Coenzyme Q_{10} Protects From Aging-Related Oxidative Stress and Improves Mitochondrial Function in Heart of Rats Fed a Polyunsaturated Fatty Acid (PUFA)-Rich Diet

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Coenzyme Q_{10} supplementation on age-related changes in oxidative stress and function of heart mitochondria in rats fed a polyunsaturated fatty acid (PUFA)-rich diet was investigated. Two groups of rats were fed for 24 months on a PUFA-rich diet, differing in supplementation or not with coenzyme Q_{10}. Animals were killed at 6, 12, or 24 months. Fatty-acid profile, hydroperoxides, α-tocopherol, coenzyme Q, catalase and glutathione peroxidase activities, and cytochromes $a+a_3$, b, $c+c_1$ and cytochrome c oxidase activity were measured. Coenzyme Q_{10}-supplemented animals showed lower hydroperoxide levels; higher content and/or activity of α-tocopherol, coenzyme Q, and catalase; and a slightly lower decrease in mitochondrial function. According to that, previously reported positive effects of coenzyme Q supplementation on the life span of rats fed a PUFA-rich diet might be a consequence, at least in part, of a lower oxidative stress level and perhaps, to a minor extent, of a smaller decrease in mitochondrial function.

MECHANISMS underlying age-related alterations are not well defined, although growing evidence supports the validity of the oxidative stress hypothesis of aging, which suggests that continuous exposure of the cell to oxidative injury contributes to the lowered functional capacity associated with age (1,2). In this process, mitochondria play a crucial role because they are not only the main generators of the primary reactive oxygen species and the most immediate targets of the oxidative damage inflicted by those species, but also because mitochondria regulate stress response and apoptosis, regulating nuclear gene expression (3,4).

Heart is a largely postmitotic tissue and one of the organs in which the potential effects of oxidative damage are most readily detectable due to its high dependence on oxidative phosphorylation to derive energy (5). In this sense, there is a link between age-associated changes in heart and clinical cardiac diseases, which are major causes of death in industrialized societies (6–8).

The relationship between dietary polyunsaturated fatty acids (PUFAs) and cardiac diseases has been widely recognized, showing different beneficial effects besides improving the lipid profile. For example, myocardial vulnerability to arrhythmia has been showed to be influenced by dietary fat with a potential benefit of dietary modification to include higher proportion of PUFA in reducing the risk of sudden cardiac death (6,9,10). The major problem of these fatty acids is that they are highly susceptible to the reactive oxygen species attack (11), subsequently increasing the oxidative damage in the organism, especially during aging (12,13). However, supplementation with antioxidants would preserve the advantages of PUFA on health while preventing their deleterious aspects.

The mitochondrial component coenzyme Q_{10} (CoQ_{10}) or ubiquinone has been used for many years as a dietary supplement intended to promote good health by trapping free radicals. Interest in ubiquinone comes from the fact that it has a pivotal role as a redox link between flavoproteins and cytochromes in the mitochondrial respiratory chain, where it also has important antioxidant properties under lipophilic conditions (14,15). Previous studies (5,14,16,17) have suggested the possible beneficial role of coenzyme Q in aging. In addition, this molecule is commonly used for treating cardiomyopathy, and there is substantial evidence that heart function improves after ubiquinone administration (15). However, no studies have explored the combination of CoQ_{10} and PUFA-rich diet on oxidative damage of rat heart during aging. According to the above-mentioned premise, the aim of the present study was to investigate, for the first time, changes during aging in lipid peroxidation and functionality of heart mitochondria, depending on supplementation or not with CoQ_{10}, in rats fed a lifelong diet rich in PUFAs.

METHODS

Experimental Protocol

One hundred twenty male Wistar rats (*Rattus norvegicus*) initially weighing 80–90 grams were maintained five per cage on a 12-h light/dark cycle, with free access to food and water. The rats were randomly assigned to two experimental groups and, according to AIN93 criteria (18), fed a semi-synthetic and isoenergetic diet containing 61% of total fatty acid as PUFA for 24 months. Table 1 shows the fatty-acid profile of the diet. Diets differed in supplementation (PUFA+CoQ_{10}) or not (PUFA) with 0.7 mg/kg/day of

Table 1. Fatty-Acid Composition of the Experimental Diet

Fatty Acids	Composition, in g/100 g
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
Total saturated	14.6 ± 0.1
Total unsaturated	85.4 ± 0.4
Total monounsaturated	24.3 ± 0.2
Total polyunsaturated	61.1 ± 0.3
α-Tocopherol (mg/kg)	1509 ± 125

Note: Results represent mean $\pm$ standard error of the mean of six samples.

