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Effect of Lifelong Coenzyme Q_{10} Supplementation on Age-Related Oxidative Stress and Mitochondrial Function in Liver and Skeletal Muscle of Rats Fed on a Polyunsaturated Fatty Acid (PUFA)-Rich Diet

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This study investigates aging-related changes in lipid peroxidation and functionality in liver and skeletal-muscle mitochondria in rats fed a diet rich in polyunsaturated fatty acids (PUFA), depending on supplementation or not with coenzyme Q_{10} (CoQ_{10}). Two groups of rats were fed for 24 months on a PUFA-rich diet, differing in supplementation or not with CoQ_{10}. At 6 and 24 months mitochondria were analyzed for fatty acid profile; hydroperoxides; α-tocopherol; $CoQ_{9;}$ $CoQ_{10;}$ cytochromes *b,* $c+c_1$, and $a+a_3$ contents; cytochrome *c* oxidase activity; and catalase activity in cytosol. Results of this study showed for the supplemented group an age-associated decrease in the peroxidizability index, an increase in catalase activity in skeletal muscle, and modulation of the aging-related changes in different mitochondrial electron-transport-chain components in skeletal muscle. These findings provide mechanisms to explain the effect of CoQ_{10} in extending the life span of animals fed a PUFA-rich diet.

AGING, common to all multicellular organisms, is characterized by an endogenous and progressive decline in physiological function that leads to morbidity and mortality (1,2). Many theories seek to explain the causes of aging, although growing evidence supports the oxidative-stress hypothesis of aging, which postulates that deleterious effects of reactive oxygen species (ROS) are responsible for this functional deterioration associated with age (1,2). Mitochondria are key in this process because they are not only the main generators of the primary ROS and the most immediate targets of the oxidative damage inflicted by those species, but they also regulate stress response and apoptosis, regulating nuclear gene expression (1–6).

There is great interest in the development of anti-aging therapies and, because oxidative stress is involved in aging, any strategy to reduce oxidative damage would have significant anti-aging effects. In this way, the impact of diet and dietary components on aging and age-associated degenerative diseases has been widely recognized (6,7). However, most of the studies have been focused on the effect of caloric restriction or antioxidant supplementation (4,7–9), and only a few studies have examined the effect of dietary lipids, despite the fact that they can modulate the membrane lipid profile [and therefore the peroxidation rate and the mitochondrial functionality (10,11)] and thus can modulate the aging process, as has been shown by our research group (12–14). In this sense, we have previously shown that, when the dietary lipid source is dispensed as virgin olive oil (monounsaturated fat–rich diet) versus sunflower oil (polyunsaturated fat–rich diet), a better general state is found at the mitochondrial function level, with lower ROS production and a delay in aging (12–14).

However, two important aspects should be kept in mind. First, the relationship between dietary polyunsaturated fatty acids (PUFA) and health, mainly with respect to cardiac disease, has been widely recognized, showing different beneficial effects besides improving the lipid profile (15,16). Second, in many countries, these PUFA constitute one of the major dietary lipid sources, and in other countries their intake is increasing (17). Therefore, if the major problem of these fatty acids, as stated above, is that they are highly susceptible to the ROS attack, especially during aging (12–14), then supplementation with antioxidants would preserve the advantages of PUFA on health while preventing their deleterious aspects.

In this field, our research group has identified an excellent candidate for this supplementation, the coenzyme Q or ubiquinone. This molecule is an essential electron carrier in the mitochondrial respiratory chain, where it also has important antioxidant properties under lipophilic conditions (18). Previous studies have suggested the possible beneficial role of coenzyme Q in aging (8,9,19). With respect to PUFA and aging, we have found that, compared to rats fed lifelong on a PUFA-rich diet alone, rats fed on the same diet but supplemented with coenzyme Q_{10} showed a significantly longer mean and maximum life span (20). However, its molecular and cellular mechanisms are still not fully understood.

Table 1. Fatty Acid Composition of the Experimental Diet

Fatty Acids	
C16:0, %	12.6 ± 0.1
C16:1n7, %	0.2 ± 0.0
C18:0, %	1.9 ± 0.0
C18:1n9, %	24.1 ± 0.1
C18:2n6, %	59.1 ± 0.2
C18:3n3, %	2 ± 0.0
SFA, %	14.6 ± 0.1
UFA, %	85.4 ± 0.4
MUFA, %	24.3 ± 0.2
PUFA, %	61.1 ± 0.3
α-tocopherol, mg/kg	1509 ± 125

Notes: Results represent mean ± standard error of the mean of six samples. SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The aim of the present study was to investigate changes during aging in lipid peroxidation and functionality in mitochondria of mitotic (liver) and postmitotic (skeletal muscle) tissues, depending on supplementation or not with coenzyme Q_{10} (CoQ_{10}), in rats fed lifelong on a PUFA-rich diet. This study attempts to establish mechanistic links between CoQ_{10} supplementation in animals fed a PUFA-rich diet and the lengthening of the life span.

