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Changes in mitochondrial and microsomal rat liver coenzyme Q_9 and Q_{10} content induced by dietary fat and endogenous lipid peroxidation

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The influence of different kinds of dietary fat (8%) and of endogenous lipid peroxidation with regard to coenzyme Q_9 (CoQ_9) and coenzyme Q_{10} (CoQ_{10}) concentrations in mitochondria and microsomes from rat liver has been investigated by means of an HPLC technique. Although the different diet fats used did not produce any effect on microsomes, it was possible to show that each experimental diet differently influenced the mitochondrial levels of CoQ_9 and CoQ_{10}. The highest mitochondrial CoQ content was found in case of a diet supplemented with corn oil. An endogenous oxidative stress induced by adriamycin was able to produce a sharp decrease in mitochondrial CoQ_9 levels in the rats to which corn oil was administered. The results suggest that dietary fat ought to be considered when studies concerning CoQ mitochondrial levels are carried out.

Coenzyme Q; HPLC; Liver mitochondrion and microsome; Malondialdehyde; Olive oil; Corn oil

1. INTRODUCTION

Ubiquinone (Coenzyme Q, CoQ) plays an important role in mitochondrial respiration, an aspect which has been studied in great detail during the past 20 years [1]. In recent years it has become clear that ubiquinone also has an important role as antioxidant, and this might explain its broad distribution in subcellular membranes [2,3]. Ubiquinone may react with free radicals, serving thereby as an effective protection against a member of harmful effects [4,5]. Moreover ubiquinone prevents the liberation of fatty acids from phospholipids and suppresses fatty acid peroxidation as a result of its ordering effect on phospholipids [6,7].

The concentration of CoQ in rat liver is known to vary depending on the nutritional status or the environmental conditions of the animal [8]. Deficiency of vitamin A [9], thyrotoxicosis [10], cold exposure [11] and coenzyme Q feeding [12] increased the coenzyme Q content in the liver. Coenzyme Q levels decreased in the livers of rats that were starved [13] or given diets deficient in protein [14].

Few studies in vivo have been made comparing CoQ levels and the possible effects induced by dietary fat on these levels. Dietary fats are known to influence lipid composition of body membranes, depending on their constitutive unsaturated and saturated fatty acid contents [15,16]. These changes modify membrane physical properties which in turn modulate membrane-associated enzymes, such as acyl-CoA-desaturases [17,18], succinate-cytochrome *c* reductase, F_1F_0-ATPase [19]. Also poly-unsaturated side chains of membrane lipids are especially susceptible to free radical-initiated oxidation, which can be generated either by the metabolism of xenobiotics or by normal cellular metabolism [20,21]. As CoQ is probably located in the inner moiety of the membrane bilayer [1,22,23], its membrane concentration could be modified after modifications of the lipid environment or as a result of both dietary fat contribution or peroxidative phenomena that take place at membrane level.

For these reasons we have undertaken a study in order to gain an understanding on whether different fatty acid contribution in the diet, with the same degree of unsaturation but distinct diene/monoene ratios (olive oil and corn oil) and of unsaponifiable material content (virgin olive oil and refined olive oil) and a subsequent peroxidative induction of membrane phospholipids, could affect the CoQ content both in mitochondria and in microsomes of the liver of rats that have been fed for 16 weeks with these controlled diets.

2. MATERIALS AND METHODS

All chemicals used were purchased from Sigma Chemical Co. Ltd., St. Louis, MO 63178, USA and all solvents were pure reagents of Merck and Carlo Erba. Different standard CoQ homologues were kind gifts from Eisai Co., Tokyo, Japan; they were stored as solutions in absolute ethanol at −20°C at concentrations ranging between 1 and 10 mM as determined spectrophotometrically at 275 nm using extinction coefficients, typical of each homolog [24,25] and according to Mayer and Isler [26].

