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The roles of coenzyme Q_{10} and vitamin E on the peroxidation of human low density lipoprotein subfractions

(fatty acids)

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ABSTRACT **The aim of our study was to investigate the relationships between the levels of coenzyme Q_{10} (CoQ_{10}) and vitamin E and the levels of hydroperoxide in three subfractions of low density lipoproteins (LDL) that were isolated from healthy donors. LDL_3, the densest of the three subfractions, has shown statistically significant lower levels of CoQ_{10} and vitamin E, which were associated with higher hydroperoxide levels when compared with the lighter counterparts. After CoQ_{10} supplementation, all three LDL subfractions had significantly increased CoQ_{10} levels. In particular, LDL_3 showed the highest CoQ_{10} increase when compared with LDL_1 and LDL_2 and was associated with a significant decrease in hydroperoxide level. These results support the hypothesis that the CoQ_{10} endowment in subfractions of LDL affects their oxidizability, and they have important implications for the treatment of disease.**

Atherogenic changes in low density lipoproteins (LDL) are likely related to oxidative events that can occur in some circulation districts, such as the subendothelial space (1). Oxidized LDL are believed to contribute to the atherogenic process by altering chemotaxis of monocytes and monocyte-derived macrophages, which can engorge the oxidatively modified particles through the scavenger receptor-mediated mechanism (2, 3). Furthermore, they promote endothelial damage and other changes also associated with atherogenesis (4). Although a relationship between oxidizability and chemical composition of LDL has been widely investigated, the relative importance of different constituents in preventing oxidation of LDL remains uncertain (5, 6). It was recently suggested that antioxidants in LDL were one of the crucial factors in determining the propensity of LDL to oxidation (7). In particular, coenzyme Q_{10} (CoQ_{10}), an important antioxidant associated with LDL, was reported to protect the particles of lipoprotein more efficiently than does vitamin E (8, 9). Recall that particles of LDL are heterogeneous and differ in size, density, and chemical composition. Recent reports have shown an increased susceptibility to oxidation in the most dense of three LDL subfractions (10, 11); this subfraction is more abundant in individuals having coronary heart disease than in healthy and normal subjects (12, 13).

To clarify the influence of an endogenous antioxidant on the susceptibility to oxidation, we assayed the levels of peroxidation products in the LDL subfractions in relation to the levels of CoQ_{10} and vitamin E. We assayed the levels of hydroperoxide in the LDL subfractions, both under basal conditions and after an exogenous radical insult, in a group of normal young volunteers before and after oral supplementation with CoQ_{10}. Because the susceptibility of LDL particles also depends on their levels of an oxidizable substrate (14), the relative amounts of free fatty acids in each LDL subfraction were also considered.

MATERIALS AND METHODS

Subjects. The subjects enrolled in this study were 10 healthy male volunteers (mean age, 26 ± 4) who were not taking any drugs. They were members of a religious community, and they had a common diet and life-style. The study was approved by the appropriate university committee and after informed consent was obtained. Plasma was collected into EDTA-containing tubes before and after oral supplementation with CoQ_{10} at a dosage of 100 mg per day for 30 days.

Isolation of LDL Subfractions. Three LDL subfractions (LDL_1 ρ = 1.030–1.033 g/ml; LDL_2 ρ = 1.033–1.040 g/ml; LDL_3 ρ = 1.040–1.045 g/ml) were isolated from freshly collected plasma by means of density-gradient ultracentrifugation during 20 hr at 14°C and at 36,000 rpm (10). After isolation, the LDL subfractions were dialyzed for 24 hr at 4°C against 0.01 M phosphate buffer (pH 7.4) containing 0.16 M NaCl and a chelating resin (Chelex 100, Sigma).

Plasma and Lipoprotein Composition. The lipid patterns in whole plasma and in each subfraction were assessed with commercially available enzymatic kits on the Synchron–Beckmann analyzer (15, 16).

Protein levels were determined by the method of Lowry *et al.* (17).

Fatty acid levels in plasma and in the LDL subfractions were determined by capillary gas chromatography after transesterification, as described by Lepage and Roy (18), on a Hewlett–Packard 5890 series II chromatograph, equipped with a 60 m × 0.32 mm i.d. fused-silica capillary column.

Determination of CoQ_{10} and Vitamin E. The levels of CoQ_{10} (in the oxidized form) and vitamin E in the LDL subfractions and in the plasma of each subject were determined using HPLC, as described (19).

Assay of Hydroperoxide. The lipid hydroperoxides were determined before and after peroxidation as induced by 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), a water-soluble azo compound, which thermally decomposes to produce peroxyl radicals (20). The assay was based on ferrous ion oxidation in the presence of xylenol orange (21). Samples (100 μg of LDL protein) of each subfraction were exposed to 2 mM AAPH (final concentration) for 120 min at 37°C in a shaking water bath. Lipid hydroperoxides were estimated spectrophotometrically at 560 nm on a Beckmann model spectrophotometer. Susceptibility to peroxidation was expressed as the increase in hydroperoxides after exposure to AAPH and in respect to basal hydroperoxides.

Statistical Analysis. The results are expressed as means ± SD. The mean differences in composition and oxidation

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Abbreviations: CoQ_{10}, coenzyme Q_{10}; LDL, low density lipoprotein(s); AAPH, 2,2-azobis(2-amidinopropane) dihydrochloride.