Materials and Methods

Experimental Protocol

One hundred twenty male Wistar rats (*Rattus norvegicus*) initially weighing 80–90 g were maintained five per cage on a 12-hour light/dark cycle, with free access to food and water. The rats were randomly assigned to two experimental groups and fed 24 months on a semisynthetic and isoenergetic diet (in g/100 g of diet): 26.7 casein, 13.53 starch, 45.29 sucrose, 1.0 vitamin mixture, 3.68 mineral mixture, 1.84 cellulose, 0.09 choline, 0.3 methionine, and 8.0 fat (containing 61% of total fatty acids as PUFA). Table 1 shows the fatty acid profile of the diet. Diets differed in the supplementation (PUFA+CoQ) or not (PUFA) with 2.5 mg/kg/day of CoQ_{10}. Twenty rats per group were killed, respectively, at 6 (young adulthood) and 24 (old age) months from the start of the experiment. Animals were handled according to the guidelines of the Spanish Society for Laboratory Animals, and the experiment was approved by the Ethics Committee of the University of Granada.

Sample Analysis

Rats were killed by cervical dislocation followed by decapitation. Liver and skeletal muscle (vastus lateralis) were removed and weighed, and their mitochondria isolated as previously described (13).

The fatty acid profile of mitochondrial membranes was measured by gas–liquid chromatography as described by Lepage and Roy (21). A gas–liquid chromatograph model HP-5890 Series II (Hewlett Packard, Palo Alto, CA) equipped with a flame-ionization detector was used to analyze the fatty acids as methyl esters. Chromatography was performed using a 60 m-long capillary column (32 mm internal diameter and 20 mm thick) impregnated with Sp 2330 FS (Supelco Inc., Bellefonte, PA). The injector and detector were maintained at 250°C and 275°C, respectively; nitrogen was used as the carrier gas, and the split ratio was 29:1. Temperature programming (for a total time of 40 minutes) was as follows: initial temperature, 160°C for 5 minutes, 6°C/minute to 195°C, 4°C/minute to 220°C, 2°C/minute to 230°C, hold 12 minutes, 14°C/minute to 160°C. The determination of the peroxidizability index (PI) was performed as described previously (22). Briefly, the PI = (% dienoic acid × 1) + (% trienoic acid × 2) + (% tetraenoic acid × 3) + (% pentanoic acid × 4) + (% hexaenoic acid × 5).

The mitochondrial membrane hydroperoxide content was determined following the procedure of Jiang and colleagues (23). *Tert*-butyl hydroperoxide was used to construct a standard curve.

CoQ_{10}, coenzyme Q_9 (CoQ_9), and α-tocopherol in mitochondrial membrane were assayed by high-performance liquid chromatography (HPLC), after extraction with ethanol/petroleum ether (60:40, vol/vol) using the method of Kröger (24). The HPLC system consisted of an apparatus equipped with a diode array detector, model 168 (Beckman Instruments, Inc., Fullerton, CA), and the column was a reverse-phase C18 Spherisorb ODS 1 of 25 × 0.46 cm. CoQ_9, CoQ_{10}, and α-tocopherol were identified by predetermining the retention times of individual standards.

Cytosolic catalase activity was determined following the method described by Aebi (25), based on monitoring (at 240 nm) the H_2O_2 decomposition, as a consequence of the catalytic activity of catalase. The activity was calculated from the first-order rate constant K (s^{-1}).

The concentration of mitochondrial cytochrome *b*, $c+c_1$, and $a+a_3$ was evaluated by measuring the reduced minus oxidized difference spectra with a λ16-Perkin Elmer double-beam spectrophotometer, as previously described (12). Cytochrome *c* oxidase (CCO) activity was assayed at 25°C using cytochrome *c* reduced by sodium dithionite, monitoring the absorbance decrease of cytochrome *c* upon oxidation at 417–409 nm every 10 seconds for 2 minutes using an extinction coefficient for cytochrome *c* of 40.7 $mM^{-1} \cdot cm^{-1}$, as previously described (12).