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Table I

Fatty acid, CoQ_9 and CoQ_{10} composition of experimental diets

	Virgin olive oil	Olive oil	Corn oil
% 16:0	8.92	11.32	12.6
% 16:1(n-7)	1.06	1.28	0.2
% 18:0	1.97	2.17	1.9
% 18:1 (n-9)	78.73	75.82	24.1
% 18:2 (n-6)	8.36	9.17	60.1
% 18:2(n-3)	0.96	0.34	1.0
% Saturated	10.89	13.49	14.6
% Unsaturated	89.11	86.61	85.4
% Monoene	79.79	77.10	24.3
% Diene	9.32	9.51	60.1
Diene/monoene	0.10	0.12	2.4
CoQ_9 (nmol/g)	≤ 3.85	3.85	234.1[a]
CoQ_{10} (nmol/g)	≤28.10	28.10	15.1[a]

Fatty acids are designed by the number of carbons followed by the number of double bonds. The positions of the first double bond relative to the methyl (n) end of the molecule is also indicated.
[a]Reference 42.

Male Wistar rats weighing 50–55 g were randomly assigned to six groups, each containing 12 animals. During 16 weeks each group was fed a basal diet containing 8% fat (Dottori Piccioni, Brescia, Italy), but in each of two groups the diet contained different fats: 'virgin' olive oil (groups VO and VO + ADR), 'refined' olive oil (RO and RO + ADR) and corn oil (CO and CO + ADR). Adriamycin (ADR) (a kind gift from Farmitalia, Milano, Italy) at a dose 20 mg/kg/day was administered intraperitoneally to the animals of VO + ADR, RO + ADR and CO + ADR groups during the last four days of the treatment period. The intraperitoneal administration was always performed at 9.00 a.m. The fatty acid composition of the three diets is shown in Table I.

Diets were stored at 4°C, and rats were fed fresh food daily. Food and water were provided ad libitum. A 12 h dark/12 h light cycle was maintained in the room, and the room temperature was 22 ± 1°C. After consuming the experimental diet for 16 weeks, the animals of each group were decapitated and exsanguinated and the livers were rapidly removed. The animals were sacrificed at 9.00 a.m. in order to avoid any circadian influence.

Rat liver mitochondria were isolated by the method of Fleischer et al. [27]. After mitochondrial isolation the remaining supernatant was recentrifuged at 105 000 × g for 60 min in order to obtain the microsomal fraction.

The mitochondria and microsomes were assayed for ubiquinone content by reverse-phase HPLC analysis as previously indicated [28], after extraction with methanol and light petroleum using the method of Kröger [29]. Separations were performed using a Spherisorb S5 ODS I 25 × 0.46 cm column with a guard column containing the same material as the main column; the mobile phase was prepared by dissolving 7.0 g $NaClO_4 \cdot H_2O$ in 1000 ml ethanol/H_2O/70% $HClO_4$ (969:30:1). The flow rate was 1 ml/min. Measurements were performed at 25°C. The HPLC system was a Waters Data Module M730-Model 721 Programmable System Controller equipped with Lambda-Max Model 481 LC Spectrophotometer. Peroxidation of mitochondrial and microsomal phospholipids was monitored measuring malondialdehyde (MDA) by the thiobarbituric acid method [30]. The protein concentration of all samples was determined with the method of Lowry et al. [31] with bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

As shown in Table II, ingestion of a diet with 8% virgin olive oil, refined olive oil and corn oil as sole fat source did not induce any difference of both CoQ_9 and CoQ_{10} microsomal fraction content. However, when during the last four days of the experimental treatment a daily intraperitoneal dose of ADR (an anthracycline antibiotic that enhances lipid peroxidation either in microsomes [32] or mitochondria [33] and inactivates respiratory chain enzymes [34,35]) was administered, a statistically significant increase of CoQ_9 concentration in microsomes ($P < 0.05$), in CO + ADR and RO + ADR groups was found, with an enhancement of 250% with regard to the same experimental groups that were not treated with ADR. In microsomes ADR did not induce changes in the CoQ_{10} content, with the only exception of the CO + ADR group, where it increased.

The measurement of MDA, one of the end products of lipid peroxidation, confirmed that the dietary fat induced higher phospholipid degradation in both microsomal and mitochondrial membranes in CO and RO groups (Table II). Adriamycin induced a statistically significant increase of MDA microsomal and mitochondrial content in all studied groups. However the MDA amount reached in the VO + ADR group is lower than that produced by the fat diet alone in CO and RO groups.