Figure 1. Effect of supplementation with coenzyme Q_{10} (CoQ_{10}) throughout life on rat weight. Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p < .05$): *polyunsaturated fatty acid (PUFA) vs PUFA+CoQ_{10} for the same period of time. **a**, 12 or 24 months vs 6 months for the same group; **b**, 24 months vs 12 months for the same group.

CoQ_{10}. Twenty rats per group were killed, respectively, at 6 (young adulthood), 12 (middle age) or 24 (old age) months from the start of the experiment. Animals were handled according to guidelines of Spanish Society for Laboratory Animals, and the experiment was approved by the Ethical Committee of the University of Granada.

Sample Analysis

Rats were killed by cervical dislocation followed by decapitation. Heart mitochondria were isolated according to Fleischer and colleagues (19). The fatty-acid profile of mitochondrial membranes was measured by gas-liquid chromatography as described by Lepage and Roy (20). 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-meter-long capillary column (32 mm inside diameter, 20 mm thickness) impregnated with Sp 2330 FS (Supelco, Inc., Bellefonte, PA). The injector and the 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 mitochondrial membrane hydroperoxide content was determined following the procedure of Jiang and colleagues (21). Tert-butyl hydroperoxide was used to construct a standard curve. When the rats were 24-months old, a second hydroperoxide determination was performed after in vitro oxidation of the samples using 2.5 mM of AAPH (2,2′-zobis (2-amidinopropane) hydrochloride), a potent generator of free radicals.

CoQ_{10}, coenzyme Q_9 (CoQ_9), and α-tocopherol in mitochondrial membrane were assayed by high-performance liquid chromatography, after extraction with ethanol/petroleum ether (60:40) using the method of Kröger (22). The high-performance liquid chromatography 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 (25 × 0.46 cm). Coenzymes and α-tocopherol were identified by predetermining the retention times of individual standards.

Catalase activity was determined following the method described by Aebi (23), based on monitoring at 240 nm the H_2O_2 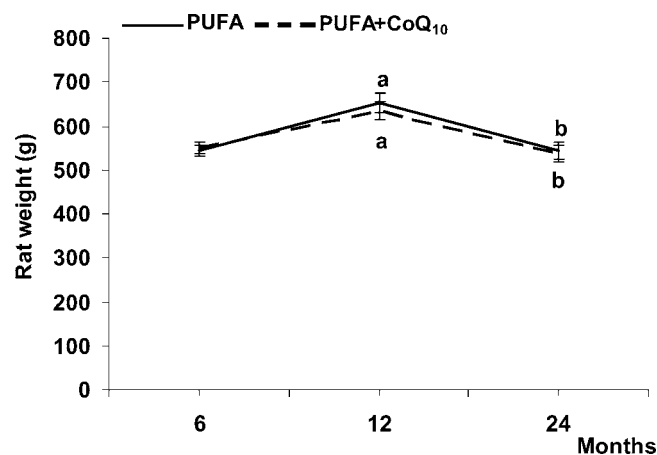decomposition, as a consequence of the catalytic activity of catalase. The activity was calculated from the first-order rate constant K (s^{-1}). For glutathione peroxidase, the technique of Flohé and Wolfgang (24) was used. Finally, the concentration of mitochondrial cytochromes b, $c+c_1$, and $a+a_3$ was evaluated by measuring the reduced minus oxidized difference spectra with a λ16-Perkin Elmer double-beam spectrophotometer, as previously described (25). 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} cm^{-1}, as previously described (25).

Statistical Analysis

Results are presented as means $\pm$ standard error of the mean (*SEM*) ($n = 20$ rats). One-way analysis of variance was used to test time-dependent changes, and the Bonferroni test was performed post hoc to evaluate differences among groups. Differences between dietary treatments for a same period of time were evaluated by the Student t test. A p value less than 0.05 was considered significant. Data were analyzed using SPSS statistical software (SPSS for Windows, version 11.0.1; 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 (Figure 1) was similar for both groups at the different time periods, with the highest value at 12 months of age.

