/day was selected to provide an adult human equivalent dose of 10 mg/kg/day¹⁹ and was based on the recommended daily intake of CoQ10 for healthy individuals²⁰ together with information provided by supplement manufacturers. The dose of 46 mg/kg/day was calculated using the dose translation formula developed by Reagan-Shaw et al¹⁹ to compensate for the difference in body surface area, oxygen utilization, caloric expenditure, basal metabolism,

Table 1 Experimental diet composition

	Control	CoQ10
Protein (g%)	17.6	17.6
Carbohydrate (g%)	61.1	61.1
Fat (g%)	3.9	3.9
kcal/g (g%)	3.5	3.5
Ingredient	g	
Soy protein	80	80
Casein, 80 mesh	120	120
L-Methionine	5	5
Corn starch	315	315
Maltodextrin 10	35	35
Sucrose	350	350
Cellulose, BW200	100	100
Guar gum	25	25
Lard	20	20
Soybean oil	25	25
Mineral mix S20001	75	75
Vitamin mix V20003	10	10
Choline bitartrate	2	2
CoQ10	0	1.80
FD&C Yellow dye #5	0.05	
FD&C Blue dye #1		0.05
Total	1162	1162

blood volume, plasma proteins, and renal function between humans and guinea pigs.

The supplementation period used in this study was based on CoQ10 supplementation studies in older humans. A previous study that supplemented aged humans with CoQ10 found a change in gene expression in skeletal muscle after 4 weeks.¹⁷ Thus, a 6-week supplementation period was selected to allow sufficient time to observe similar changes in our aged guinea pigs.

Animals were provided with a weighed portion of food on a daily basis with any remaining food weighed prior to the next portion of food being provided; animals were maintained on a 12-hour light/dark photo-period with a room temperature of $21 \pm 2^\circ\text{C}$ and weighed weekly. During the supplementation period at weeks 0, 2, 4, and 6, 500 μL of blood was collected from the saphenous vein into EDTA coated tubes for measurement of plasma CoQ10 concentration and DNA abasic sites in white blood cells (WBC).

At the end of the supplementation period animals were weighed and euthanized with sodium pentobarbital (70 mg/kg). The soleus muscle was then rapidly excised, frozen in liquid nitrogen, and stored at -80°C until analysis of CoQ10, GSH and MDA concentrations, activities of catalase and GPx, DNA abasic sites, and the expression of cytochrome *c* oxidase subunits III and VIa. The soleus muscle was chosen as it has a high concentration of mitochondria compared to other hind limb muscles.²¹

Blood processing

Immediately after blood collection, samples were centrifuged at 1200 g for 10 minutes at 4°C . The plasma was then removed and stored at -80°C until biochemical analysis of CoQ10. The buffy coat containing WBC was also removed and stored at -80°C until biochemical analysis of DNA abasic sites.

Muscle homogenates

A 10% w/v muscle homogenate using 100 mM phosphate buffer, pH 7.4, was prepared from each sample in a FastPrep homogenizer (MP Biomedicals, Solon, OH, USA). Homogenates were centrifuged at 12000 g for 15 minutes at 4°C . The supernatant was removed, placed into a clean tube, and placed on ice until use. Samples were analysed for DNA abasic sites, CoQ10, glutathione (GSH) and malondialdehyde (MDA) concentrations, and glutathione peroxidase (GPx) and catalase activities.

Quantification of CoQ10

Plasma and muscle samples were assayed for CoQ10 concentrations via HPLC using methods described previously.²² Briefly, 100 μL of plasma or muscle homogenate were mixed with 850 μL of cold 1-propanol and 50 μL of internal standard (CoQ9, 2 mg/100 mL).