Microsomal CoQ_9 increase might be related, according to Demant calculations [36], with the possibility that one molecule of reduced ubiquinone could be able

Table II

Effect of dietary fat and endogenous lipid peroxidation (induced by adriamycin) on CoQ_9, CoQ_{10} and malondialdehyde (MDA) content in rat liver mitochondria and microsomes

	Mitochondria			Microsomes		
GROUP	CoQ_9 (pmol/mg)	CoQ_{10} (pmol/mg)	MDA (nmol/mg)	CoQ_9 (pmol/mg)	CoQ_{10} (pmol/mg)	MDA (nmol/mg)
VO	2544 ± 326^{bc}	378 ± 70^{b}	2.75 ± 0.13^{a}	238 ± 80^{a}	78.5 ± 14.7^{ab}	2.41 ± 0.50^{a}
VO + ADR	2173 ± 106^{ab}	231 ± 20^{a}	3.61 ± 0.32^{b}	289 ± 79^{a}	53.6 ± 14.9^{a}	3.28 ± 0.43^{ab}
CO	3343 ± 267^{d}	418 ± 43^{b}	3.64 ± 0.33^{b}	255 ± 40^{a}	62.0 ± 10.5^{a}	2.76 ± 0.25^{a}
CO + ADR	2211 ± 219^{ab}	243 ± 30^{a}	4.69 ± 0.31^{c}	640 ± 100^{b}	97.0 ± 16.8^{bc}	3.67 ± 0.27^{bc}
RO	2426 ± 216^{bc}	303 ± 27^{ab}	3.98 ± 0.12^{b}	318 ± 44^{a}	156.2 ± 61.1^{c}	3.78 ± 0.34^{bc}
RO + ADR	2845 ± 264^{cd}	337 ± 45^{b}	4.92 ± 0.27^{c}	768 ± 103^{b}	115.5 ± 11.1^{c}	4.20 ± 0.25^{c}

Values are means ± SEM per mg protein, $n = 8$. Means within a column not followed by the same superscript letter are significantly different at $P < 0.05$ or less, by a least significant difference analysis.

to protect more than 1300 molecules of poly-unsaturated fatty acids (650 nmol/mg protein) as was suggested in the case of peroxidation initiated by the adriamycin-Fe^{3+} complex.

On the other hand, in mitochondria, the sole diet produced outstanding differences. While CoQ_9 levels were extremely similar between VO and RO groups (with a concentration of 2.54 ± 0.32 nmol/mg mitochondrial protein and 2.42 ± 0.21 nmol/mg mitochondrial protein, respectively), it was significantly increased ($P<0.05$) in the CO group (3.34 ± 0.26 nmol/mg mit. prot.). However, when these groups underwent an oxidative stress induced by ADR, a significant decrease of CoQ_9 content in the CO + ADR group but not in the VO + ADR group was found.

Dietary fats are known to produce changes both in microsomal and mitochondrial protein content; according to this possibility some of the previous results could not reflect the real CoQ concentration. If data are referred to as nmol of mitochondrial or microsomal CoQ per g of wet liver (Fig. 1), it is observed that CoQ_9 and CoQ_{10} levels in either mitochondria or microsomes do not reflect dietary CoQ contribution (Table I).

In fact, taking into account that the CO group has taken in 60-fold CoQ_9 with respect to RO and VO groups, the three groups display the same CoQ_9 level in microsomes and only the RO group presents higher CoQ_9 and CoQ_{10} concentrations with respect to VO and CO groups ($P<0.05$). Mitochondrial CoQ_9 content was completely different in each experimental group, with the highest level in CO (56.44 ± 6.45 nmol/g wet liver) and the lowest amount in VO (20.87 ± 3.35 nmol/g wet liver).

During the last four experimental days, the rats of the CO + ADR group took on about 700 nmol of CoQ_9 while, in the same period, they lost 25 nmol of mitochondrial Q_9 per g of wet liver, meaning a total loss of 300 nmol of CoQ_9 for the whole liver mitochondrial fraction. It is evident that the CoQ_9 contribution of this diet might not be sufficient for maintaining the normal membrane levels. A damage in the metabolic CoQ biosynthetic pathway induced by the oxidative stress is unlikely because in VO and RO groups the dietary CoQ_9 contribution was 60 times lower but no decrease of CoQ_9 mitochondrial content was observed. The most likely explanation for the CoQ loss in the CO + ADR group might be in an enhanced destruction.