Mitochondrial-Lipid Profile

Table 2 shows the effect of supplementation with CoQ_{10} (0.7 mg/kg/day) on heart mitochondrial-lipid profile. There were no statistically significant differences between groups for the different periods of time. In relation

Table 2. Effect of Supplementation With Coenzyme Q_{10} (CoQ_{10}) Throughout Life on Rat Heart Mitochondrial Fatty Acid Profile

	6 Months		12 Months		24 Months	
Fatty Acid Indexes	PUFA	PUFA+CoQ_{10}	PUFA	PUFA+CoQ_{10}	PUFA	PUFA+CoQ_{10}
Saturated fatty acids	62.9 ± 1.9	60.8 ± 1.2	72.0 ± 1.5^a	70.5 ± 1.3^a	68.5 ± 1.3^a	66.3 ± 1.8^a
Monounsaturated fatty acids	6.9 ± 1.0	7.6 ± 1.4	4.3 ± 0.5^a	4.0 ± 0.4^a	3.2 ± 0.3^a	3.4 ± 0.3^a
Polyunsaturated fatty acids (PUFA)	30.2 ± 1.5	31.5 ± 2.9	23.6 ± 1.2^a	25.2 ± 1.2^a	28.4 ± 1.0^b	30.6 ± 1.6^b
Unsaturation index (UI)	1.6 ± 0.1	1.8 ± 0.1	1.1 ± 0.1^a	1.3 ± 0.1^a	1.4 ± 0.0^b	1.5 ± 0.1^b

Note: Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p < .05$): [a]12 or 24 months vs 6 months for the same group; [b]24 months vs 12 months for the same group.

to time-dependent changes for each group, the percentage of saturated fatty acids was higher in both experimental groups at 12 and 24 months, values peaking at 12 months of age. Monounsaturated fatty acids were lower at advanced periods of time compared to 6 months. The percentage of PUFAs was lower at 12 months, although at 24 months the values were similar to those found at 6 months. Finally, the unsaturation index, a measurement of the average number of double bonds, followed the same pattern as PUFAs.

Hydroperoxide Levels

Hydroperoxide levels in heart mitochondria are shown in Figure 2. For all study periods, the PUFA group showed higher values than did the group supplemented with CoQ_{10}. In both groups, the rats killed at 6 months showed lower hydroperoxide levels than those killed at 12 and 24 months. The value obtained for this parameter at 24 months in PUFA group was similar to that found at 12 months, but in the group supplemented with CoQ_{10} this value was smaller. The hydroperoxide values after in vitro induction of oxidative damage in mitochondria from 24-month-old rats with AAPH are shown in Figure 3. The rats not supplemented with CoQ_{10} showed significantly higher values than did supplemented animals.

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

At 24 months, both groups showed higher levels of α-tocopherol than at 6 and 12 months (Table 3) and only the
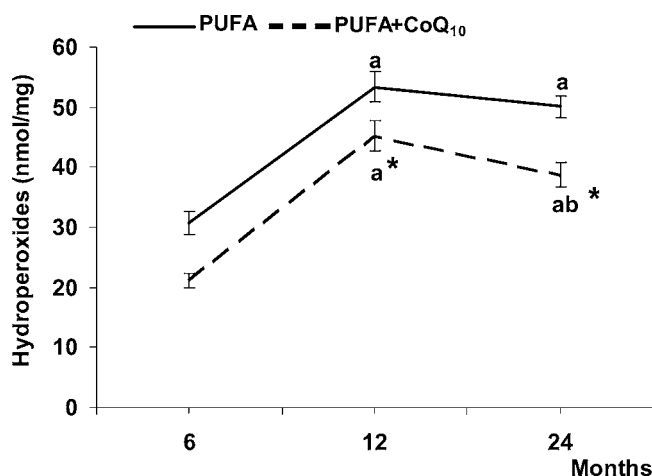
PUFA+CoQ_{10} group showed higher values at 12 months than at 6 months. Animals supplemented with CoQ_{10} showed higher values than did the nonsupplemented group at 6 and 12 months. CoQ_9 (Table 3) was higher in supplemented animals at 12 and 24 months. Regarding the aging effect, the PUFA group showed lesser values at 12 months compared to 6 months, and higher values at 24 months compared to 12 months. The PUFA+CoQ_{10} group showed only higher values at 24 months than at 6 months. CoQ_{10} (Table 3) was higher in the supplemented rats at 12 and 24 months. The aging effect in the PUFA group was observed only at 24 months, registering higher values than those at 6 and 12 months. For the supplemented group, higher values were found at 12 months compared to 6 months, and at 24 months compared to 6 and 12 months.