Statistical Analysis

Results are presented as means ± standard error of the mean (*SEM*) ($n = 20$ rats). Except for the lipid profile (expressed as percentage), results are expressed per gram of wet tissue. Significant interaction terms were evaluated by a Student *t* test. Results were considered significant at a p value of $< .05$. Data were analyzed using the SPSS statistical software package (SPSS for Windows, 13.0, 2004; SPSS Inc., Chicago, IL).

Results

Food Intake and Rat Weight

Dietary intake did not significantly vary between groups during the experiment (data not shown). Body weight was similar for both groups for the different time intervals (PUFA vs PUFA+CoQ, for 6 months: 543.1 ± 12.2

Table 2. Effect of Lifelong Supplementation with Coenzyme Q_{10} on Liver and Skeletal Muscle Mitochondrial Fatty-Acid Profile of Rats Fed a PUFA-Rich Diet

		6 Months		24 Months	
Fatty Acid Index	Tissue	PUFA	PUFA+CoQ	PUFA	PUFA+CoQ
SFA, %	Liver	45.4 ± 1.4	47.6 ± 1.7	48.2 ± 1.1	49.3 ± 0.9
	Skeletal muscle	64.7 ± 2.1	67.6 ± 1.1	62.7 ± 1.4*	70.0 ± 1.8
MUFA, %	Liver	20.7 ± 1.3*	15.3 ± 1.3†	17.6 ± 0.7*	24.4 ± 1.9
	Skeletal muscle	17.5 ± 1.9	15.5 ± 1.3	19.0 ± 1.6	15.9 ± 0.9
PUFA, %	Liver	33.8 ± 0.9	37.0 ± 2.2†	34.8 ± 0.7*	26.3 ± 1.7
	Skeletal muscle	18.8 ± 0.9	18.3 ± 0.7†	19.8 ± 0.3*	15.0 ± 0.9
PUFA n-6, %	Liver	32.9 ± 0.9	35.9 ± 2.1†	33.3 ± 0.8*	24.0 ± 1.8
	Skeletal muscle	18.5 ± 0.9	17.6 ± 0.6†	19.4 ± 0.4*	14.6 ± 0.9
PUFA n-3, %	Liver	0.9 ± 0.1†	1.1 ± 0.0†	1.6 ± 0.0*	2.3 ± 0.2
	Skeletal muscle	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
PI	Liver	72.3 ± 1.8	78.0 ± 3.8†	79.0 ± 1.8*	64.8 ± 3.5
	Skeletal muscle	29.0 ± 1.1†	30.6 ± 1.2†	32.1 ± 0.7*	24.4 ± 1.5

Notes: Results are mean ± standard error of the mean of 20 animals.

*$p < .05$ (PUFA vs PUFA+CoQ for the same time period).

†$p < .05$ (6 months vs 24 months for the same group).

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; PI = peroxidizability index.

vs 549.9 ± 13.2; for 24 months: 544.25 ± 19.73 vs 537.8 ± 18.5).

Mitochondrial-Lipid Profile

Table 2 shows the effect of supplementation with CoQ_{10} (2.5 mg/kg/day) on fatty acid profile in mitochondrial membranes of liver and skeletal muscle. The sum of the total saturated fatty acids did not show changes associated with age and registered differences only between groups ($p < .05$) in skeletal muscle at 24 months of age. Total monounsaturated fatty acids (MUFA) (Table 2) showed, although only in liver, an increase associated to age in PUFA+CoQ group, this group having a lower proportion of MUFA at 6 months and higher at 24 months with respect to nonsupplemented rats. The sum of total PUFA (Table 2) revealed, in both tissues, an age-associated decrease in the group supplemented with CoQ_{10}. This group showed a lower proportion at 24 months with respect to nonsupplemented rats ($p < .05$) for these fatty acids. PUFA n-6 followed in both tissues the same pattern as PUFA. However, PUFA n-3 registered an age-associated increase in both groups in liver, the PUFA group showing the lowest proportion at 24 months ($p < .05$).

Finally, the PI in both tissues indicated a statistically significant age-associated decrease in the PUFA+CoQ group, which presented the lowest value at 24 months ($p < .05$). In skeletal muscle, an increase in this index was associated with age in the PUFA group.

Hydroperoxide Levels

In liver (Figure 1) only the PUFA group showed higher hydroperoxide levels associated with age; these levels statistically differed with respect to PUFA+CoQ group at 24 months. In skeletal muscle (Figure 1), both groups reached higher levels associated with age, the PUFA group presenting the highest values ($p < .05$) in both time periods.