HPLC analyses were performed on a Shimadzu (LC-10AD) using a reversed-phase Microsorb-MV column (4.6 mm \times 15 cm; 5 mm bead size) with a reversed-phase C18 guard column (4.6 \times 10 mm; 5 mm bead size). The mobile phase was a mixture of sodium acetate trihydrate (6.8 g), 15 mL glacial acetic acid, 15 mL 2-propanol, 695 mL methanol and 275 mL hexane, pH 6, added at a flow rate of 1 mL/min.

Oxidized and reduced CoQ10 were monitored by coulometric detection. Total CoQ10 was determined by summing the oxidized (Ubiquinone-10) and reduced (Ubiquinol-10) forms. CoQ10 in plasma samples was expressed as nmol/L and in muscle samples it was expressed as pmol/mg protein.

Glutathione assay

Total GSH in muscle was determined using the Northwest Life Science Specialities assay kit (NWLSS, USA), which is based on the principle that 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) reacts with GSH causing a color increase which is measured spectrophotometrically at 405 nm.

The assay was conducted using a Cary 300 UV-Vis spectrophotometer (Varian, Australia).

Glutathione peroxidase assay

The activity of GPx in muscle was determined using the Northwest Life Science Specialities assay kit, which is based on a modification of the method of Paglia & Valentine.²³ GPx oxidizes reduced glutathione (GSH) as it breaks down H_2O_2 forming GSSG. GSSG is then reduced by glutathione reductase and NADPH forming NADP^+ (leading to a decrease in absorbance at 340 nm) and recycling the GSH.

GPx activity was measured spectrophotometrically using a Cary 300 UV-Vis spectrophotometer (Varian). One unit of GPx is defined as the amount of enzyme necessary to catalyse the oxidation (by H_2O_2) of 1.0 μMole GSH to GSSH per minute.

Catalase activity

Catalase activity in muscle was determined using the Northwest Life Science Specialities assay kit, which

is based on a modification of the method of Beers & Sizer.²⁴

Catalase activity was measured by observing the consumption of H₂O₂ substrate. Catalase activity was measured spectrophotometrically at 240 nm using a Cary 300 UV-Vis spectrophotometer (Varian). One unit of catalase activity is defined as the amount of enzyme that will break down 1.0 μMole H₂O₂ substrate per minute.

Malondialdehyde assay

MDA, an end product of lipid peroxidation and marker of oxidative damage, was determined in muscle using the Northwest Life Science Specialities assay kit. The assay is based on the principle that thiobarbituric acid (TBA) reacts with MDA to form a TBA-MDA adduct, which is measured spectrophotometrically at an absorbance of 532 nm.

The assay was conducted using a Cary 300 UV-Vis spectrophotometer (Varian).

Protein carbonyl assay

Protein carbonyls, formed by oxidative damage to proteins, were determined in muscle using the the Biocell PC test kit (BioCell, New Zealand). The assay is based on the principle that the quantity of protein carbonyls in a protein sample can be determined by derivatising with dinitrophenylhydrazine (DNP) and measuring bound DNP immunologically.

The assay was conducted using a Perkin-Elmer Fusion-Alpha HT universal microplate analyzer.

Oxidative damage to DNA

Abasic sites, an end product of oxidative damage to DNA, were determined in genomic DNA from muscle and white blood cells (WBC) using a DNA damage quantification kit (Dojindo Molecular Technologies, USA) according to the manufactures instructions. The kit measures the number of abasic sites present in DNA as a measure of oxidative damage.

Reverse transcription-real-time PCR measurement of mRNA

The gene expression of two cytochrome *c* oxidase (complex IV) subunits in the mitochondrial electron transport chain of muscle were determined. Complex IV was chosen based on previous reports showing that CoQ10 supplementation increased the gene expression of cytochrome *c* oxidase subunits in the hearts of mice.¹⁶

Cytochrome *c* oxidase is made up of 13 subunits; three mitochondrial DNA encoded and 10 nuclear DNA encoded.²⁵ Subunit III was chosen as an example of mitochondrial

encoded DNA, and subunit VIa was chosen as an example of nuclear encoded DNA.