Catalase and Glutathione Peroxidase Activity

Both cytosolic antioxidant enzymes (Table 4) showed a gradual increase in their activities with age in the two experimental groups, with higher values at 12 months than at 6 months and at 24 months than at 6 and 12 months. The CoQ_{10}-supplemented rats showed higher values at 24 months for catalase and at 6 months for glutathione peroxidase.

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

The concentration of these cytochromes is shown in Table 4. In general, the highest values for all studied cytochromes in both groups were found at 12 months, when the
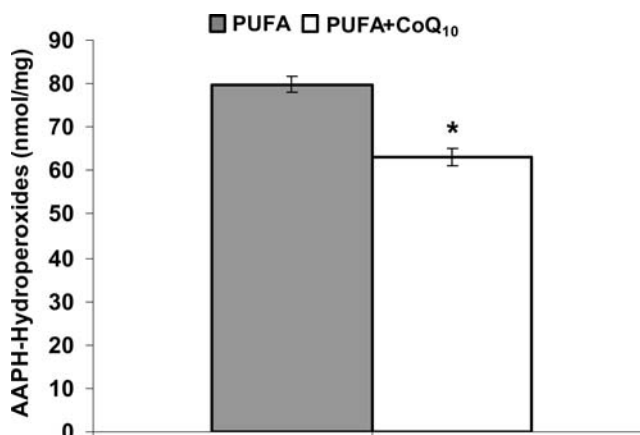

Figure 2. Effect of supplementation with coenzyme Q_{10} (CoQ_{10}) throughout life on hydroperoxide levels on rat heart mitochondria. Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p < .05$): *polyunsaturated fatty acid (PUFA) vs PUFA+CoQ_{10} for the same period of time. **a**, 12 or 24 months vs 6 months for the same group; **b**, 24 months vs 12 months for the same group.

Figure 3. Effect of supplementation with coenzyme Q_{10} (CoQ_{10}) throughout life on 2,2′-zobis (2-amidinopropane) hydrochloride (AAPH)-induced hydroperoxide levels in heart mitochondria of 24-month-old rats. Results are mean $\pm$ standard error of the mean of 20 animals. *Indicates statistical significances ($p < .05$) between groups.

Table 3. Effect of Supplementation With Coenzyme Q_{10} (CoQ$_{10}$) Throughout Life on Rat Heart Mitochondrial Levels of α-Tocopherol, Coenzyme Q_9, and CoQ$_{10}$, and on Activity of Cytosolic Antioxidant Enzymes, Catalase, and Glutathione Peroxidase

Antioxidants	6 Months		12 Months		24 Months	
	PUFA	PUFA+CoQ$_{10}$	PUFA	PUFA+CoQ$_{10}$	PUFA	PUFA+CoQ$_{10}$
α-Tocopherol, nmol/mg	1.1 ± 0.1	1.5 ± 0.1*	1.4 ± 0.2	2.1 ± 0.1*,a	3.8 ± 0.2a,b	3.6 ± 0.2a,b
Coenzyme Q_9, nmol/mg	4.9 ± 0.3	5.1 ± 0.3	3.9 ± 0.3a	6.0 ± 0.5*	5.6 ± 0.3b	7.0 ± 0.3*,a
CoQ$_{10}$, pmol/mg	456.2 ± 30.2	440.8 ± 31.9	403.2 ± 48.1	587.8 ± 39.7*,a	556.2 ± 52.0a,b	738.4 ± 57.9*,a,b
Catalase, k (s^{-1}/mg^{-1})	0.04 ± 0.01	0.06 ± 0.01	0.17 ± 0.03a	0.13 ± 0.02a	0.31 ± 0.05a,b	0.64 ± 0.11*,a,b
Glutathione peroxidase, U/mg	109.9 ± 13.5	179.6 ± 17.7*	230.7 ± 12.2a	204.3 ± 13.4	324.7 ± 22.6a,b	294.8 ± 31.1a,b

Note: Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p < .05$): *Polyunsaturated fatty acid (PUFA) vs PUFA+CoQ$_{10}$ for the same period of time: [a]12 or 24 months vs 6 months for the same group; [b]24 months vs 12 months for the same group.

nonsupplemented group registered the highest values ($p < .05$). At 24 months, cytochromes b and $c+c_1$ showed higher levels than at 6 months. However, cytochrome $a+a_3$ registered a lower concentration at 24 months than at 6 months, with the nonsupplemented group showing lower levels at 24 months than did the supplemented group for the same period.