Concentration of α-Tocopherol, CoQ_9, and CoQ_{10}

At 6 months, the PUFA group showed a lower α-tocopherol concentration than did the supplemented rats in both tissues (Table 3). However, at 24 months, the same group showed the highest concentration ($p < .05$), although only in skeletal muscle. With respect to the effect of age, liver increased in the α-tocopherol concentration in

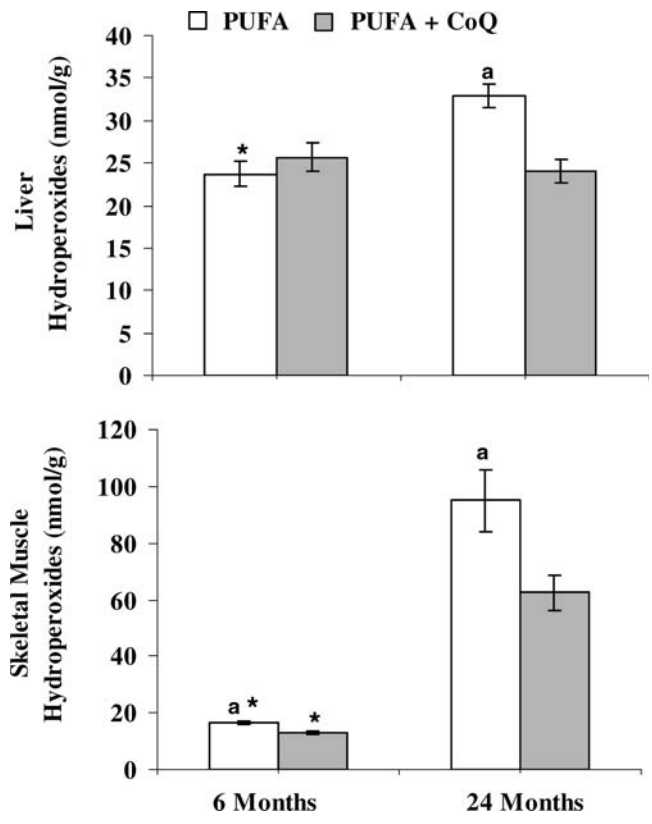

Figure 1. Effect of lifelong supplementation with coenzyme Q_{10} (CoQ_{10}) on hydroperoxide levels in liver and skeletal muscle mitochondria of rats fed a polyunsaturated fatty acid (PUFA)-rich diet. Results are mean ± standard error of the mean (*SEM*) of 20 animals. Statistical significance ($p < .05$): *6 months vs 24 months for the same group. [a]PUFA vs PUFA+CoQ for the same period of time.

Table 3. Effect of Lifelong Supplementation with Coenzyme Q_{10} on Liver and Skeletal Muscle Mitochondrial Levels of α-Tocopherol, Coenzyme Q_9, and Coenzyme Q_{10} of Rats Fed a PUFA-Rich Diet

		6 Months		24 Months	
	Tissue	PUFA	PUFA+CoQ	PUFA	PUFA+CoQ
α-Tocopherol, nmol/g	Liver	7.5 ± 0.5*†	10.9 ± 1.0†	9.7 ± 0.8	8.4 ± 0.5
	Skeletal muscle	0.6 ± 0.1*†	1.7 ± 0.2†	4.0 ± 0.3*	2.8 ± 0.4
Coenzyme Q_9, pmol/g	Liver	3871.7± 275.5†	4103.4 ± 545.9†	6486.6 ± 345.8	6737.9 ± 647.5
	Skeletal muscle	871.2 ± 10.0*†	1536.6 ± 218.9	4444.4 ± 528.4*	1637.3 ± 209.7
Coenzyme Q_{10}, pmol/g	Liver	454.7 ± 39.2*†	3562.3 ± 466.0†	1626.5 ± 189.0*	6048.9 ± 985.3
	Skeletal muscle	56.7 ± 5.3*†	89.1 ± 10.0	273.4 ± 33.6*	97.3 ± 19.0
Ratio CoQ_9/CoQ_{10}	Liver	8.9 ± 0.4*†	1.1 ± 0.1	5.4 ± 0.8*	1.5 ± 0.3
	Skeletal muscle	16.0 ± 0.9	18.3 ± 1.2	18.6 ± 1.5	19.4 ± 2.5

Notes: Results are mean ± standard error of the mean of 20 animals.
*$p < .05$ (PUFA vs PUFA+CoQ for the same time period).
†$p < .05$ (6 months vs 24 months for the same group).
PUFA = polyunsaturated fatty acid; CoQ = coenzyme Q.

nonsupplemented animals and decreased in supplemented animals, whereas skeletal muscle had higher concentrations associated with age in both groups.