RNA was extracted from the soleus muscle using TRI reagent (Molecular Research Centre, USA) following the manufactures instructions. Total RNA concentration was determined spectrophotometrically at 260 nm and then first-strand cDNA was generated from 1 μg RNA using AMV RT (Promega, Australia). The cDNA was stored at -20°C for subsequent analysis.

Primer sequences for the selected genes were designed using primer3. Real-time PCR primers for subunit III were: Forward primer: 5'-AGG ATT TTG ACC ACC AGC AG-3'; Reverse primer: 5'-TCA GAT GGG GTT TAT GGC TC-3'. Real-time PCR primers for subunit VIa were: Forward primer: 5'TGT GAG GGC CCT GAG TAG G-3'; Reverse primer: 5'-ATT CAT CCC CTA CCA CCA CC-3'. Real-time PCR primers for beta-actin were: Forward primer: 5'-GCT GTC CGG AGA CAC TCT TC-3'; Reverse primer: 5'-TGC TGA TCG TAT GCA AAA GG-3'.

Real-time PCR was performed using MiniOpticon System (BioRad, Australia) with PCR reactions performed using iQ SYBR Green Supermix (BioRad, Australia). To compensate for variations in input RNA amounts and efficiency of reverse transcription, Beta-actin was quantified and all results were normalized to these values. Fluorescent emission data were captured and mRNA levels were analyzed using the critical threshold (C_t) value. The relative expression of the gene of interest was calculated using the expression $2^{-\Delta CT}$ and reported as arbitrary units.

Statistical analysis

All data was found to be normally distributed. Comparisons between groups for the muscle markers of antioxidant status, oxidative damage, and gene expression were made by independent *t*-tests. Comparisons between groups for animal body weight data, plasma CoQ10 concentration, and WBC DNA oxidative damage were analyzed using repeated measures ANOVA. A post hoc pair-wise comparison using a Student Newman-Keuls test was also carried out.

Significance was established at the 95% confidence level ($P < 0.05$). All data are shown as the mean ± SEM.

Results

Body weight and food intake

No adverse effects were observed in either group of guinea pigs throughout the study. Body mass was not significantly different between groups throughout the CoQ10 supplementation period ($P > 0.05$). Average food intake for the

supplemented group was 30.1 ± 1.5 g/day and the control group 29.7 ± 1.6 g/day. This diet provided the supplemented guinea pigs with a CoQ10 dose of 46.6 ± 2.3 mg/kg/day.

Effect of CoQ10 supplementation on plasma concentration of CoQ10

CoQ10 supplementation at a dose equivalent to 10 mg/kg/day in humans significantly increased the plasma concentration of ubiquinol-10, ubiquinone-10, and total CoQ10 at weeks 2, 4 and 6 ($P < 0.05$, Table 2). The concentrations of ubiquinol-10, ubiquinone-10, and total CoQ10 increased from 64 ± 4 nmol/L, 9 ± 2 nmol/L and 73 ± 3 nmol/L at week 0 to 511 ± 15 nmol/L, 70 ± 4 nmol/L and 581 ± 15 nmol/L at week 6 respectively.

Effect of CoQ10 supplementation on WBC DNA abasic sites

CoQ10 supplementation caused a significant decrease in WBC DNA abasic sites at 4 and 6 weeks ($P < 0.05$, Table 2). The number of WBC DNA abasic sites decreased from 17 ± 0.5 abasic sites/100000 base pairs at week 0 to 13 ± 0.4 abasic sites/100000 base pairs at week 4 and 10 ± 0.4 abasic sites/100000 base pairs at week 6.

Effect of CoQ10 supplementation on markers of skeletal muscle antioxidant status

CoQ10 supplementation did not significantly alter GSH concentration, or the activities of catalase and GPx ($P > 0.05$,

Table 3). Ubiquinol-10, ubiquinone-10 and total CoQ10 concentrations in skeletal muscle were also not significantly different between groups ($P > 0.05$, Table 3).