CCO Activity

CCO activity is show in Figure 4. In both groups, the activity of this enzyme showed a gradual decrease with age, with lesser values at 12 months than at 6 months and at 24 months than at 6 and 12 months. For all study periods, the CoQ$_{10}$-supplemented animals showed higher values ($p < .05$) at 6, 12, and 24 months, respectively, than did their nonsupplemented counterparts.

DISCUSSION

The aim of this study was to investigate possible changes during aging in lipid peroxidation and functionality of heart mitochondria, depending on a lifelong supplementation or not with CoQ$_{10}$, in rats fed a diet rich in PUFAs throughout their life. Thus, we tried to test whether CoQ$_{10}$ supplementation might attenuate aging-related oxidative alterations observed in the heart of rats fed a PUFA-rich diet (12). Confirmation of that effect enables the preservation of beneficial aspects of PUFA on health, such as those related to cardiac diseases (6,9,10).

Prior to the analysis of possible effects of CoQ$_{10}$ supplementation, it was necessary to assess adaptation of the rats to such supplementation. In the present study, we found that supplementation led to higher CoQ$_{10}$ levels at 12 and 24 months of age in heart mitochondria. Considering that, besides plasma, liver, and spleen, most of the tissues are resistant to increased amounts of coenzyme Q from exogenous sources (26), we can state that our lifelong supplementation schedule based on a low CoQ$_{10}$ concentration (0.7 mg/kg/day) led to a good adaptation pattern.

The analysis of hydroperoxide levels as an indicator of lipid peroxidation reveals that, regardless of the dietary manipulation, cardiac mitochondrial lipid peroxidation was higher at 12 and 24 months than at 6 months of life. This result agrees with previously reported data (12,27,28), and is consistent with the free radical theory of aging of Harman (1). However, there were important differences between experimental groups with respect to this parameter. Animals fed a PUFA-rich diet and supplemented throughout life with CoQ$_{10}$ (0.7 mg/kg/day) showed lower values for all the periods studied. In addition, although both groups reached the highest hydroperoxide value at 12 months, the non-supplemented animals maintained this value until 24 months of age, whereas the supplemented animals were able to decrease it significantly. Thus, at 24 months of age, the supplemented animals reached a hydroperoxide value close to that of nonsupplemented animals at 6 months of life.

There are different ways through which CoQ$_{10}$ could be able to cause these differences in hydroperoxide levels, such as changes in the mitochondrial fatty-acid profile and therefore its susceptibility to lipid peroxidation, increasing antioxidant defenses or acting on the free radicals sources. We have studied, partially, all these ways.

It has been shown that modifications of the mitochondrial fatty-acid profile can modulate the susceptibility of the mitochondrial membranes to lipid peroxidation during aging (12). In this sense, administration of CoQ$_{10}$ has been shown capable of modifying the phospholipid fatty-acid composition in monocytes and granulocytes (15). However, under our experimental conditions, the study of the mitochondrial fatty-acid profile shows that lifelong CoQ$_{10}$ supplementation was not able to modify this parameter significantly. Both experimental groups registered higher levels of saturated fatty acids associated with age and lower monounsaturated

Table 4. Effect of Supplementation with Coenzyme Q_{10} (CoQ$_{10}$) Throughout Life on Rat Heart Mitochondrial Levels of Cytochromes $a+a_3$, b, and $c+c_1$ in Rat Heart Mitochondria