CoQ_9 and CoQ_{10} concentrations (Table 3) in liver registered higher concentrations associated with age in both groups, but in skeletal muscle this result was found only in nonsupplemented rats. At 6 months, both CoQ_9 and CoQ_{10} showed lower concentrations in the skeletal muscle of nonsupplemented rats, but in liver only CoQ_{10} showed this behavior. At 24 months, the CoQ_{10} group was the only one with statistically significant differences in the two tissues, with the nonsupplemented rats presenting the lowest value in liver and the highest value in skeletal muscle ($p < .05$). The ratio CoQ_9/CoQ_{10} showed in liver a decrease associated with age in nonsupplemented animals and, in the same tissue, lower values along the study in supplemented animals.

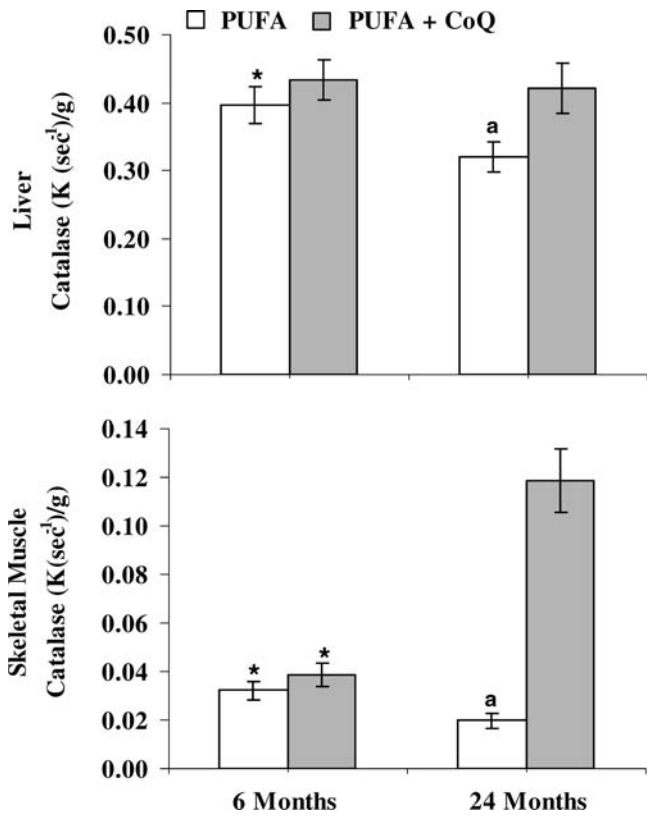

Figure 2. Effect of lifelong supplementation with coenzyme Q_{10} (CoQ_{10}) on catalase activity in liver and skeletal muscle mitochondria of rats fed a polyunsaturated fatty acid (PUFA)-rich diet. Results are mean ± standard error of the mean (*SEM*) of 20 animals. Statistical significance ($p < .05$): *6 months vs 24 months for the same group. [a]PUFA vs PUFA+CoQ for the same period of time.

Catalase Activity

In both tissues (Figure 2), we found lower cytosolic catalase activity associated with age in the PUFA group, and only in skeletal muscle higher activity with age in the supplemented rats. The PUFA+CoQ group showed the highest activity at 24 months in both tissues.

Concentrations of Cytochromes $a+a_3$, b, and $c+c_1$

The concentration of these cytochromes is shown in Table 4. For these mitochondrial electron transport chain (METC) components, both tissues showed the highest levels in old animals from both groups ($p < .05$). Between groups, only skeletal muscle at 24 months of age showed some statistically significant differences with regard to supplemented rats, having lower concentrations of cytochromes *b* and $c+c_1$ than in nonsupplemented rats ($p < .05$).

CCO Activity

CCO activity is presented in Figure 3. No differences were found between the two tissues concerning dietary treatment. In liver, we detected the highest CCO activity for both groups at 24 months ($p < .05$), and the opposite was found in skeletal muscle, with lower values at 24 months ($p < .05$).

Discussion

A previous study has shown that lifelong supplementation with a low dosage of CoQ_{10} lengthens both the mean and maximum life span of rats fed a PUFA-rich diet (20). However, the possible mechanisms of action in different tissues were not studied.