Effect of CoQ10 supplementation on markers of oxidative damage in skeletal muscle

MDA concentrations, protein carbonyl levels and DNA abasic sites in the soleus muscle of control and CoQ10 supplemented guinea pigs were not significantly different between groups (Table 4).

Effect of CoQ10 supplementation on skeletal muscle gene expression

CoQ10 supplementation did not significantly alter the expression of cytochrome *c* oxidase subunit III ($123 \pm 56\%$ of control) or subunit VIa ($176 \pm 60\%$ of control).

Discussion

In the present study baseline plasma total CoQ10 levels were approximately ten times lower than those previously reported in young guinea pigs.²⁶ The decreased amount of plasma CoQ10 observed in 26 month old guinea pigs suggests there is an age associated decline in plasma CoQ10 concentration.

Unfortunately this previous study in young guinea pigs did not measure markers of oxidative stress, therefore it is not possible to determine if the age associated decline in CoQ10 levels found in the old guinea pigs used in the current study

Table 2 Plasma CoQ10 concentration and WBC DNA abasic sites. Plasma concentration of ubiquinol-10, ubiquinone-10, total CoQ10 and WBC DNA abasic sites of aged guinea pigs supplemented with CoQ10 compared to control animals. Values are expressed as mean \pm SEM

	Week of supplementation			
	0	2	4	6
Ubiquinol-10 (nmol/L)				
Control	61 ± 3	54 ± 4	59 ± 4	62 ± 7
CoQ10 supplemented	64 ± 4	312 ± 9^b	436 ± 16^b	511 ± 15^b
Ubiquinone-10 (nmol/L)				
Control	8 ± 1	7 ± 2	8 ± 1	7 ± 1
CoQ10 supplemented	9 ± 2	42 ± 2^b	60 ± 4^b	70 ± 4^b
Total^a CoQ10 (nmol/L)				
Control	69 ± 4	61 ± 5	67 ± 5	69 ± 8
CoQ10 supplemented	73 ± 3	354 ± 7^b	496 ± 18^b	581 ± 15^b
DNA oxidation (abasic sites/100000 base pairs)				
Control	17 ± 0.2	17 ± 0.9	16 ± 0.5	17 ± 0.2
CoQ10 supplemented	17 ± 0.5	16 ± 0.5	13 ± 0.4^b	10 ± 0.4^b

Notes: ^aTotal CoQ10 = ubiquinol-10 + ubiquinone-10. ^bSignificantly different between control and treatment groups ($P < 0.05$).

Table 3 Antioxidant markers in skeletal muscle. Antioxidant markers in the soleus muscle of aged guinea pigs supplemented with CoQ10 compared to control animals. Values are expressed as mean \pm SEM

Marker	Units	Control	CoQ10 supplemented
Ubiquinol-10	pmol/mg protein	26 \pm 1	25 \pm 1
Ubiquinone-10	pmol/mg protein	17 \pm 1	18 \pm 2
Total CoQ10 ^a	pmol/mg protein	43 \pm 1	43 \pm 2
Glutathione	μ g/mg muscle wet weight	1.37 \pm 0.21	1.40 \pm 0.09
Glutathione peroxidase	Units/mg wet weight	0.0046 \pm 0.0005	0.0049 \pm 0.0003
Catalase	Units/mg wet weight	0.88 \pm 0.3	0.91 \pm 0.2

Note: ^aTotal CoQ10 = ubiquinol-10 + ubiquinone-10.

results from an increase in oxidative stress relative to young guinea pigs. However, the lower baseline levels of plasma CoQ10 observed in aged guinea pigs from the current study were reversed with CoQ10 supplementation to the point where after six weeks of supplementation plasma CoQ10 levels return to those previously reported in young guinea pigs.²⁶