Fatty acids in animal tissues reflect the fatty acids in the animal's diet [15,16]. The effect is most pronounced when the diet is high in a dietary fat consisting primarily of poly-unsaturated fatty acids [37]. The administration of diets particularly rich in linoleic and oleic acids also induces a clear enhancement of linoleic and oleic content in the phospholipids of microsomes [38,39] and mitochondria, respectively [37]. These changes in phospholipid dienoic/monoenoic ratios are correlated with membrane fluidity modifications [39]. It could be

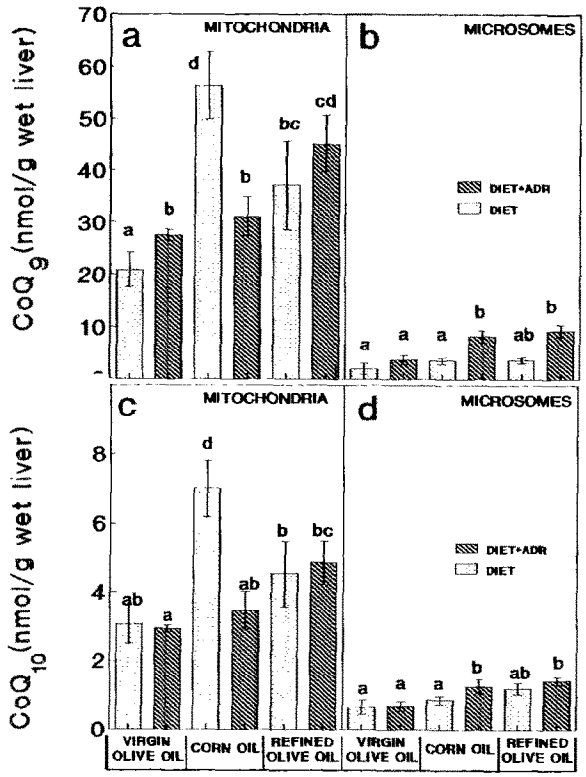

Fig. 1. Effect of dietary fat and endogenous lipid peroxidation (induced by adriamycin) on CoQ_9 and CoQ_{10} content in rat liver mitochondria and microsomes. Values are means ± SEM per g wet liver, $n=8$. Means of values not followed by the same letter (simple letter for stippled bars, circled letter for hatched bars) are significantly different at $P<0.05$ or less, by a least significant difference analysis.

possible that, in mitochondria, a mechanism exists to modify CoQ_9 and CoQ_{10} levels as a direct function of the dienoic/monoenoic ratio.

This hypothesis would be in accordance with the high level of CoQ in the CO group and also with the dramatic fall after adriamycin (CO + ADR group). In fact, Sevanian et al. [40] have indicated that lipid peroxidation produces a general increase in membrane viscosity which is associated with enhanced phospholipase A2 attack. In our studies, the lowering of mitochondrial CoQ_9 and CoQ_{10} content in CO + ADR group, that underwent an oxidative stress with ADR, could be ascribed to an increase of membrane viscosity [40]. On the contrary, an increase of CoQ_9 and CoQ_{10} was found in microsomes from all groups after oxidative stress. However the CoQ concentration in microsomes was so low that microsomal viscosity is unlikely to represent an obstacle for CoQ increase.

We have previously collected experimental evidence [23,41] that CoQ levels in mitochondria are physiologically not saturating for a maximum electron-transfer rate. Dilution of the mitochondrial inner membrane with excess phospholipids, so that the CoQ con-

centration in the membrane is lowered, proportionally reduces electron transfer turnover from NADH or succinate to cytochrome *c* [23,41]. On the contrary, the addition of exogenous CoQ enhances the respiratory turnover above the physiological rate but without reaching theoretical V_{max}, owing to the limited miscibility of ubiquinones with the membrane phospholipids [23,41]. The results of this study suggest that this miscibility may depend on the lipid composition of the membrane.

These results let us conclude that the mitochondrial CoQ content is dependent upon the unsaturation level of dietary fats. The data suggest to consider dietary fats when both in vitro and in vivo studies are carried out with the aim of studying mitochondrial CoQ content and behaviour.

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