Cytochrome Levels	6 Months		12 Months		24 Months	
	PUFA	PUFA+CoQ$_{10}$	PUFA	PUFA+CoQ$_{10}$	PUFA	PUFA+CoQ$_{10}$
Cytochrome $a+a_3$, nmol/mg	0.29 ± 0.02	0.29 ± 0.03	0.62 ± 0.05a	0.50 ± 0.03*,a	0.18 ± 0.02a,b	0.28 ± 0.02*,b
Cytochrome b, nmol/mg	0.24 ± 0.01	0.22 ± 0.01	0.59 ± 0.04a	0.45 ± 0.03*,a	0.34 ± 0.02a,b	0.38 ± 0.03a
Cytochrome $c+c_1$, nmol/mg	0.32 ± 0.02	0.31 ± 0.01	1.02 ± 0.08a	0.84 ± 0.02*,a	0.60 ± 0.07a,b	0.49 ± 0.04a,b

Notes: Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p < .05$): *Polyunsaturated fatty acid (PUFA) vs PUFA+CoQ$_{10}$ for the same period of time: [a]12 or 24 months vs 6 months for the same group; [b]24 months vs 12 months for the same group.

fatty acids. This result agrees with previous data reported by our research group (12) for heart in aged rats fed a PUFA-rich diet.

The enzymatic and nonenzymatic components of the antioxidant system studied followed a similar response in both groups with higher levels related to age (except coenzyme Q levels in nonsupplemented animals). Previously, increased cardiac antioxidant levels associated with age have been reported (12,29,30). It has been postulated that a compensatory antioxidant defense system exists to counteract oxidative stress associated with aging, and therefore some antioxidants would be expected to accumulate, such as α-tocopherol, at the sites in which it is needed (30).

Despite the similar pattern followed by both experimental groups with respect to the antioxidant system, there were important differences between them. For the nonenzymatic antioxidants, α-tocopherol levels were higher, at least at 6 and 12 months of age, in the supplemented animals, and this group also showed a gradual increase associated with age in coenzyme Q content (both CoQ_9 and CoQ_{10}), whereas the nonsupplemented group showed no age-associated changes in coenzyme Q content. Results from the present study agree with those of other groups. For example, Kwong and colleagues (26) reported that supplementation with CoQ_{10} (150 mg/kg/day) for 13 weeks increased the level of this coenzyme and its homologue, CoQ_9. Also, Lass and colleagues (31), working with mice, demonstrated that a similar supplementation (123 mg/kg/day) resulted in a concomitant increase in the level of α-tocopherol in heart mitochondria. It is of interest to note that, although our study did not use high CoQ_{10} dosages, as other studies (26,31), we found the mentioned increase in α-tocopherol and CoQ_9 concentration. The aforementioned suggest that both acute supplementation of high dosages and chronic administration of low dosages of oQ_{10} are able to induce levels of α-tocopherol and CoQ_9.

It is noteworthy that the higher levels of α-tocopherol shown in nonsupplemented 24-month-old animals were not accompanied by a similar pattern in the coenzyme Q contents, and several results indicate that these two lipid-soluble antioxidants are in fact more efficient when acting together (14). Thus, it seems to indicate that old mitochondrial membrane from supplemented animals could be more prepared against oxidative injury than is that from nonsupplemented animals. To test this hypothesis, we have induced in vitro oxidative stress against these old membranes using the potent free radical generator AAPH. The results indicate that, despite the similar PUFA content of these membranes (and hence a theoretically similar susceptibility to oxidative damage), mitochondrial membranes from old supplemented animals registered lower AAPH-induced hydroperoxide values and therefore proved to be more resistant against oxidative damage. This result is important because one of the problems with aged cardiac mitochondria is that they are more susceptible to oxidative damage and therefore predispose the heart to greater injury during oxidative insults (3).

With respect to enzymatic antioxidants, both showed the highest activities at 24 months, which we believe is due to an increase in free radical generation at this age, because it has been suggested to be the most important factor for the
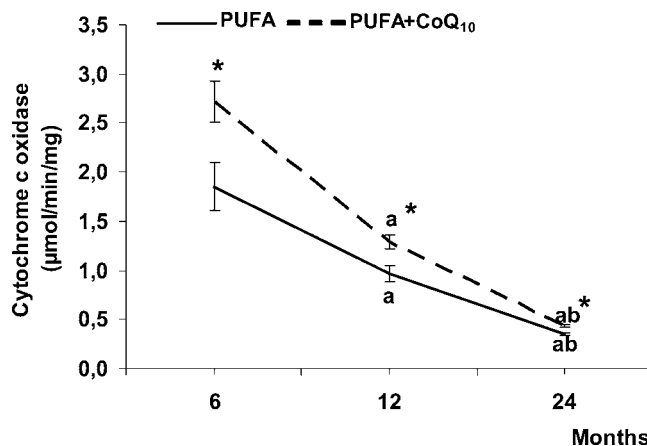