The increase in total plasma CoQ10 levels with dietary supplementation was the results of increased plasma concentrations of both ubiquinol-10 and ubiquinone-10. The ratio of ubiquinol-10 to ubiquinone-10 was found to be 88:12 which is similar to the ratio found by Yamashita and Yamamoto²⁷ and remained constant for the duration of the study in both CoQ10 supplemented and control groups. These results are in agreement with the findings of previous studies. Kwong et al¹² reported an increase in CoQ10 concentration in the plasma of 14 month old rats after 4 and 13 weeks of CoQ10 dietary supplementation at a dose of 150 mg/kg/day (equivalent to 24 mg/kg/day in humans). In another study, CoQ10 concentration increased in the plasma of three month old mice after supplementation with 148 or 645 mg/kg/day (equivalent to 24 or 105 mg/kg/day in humans) for 11 weeks.²⁸ In a human study, CoQ10 supplementation at a dose 3 mg/kg/day for 28 days significantly increased plasma CoQ10 concentration.²⁹

However, in the present study, CoQ10 supplementation at a dose equivalent to 10 mg/kg/day in humans did not alter the ubiquinol-10, ubiquinone-10 or total CoQ10 concentrations in skeletal muscle. In contrast, it was shown that a dose of 645 mg/kg/day (equivalent to 105 mg/kg/day in humans) for 11 weeks increased CoQ10 levels in skeletal muscle of three month old mice.²⁸

In another study using three month old mice, CoQ10 doses of 93 or 371 mg/kg/day (equivalent to 15 or 60 mg/kg/day in humans) for 3.5 and 17.5 months increased CoQ10 concentrations in skeletal muscle.²

Differences in the supplementation regimes are a likely explanation for the difference between our results and previous findings. In the two aforementioned studies, the supplementation periods were longer, and the CoQ10 doses administered were higher than the present study. Indeed, Sohal and Forster³⁰ indicate that although CoQ10 uptake is a complex process, supplementation at high doses for relatively long periods can result in increased skeletal muscle CoQ10 concentration. Further studies are therefore warranted to determine minimum dosage and supplementation periods for increasing CoQ10 levels in skeletal muscle.

Our results show that dietary supplementation with CoQ10 decreased WBC DNA oxidation. It appears that the increased levels of plasma CoQ10 in the present study either directly or indirectly protected WBC DNA from oxidative damage as abasic site numbers were lower in CoQ10 supplemented animals. Such findings have been reported previously. Exposure of human lymphocytes to CoQ10 *in vitro* was protective against hydrogen peroxide induced DNA strand breaks.¹⁵ Another study found that CoQ10 supplementation in humans at a dose of 3 mg/kg/day for 28 days decreased the formation of 8-hydroxydeoxy-guanosine, a marker of oxidative damage, in human lymphocyte DNA.²⁹

In contrast, the results from the present study show that dietary CoQ10 supplementation did not alter soleus DNA abasic sites indicating that skeletal muscle DNA oxidation may not be affected by CoQ10 supplementation. The concentration

Table 4 Oxidative damage markers in skeletal muscle. Markers of oxidative damage in the soleus muscle of aged guinea pigs supplemented with CoQ10 compared to control animals. Values are expressed as mean \pm SEM

Marker	Units	Control	CoQ10 supplemented
Malondialdehyde	nM/mg muscle wet weight	35 \pm 3	41 \pm 3
Protein carbonyl	nmol/mg protein	2.4 \pm 0.4	2.5 \pm 0.4
DNA oxidation	abasic sites/100000 base pairs	19.5 \pm 0.3	19.7 \pm 0.3

of soleus MDA and protein carbonyls was also not different between groups indicating that CoQ10 supplementation may have had no effect on lipid or protein oxidation.