Table 4. Effect of Lifelong Supplementation With Coenzyme Q_{10} on Liver and Skeletal Muscle Mitochondrial Levels of Cytochromes *b*, $c+c_1$, and $a+a_3$ of Rats Fed a PUFA-Rich Diet

		6 Months		24 Months	
	Tissue	PUFA	PUFA+CoQ	PUFA	PUFA+CoQ
Cytochrome *b*, nmol/g	Liver	1.02 ± 0.09*	1.13 ± 0.11*	2.31 ± 0.17	2.49 ± 0.38
	Skeletal muscle	0.10 ± 0.02*	0.14 ± 0.02*	0.49 ± 0.07†	0.33 ± 0.02
Cytochrome $c+c_1$, nmol/g	Liver	1.56 ± 0.11*	1.58 ± 0.17*	2.57 ± 0.37	3.22 ± 0.38
	Skeletal muscle	0.12 ± 0.01*	0.13 ± 0.01*	0.89 ± 0.16†	0.37 ± 0.06
Cytochrome $a+a_3$, nmol/g	Liver	0.86 ± 0.09*	1.15 ± 0.22*	2.34 ± 0.22	2.53 ± 0.28
	Skeletal muscle	0.12 ± 0.01*	0.15 ± 0.01*	0.29 ± 0.03	0.22 ± 0.03

Notes: Results are mean ± standard error of the mean of 20 animals.
*$p < .05$ (6 months vs 24 months for the same group).
†$p < .05$ (PUFA vs PUFA+CoQ for the same time period).
PUFA = polyunsaturated fatty acid; CoQ = coenzyme Q.

Our work focused on liver (mitotic tissue) and skeletal muscle (postmitotic tissue) because it is known that each tissue ages differently, depending on its capacity to repair damage or to replace cells (12,13). Therefore, to determine the possible mechanisms of action of CoQ_{10}, we must compare the effects of this molecule in tissues with different regenerative capacity, attempting to find a similar response. Liver is a critical organ in the protection from oxidative damage and is key in the breakdown of potentially toxic lipophilic toxins (26). Some age-associated changes in liver physiology and function have been described (26), and many of these changes have been suggested as a consequence of oxidative stress. Skeletal muscle is critical for health maintenance in the elderly population. Aging in muscle involves decreased mass, strength, and contraction rate, processes that may be triggered by ROS generated throughout life (27).

The election of CoQ_{10} for supplementation of the diet instead of CoQ_9, predominant in rat, was due to several factors. Among these are included the ostensible better antioxidative capacity of CoQ_{10} or the fact that superoxide anions' radical generation has been directly correlated with the amounts of mitochondrial CoQ_9 and are inversely related to CoQ_{10} content (8,9).

Prior to the analysis of possible effects of CoQ_{10} supplementation, it was necessary to assess the adaptation to such supplementation in rats. Several groups have carried out studies based on CoQ supplementation in rodents (8,9,20,28–30). In general, results show that besides plasma, liver, and spleen, most tissues are resistant to increased amounts of CoQ from exogenous sources, with skeletal muscle being one of the tissues more resistant to dietary CoQ (18,30). For example, in one noteworthy report (29), tritium-labeled dietary CoQ was detected after 1 day in blood, liver, spleen, and heart, although no uptake was visible in skeletal muscle, among other tissues. In this light, we can state that the lifelong supplementation schedule in rats, based on a low CoQ_{10} rates (2.5 mg/kg/day), led to a good adaptation pattern with higher CoQ levels for the periods studied in liver and in skeletal muscle of rodents at least 6 months of age. However, skeletal muscle maintains these values throughout the study, which could indicate a limited uptake of exogenous CoQ by this tissue under normal conditions, probably due to its cellular location and functional requirements, as it has been indicated in other studies (for review, see 18).

Contradictory results concerning free-radical damage and aging have been reported in recent years. However, when the artifacts are avoided and the appropriate biomarkers are used, overall, it appears that free-radical damage increases during aging (31). In the present study, lipid peroxidation values show an age-associated increase in nonsupplemented animals in both tissues; this finding is consistent with the