Figure 4. Effect of supplementation with coenzyme Q_{10} (CoQ_{10}) throughout life on cytochrome c oxidase activity in rat heart mitochondria. Results are mean $\pm$ standard error of the mean of 20 animals. Statistical significances ($p <$ .05): *polyunsaturated fatty acid (PUFA) vs PUFA+CoQ_{10} for the same period of time. **a**, 12 or 24 months vs 6 months for the same group; **b**, 24 months vs 12 months for the same group.

induction of the activities of these enzymatic antioxidants (2). Increased catalase activity has been associated with greater resistance to oxidative damage (2), and it has been suggested that catalase can function as a major pathway for detoxifying H_2O_2 in cardiac tissue (32). With our data alone, it is difficult to ascertain the mechanisms by which CoQ_{10} supplementation can increase the activity of this antioxidant enzyme, and thus this issue needs further study. Nevertheless, it has been showed that CoQ_{10} administration was able to increase in mice heart the gene expression of glutathione S-transferase, another element of the antioxidant system family (5). We can not rule out that a similar interaction could exist between CoQ_{10} and catalase.

The third possible way by which coenzyme Q could modulate lipid peroxidation levels is by acting on the free radical source, the mitochondrial electron transport chain (METC). Aging has been associated with decay in mitochondrial respiratory chain activity (28) and an increased rate of mitochondrial $O_2^{\cdot-}/H_2O_2$ generation (33). In addition, it has been observed that CCO activity decreases with age (16), and this decrease has been correlated with the concomitant increase in the flux of mitochondrial $O_2^{\cdot-}$ and H_2O_2 generation (33). Thus, it has been suggested that there could be an obstruction or partial blockage of electron flow through some respiratory complexes associated with age; therefore, a greater number of free radicals could be generated at these sites along the METC (12,33). Results related to cytochrome content and CCO activity obtained in our study agree with the aforementioned. We have detected higher levels of cytochrome (at least in cytochrome b and $c+c_1$) associated with age in parallel to lower CCO activity. As has been suggested, this situation could give rise to a possible partial blockage of electron flow and therefore higher free radical production. In fact, we have found a good inverse correlation ($r = -561$; $p <$ .01 for nonsupplemented animals and $r = -0.399$; $p <$ .01 for supplemented animals) between hydroperoxide content and CCO activity. Thus, taking together the fact that the antioxidant enzymes activity increase

at 24 months compared with 12 months and the fact that CCO activity was the lowest at the oldest age, despite of the similar hydroperoxides values between the two ages, we can suggest that at 24 months of life the generation of free radicals was higher than at 12 months and therefore the oxidative insult.

In contrast, CoQ_{10} supplementation appears to improve these age-associated alterations or changes in the METC. Besides a slight effect on cytochromes content, mostly at 12 months of life, CoQ_{10} supplementation can increase CCO activity, with respect to that in nonsupplemented animals, throughout life. In addition, CoQ_{10} supplementation led to a higher content of this molecule and its homologue (CoQ_9) at the mitochondrial membrane level (nonsupplemented animals did not show this effect). This should be important for the METC function, because, as has been widely reported, under physiological conditions, the mitochondrial concentration of coenzyme Q does not saturate the enzymes that use it as a substrate (34). It should also be noted that CoQ_{10} supplementation could modulate the CCO activity either by its effects on lipid peroxidation (allowing a better environment for this activity), and/or by its effect on the inducement of several genes encoding some subunits of this METC complex (5).

In conclusion, on the basis of these results we can suggest that previously reported positive effects of CoQ_{10} supplementation on mean and maximal life span of rats fed a PUFA-rich diet might be a consequence, at least in part, of a lower oxidative stress level and perhaps, to a minor extent, to a smaller decrease in mitochondrial function. In addition, these results could lead to a better understanding of the beneficial effects on heart function after administration of CoQ_{10}, although investigation of different diets and further studies in humans are still needed to elucidate this protective role of CoQ_{10}.

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