These results are supported by the work of others showing that prolonged CoQ10 intake failed to modulate levels of oxidative stress in skeletal muscle from 25 month old mice.² Specifically, rates of superoxide production in skeletal muscle submitochondrial particles were unchanged after CoQ10 supplementation at a dose of 93 or 371 mg/kg/day (equivalent to 15 or 60 mg/kg/day in humans) beginning at 3.5 months of age.² Although an earlier finding by Lass and Sohal¹³ showed that CoQ10 intake decreased mitochondrial superoxide generation in skeletal muscle, the rate of superoxide generation was inversely related to mitochondrial α -tocopherol content but unrelated to CoQ10 content. The authors hypothesized that CoQ10 had a sparing effect on α -tocopherol. Taken together, these findings suggest that CoQ10 intake may not directly affect oxidative stress or directly reduce oxidative damage in skeletal muscle.

Our results show that at baseline muscle total CoQ10 levels observed in 26 month old guinea pigs parallels the decrease plasma CoQ10 levels found in the current study and supports the idea there is an age associated decline CoQ10 concentration in a range of tissues. However, in the present study CoQ10 supplementation for 6 weeks at a dose equivalent to 10 mg/kg/day in humans did not increase ubiquinol-10, ubiquinone-10 or total CoQ10 concentrations in the soleus muscle.

As previously discussed, differences in the supplementation regimes are a likely explanation for the discrepant findings. In the present study, the concentration of GSH in aged skeletal muscle was not affected by CoQ10 intake. In a study using mice, supplementation with CoQ10 did not change the concentration of total GSH in the liver, heart, kidney and brain.² Although Sohal et al² did not study skeletal muscle, their results are similar to the present study as they show no effect of CoQ10 supplementation on total GSH concentration in various tissues. The activities of catalase and GPx in the soleus muscle of aged guinea pigs were also not affected by CoQ10 supplementation. These findings are supported by other studies that have supplemented animals with CoQ10 and found no effect on skeletal muscle catalase and GPx activity.^{2,12} These results indicate that CoQ10 supplementation may not alter the capacity of aged skeletal muscle to eliminate hydrogen peroxide.

In our study, the expression of cytochrome *c* oxidase subunits III and VIa in the soleus muscle were not different between control and treatment groups. In contrast, in a life

span study, Lee et al¹⁶ found that dietary supplementation with CoQ10 (100 mg/kg from 14 months of age) upregulated the expression of genes encoding cytochrome *c* oxidase subunits and ATP synthase in the hearts of mice. The authors hypothesized that the pro-oxidant form of CoQ10 results in the production of hydrogen peroxide which may then function as a second messenger to inform the nucleus and the mitochondria to up- or downregulate genes.³¹ The mechanism to explain the conflicting results is not clear, however, it may be due to differences between the supplementation regimes, species, and tissue. It is interesting to note that Sohal et al² reported no effect of CoQ10 intake on the activities of mitochondrial NADH-ferricytochrome *c* reductase (complex I/III) and ferrocytochrome *c* oxidase (complex IV) in skeletal muscle of 25 month old mice fed 93 or 371 mg CoQ10/kg/day (equivalent to 15 or 60 mg/kg/day in humans) since the age of 3.5 months.³⁰

Conclusion

These experiments have shown that dietary supplementation with CoQ10 at a dose equivalent to 10 mg/kg/day in humans increased CoQ10 concentration in plasma and decreased WBC DNA abasic sites.

In contrast, CoQ10 supplementation did not change MDA concentration, protein carbonyl levels, or DNA abasic sites in aged skeletal muscle. In addition, supplementation did not alter muscle GSH concentration, the activities of catalase and Gpx, or the gene expression of cytochrome *c* oxidase subunits III and VIa.

Although the relevance of these findings to humans is yet to be established, the results indicate that dietary supplementation with CoQ10 at a dose of 10 mg/kg/day may be capable of increasing antioxidant protection and reducing oxidative damage in the plasma, but may have no effect in skeletal muscle.

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Disclosure

The authors report no conflicts of interest in this work.

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