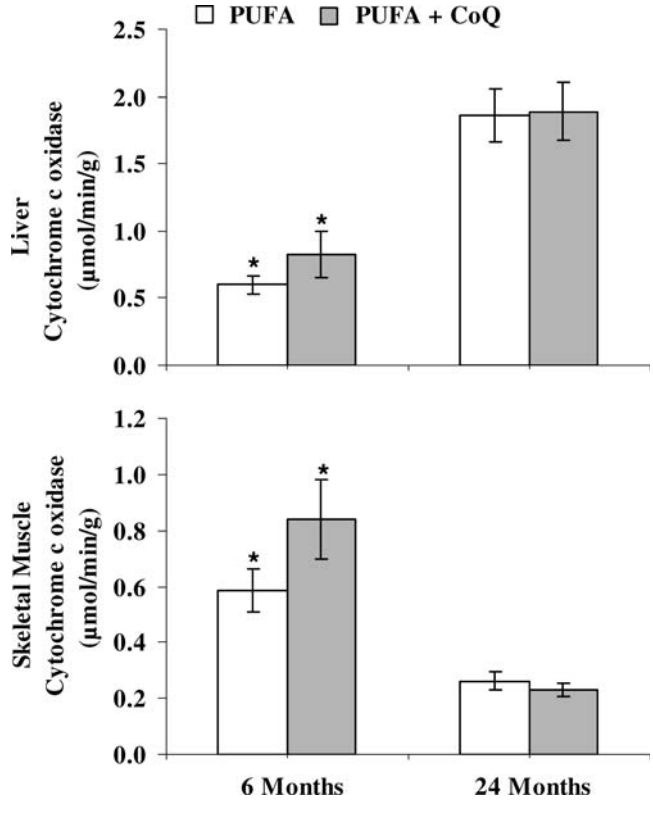

Figure 3. Effect of lifelong supplementation with coenzyme Q_{10} (CoQ_{10}) on cytochrome *c* oxidase activity in liver and skeletal muscle mitochondria of rats fed on a polyunsaturated fatty acid (PUFA)-rich diet. Results are mean ± standard error of the mean (*SEM*) of 20 animals. Statistical significance ($p < .05$): *6 months vs 24 months for the same group. PUFA vs PUFA+CoQ for the same period of time.

free radical theory of aging of Harman (2). However, animals supplemented with our dosage of CoQ_{10} showed an age-associated increase only in hydroperoxides of mitochondrial skeletal muscle; this group registered lower values for this parameter at 6 and 24 months of age with respect to the nonsupplemented animals.

There are at least three possible ways by which CoQ_{10} could cause these differences in hydroperoxide levels between the studied groups: (i) changes in the mitochondrial fatty-acid profile (and, therefore, changes in its susceptibility to lipid peroxidation), (ii) increase in the antioxidant defenses, and (iii) modulation of the free radical sources. We have examined, partially, these ways.

It has been shown that changes in the mitochondrial fatty-acid profile can modulate the susceptibility of the mitochondrial membrane to lipid peroxidation during aging (13). Also, it has been demonstrated that the maximum life span in vertebrates is inversely correlated with the rate of endogenous free-radical generations and the degree of unsaturation and/or the PI of membrane fatty acids (1,32). To our knowledge, there is only one study that supports an effect of ubiquinone in rat liver fatty-acid profile, although that study used a higher dosage and lasted only 38 days (33); thus, it is difficult to compare those data with our data. Our results indicate a clear effect of CoQ_{10} supplementation on mitochondrial membrane fatty-acid profile at 24 months of age in both tissues. From the standpoint of relating aging and oxidative stress, the most informative result with respect to the mitochondrial fatty-acid profile is the age-associated decrease in the PUFA percentage and, therefore, the PI. If the PUFA content and PI of membranes are inversely correlated with lipid peroxidation and maximum life span (1,32), it could be a first possible mechanism through which CoQ_{10} supplementation lowers the hydroperoxide values in liver and skeletal muscle and lengthens both the mean and maximum life span of rats fed a PUFA-rich diet, as previously reported (20). Also, these results are important for being the first evidence, to our knowledge, that lifelong supplementation with a low dosage of CoQ_{10} can alter the mitochondrial fatty-acid profile in liver and skeletal muscle. It has been shown that CoQ_{10} induces an overexpression of different lipid-related genes (27,34), so it could be speculated that CoQ_{10} supplementation is able to modulate the gene expression of enzymes involved in the homeostatic regulation of the double-bond content of tissues' fatty acids, to establish the best conditions for membrane proteins, membrane transport, etc., and decrease oxidative damage. However, this issue needs further study.

A second plausible mechanism involves the antioxidant system. The increase in some nonenzymatic antioxidants with aging has been shown (13,31,35), attempting to reduce oxidative damage. Also, it has been suggested that these antioxidants accumulate at the sites where they are needed to protect against the development of age-associated changes (35). Our results agree with the aforementioned, as nonsupplemented animals in both tissues increased their age-associated hydroperoxide values (higher in skeletal muscle).. At the same time, these animals presented higher α-tocopherol values; this increase was greater in skeletal muscle as well. In contrast, supplemented animals showed no increase in hydroperoxide values in liver and, therefore, showed no increase in α-tocopherol values. In addition, in skeletal muscle, animals supplemented with CoQ_{10} had lower increases in both parameters—hydroperoxides and α-tocopherol—than did nonsupplemented animals. In contrast, several studies indicate that α-tocopherol and CoQ are more efficient as antioxidants when acting together (28,30). It might be concluded that an increase in α-tocopherol should be accompanied by a similar increase in CoQ, as in our study in nonsupplemented animals. To understand the different behavior of CoQ in supplemented animals, it is necessary to consider two aspects: first, that supplementation with CoQ results in greater CoQ content, mainly in liver; and, second, that the increase in skeletal muscle α-tocopherol in this group was approximately 4-fold lower than in the nonsupplemented group.

With respect to catalase activity, the results in our nonsupplemented group showed an age-associated decrease in liver and skeletal muscle, in agreement with previous data reported by our research group (13). However, rats supplemented lifelong with a low dosage of CoQ_{10} did not show lower catalase activity in liver or a significant increase in the activity of this antioxidant enzyme in skeletal muscle. Previously, our research group reported a similar effect of CoQ_{10} supplementation on heart catalase activity in rat (28). Increased catalase has been associated with greater resistance to oxidative damage (6) and, in this sense, catalase seems to be important in overall antioxidant enzymatic defense systems with respect to life span (36). This theory has been very recently reinforced by the demonstration that overexpression of catalase in heart, both in mitochondria and cytosol, and in skeletal muscle, prevents ROS production and increases life span in transgenic animals (37). With only our data, it is difficult to explain how CoQ_{10} supplementation can affect catalase activity. Nevertheless, CoQ_{10} administration has been able to increase the gene expression of glutathione *S*-transferase in mice heart (38) and to modulate phospholipid hydroperoxide glutathione peroxidase gene expression in prostate cells lines (39). Therefore, we can not rule out that a similar interaction could exist between CoQ_{10} and catalase. Thus, this effect on catalase activity in postmitotic tissues could be a second possible mechanism by which CoQ_{10} supplementation affects oxidative stress and life span.

A third possible way by which CoQ could modulate lipid-peroxidation levels is by acting on the free-radical source, the METC. Aging has been associated with alterations in concentrations and/or activity of different components of the METC that are more prevalent and severe in the postmitotic tissue (12,28). In general, our results indicate an age-associated increase in practically all the METC components studied in both tissues. However, the greatest difference between tissues was in CCO activity, which increased in liver, as it did in the rest of the METC components, but decreased in skeletal muscle. Similar decreases in the activity of this METC complex in skeletal muscle have been previously reported by us and other groups (5,12,40). Decreases in CCO activity and increases in other METC components have been correlated with the concomitant increases in the flux of mitochondrial O_2^- and H_2O_2

generation (12,19). Thus, it has been suggested that there could be an obstruction or partial blockage of electron flow through some respiratory complexes associated with age, and therefore a greater number of free radicals could be generated at these sites along the METC (12,19). Results related to cytochrome content and CCO activity obtained in skeletal muscle agree with this scenario. In nonsupplemented animals, there was an age-associated increase in cytochrome contents and CoQ_9, in parallel with a decrease in CCO activity, a situation that could partially block the electron flow and thereby boost free-radical production. A previous study in heart tissue of rats showed that CoQ_{10} supplementation increased CCO activity but had no effect on cytochrome content (28). In skeletal muscle, CoQ_{10} supplementation appeared not to influence CCO activity but clearly affected cytochromes (at least *b* and $c+c_1$) and the CoQ_9 content, modulating the age-associated increase observed in these METC components. These effects could relieve the obstruction or partial blockage of electron flow and thereby reduce free-radical generation. However, this speculation requires more investigation.

In contrast, levels of CoQ_{10} have been correlated with levels of CoQ_9 (28), possibly due to the antioxidative protection of mitochondrial CoQ_9 by CoQ_{10} (32). This correlation is in agreement with the decrease associated with age in the ratio CoQ_9/CoQ_{10} observed in nonsupplemented animal liver and with the same ratio shown by the two groups in skeletal muscle. In addition, this fact, together with the relationship between α-tocopherol and CoQ_{10} previously mentioned, and the resistance of skeletal muscle to uptake exogenous CoQ, could help to explain the pattern shown by this tissue in the supplemented group with respect to CoQ content.

Conclusion

The results of this study reveal three possible mechanistic links between life-span extension and CoQ_{10} supplementation in animals fed a PUFA-rich diet: (i) through a decrease in the degree of unsaturation or PI in mitochondrial membranes; (ii) through an increase in catalase activity in postmitotic tissues; and (iii) through the modulation of the age-associated changes in different METC components in postmitotic tissues.

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