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Table 1. Lipid, CoQ_{10}, and vitamin E levels and fatty acid composition in plasma

Substance	Plasma composition
Cholesterol, mg/dl	162.8 ± 28.3
Triglycerides, mg/dl	88.4 ± 21.8
Phospholipids, mg/dl	174.8 ± 24.7
CoQ_{10}, μg/ml	
Before supplementation	0.6 ± 0.1
After supplementation	1.5 ± 0.3*
Vitamin E, μg/ml	10.5 ± 3.1
Fatty acids, %	
14:0	
16:0	21.7 ± 0.7
16:1	1.5 ± 0.3
18:0	8.3 ± 1.1
18:1	17.4 ± 0.61
18:2	32.7 ± 1.0
20:4	6.6 ± 0.5
Unsaturated	67.1 ± 1.5
Saturated	32.9 ± 1.5
Polyunsaturated	45.8 ± 2.7
Unsaturation index	4.3 ± 0.3

*Data are statistically significant at $P < 0.01$.

parameters in the LDL subclasses were evaluated by ANOVA using STAT VIEW II ANOVA tests on a Macintosh LC computer.

RESULTS

Whole Plasma. The levels of lipids, CoQ_{10}, and vitamin E in plasma are shown in Table 1. No significant difference in the lipid patterns was found after CoQ_{10} supplementation in comparison with the basal levels.

LDL Subfractions Before CoQ_{10} Supplementation. The results on the lipid and apoprotein compositions are in accordance with de Graaf *et al.* (10). The fatty acid composition of LDL subfractions showed a higher percentage of the unsaturated fatty acids in LDL_1 and LDL_2 subfractions as compared with LDL_3 subfraction (Table 2). In particular, the levels of arachidonic and linoleic acids were significantly higher in LDL_1 and LDL_2 subfractions in comparison with LDL_3 subfraction, and LDL_3 subfraction had the highest level of palmitic acid. The levels of CoQ_{10} and vitamin E were significantly lower in LDL_3 subfraction than in LDL_1 and LDL_2 subfractions in all subjects ($P < 0.01$; Figs. 1 and 2). This study of LDL peroxidation showed increased "basal" levels of hydroperoxides in LDL_3 subfraction with respect to LDL_1 and LDL_2 subfractions ($P < 0.01$; Fig. 3).

After exposure to AAPH, the levels of hydroperoxide were slightly higher in LDL_3 in comparison with the other two subfractions, although the differences were not significant

Table 2. Fatty acid composition of LDL subfractions

Fatty acid	Fatty acid composition, %		
	LDL_1	LDL_2	LDL_3
16:0	21.6 ± 0.7	20.4 ± 1.2	21.2 ± 0.9
16:1	1.5 ± 0.3	2.3 ± 0.8	0.8 ± 0.1
18:0	9.5 ± 1.1	11.8 ± 5.2	41.4 ± 12.3*
18:1	17.4 ± 0.6	16.1 ± 2.5	8.5 ± 3.6
18:2	33.7 ± 1.0	30.1 ± 4.4	13.1 ± 6.7*
20:4	6.6 ± 0.5	5.7 ± 0.9	2.0 ± 0.2†
Unsaturated	67.2 ± 1.5	64.4 ± 5.2	32.2 ± 14.2*
Saturated	32.8 ± 1.5	35.6 ± 5.2	67.8 ± 14.2*
Polyunsaturated	44.5 ± 2.8	43.9 ± 2.9	22.5 ± 4.4
Unsaturation index	4.3 ± 0.3	4.2 ± 0.6	1.4 ± 0.6*

*Data are statistically significant at $P < 0.01$.
†Data are statistically significant at $P < 0.05$.

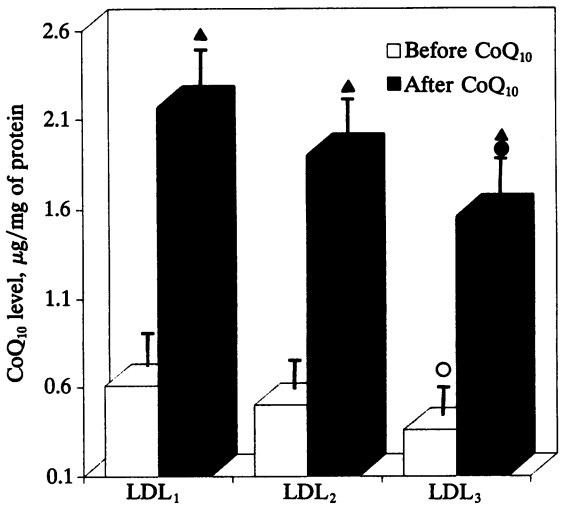

FIG. 1. CoQ_{10} levels in LDL subfraction before and after CoQ_{10} supplementation. ○, LDL_3 vs. LDL_1 and LDL_2, $P < 0.001$; ●, LDL_3 vs. LDL_1 and LDL_2, $P < 0.01$; ▲, LDL_1, LDL_2, and LDL_3 vs. LDL_1, LDL_2, and LDL_3 after CoQ_{10} supplementation, $P < 0.01$.

(Fig. 4). No relevant differences in lipid peroxidation susceptibility were found among the three LDL subfractions. Susceptibility was considered as an increase in levels of lipid hydroperoxides after incubation with AAPH in comparison with basal levels (Fig. 5).

LDL Subfractions After CoQ_{10} Supplementation. No significant difference was found in lipoprotein composition after CoQ_{10} supplementation. The levels of CoQ_{10} were significantly increased in all three subfractions ($P < 0.01$) of every subject. LDL_3 showed the highest increase in the level of CoQ_{10} after supplementation, and the posttreatment level was lower than the corresponding levels in LDL_1 and LDL_2 subfractions (Fig. 1).

A small increase in the level of vitamin E was observed in all three subfractions and was statistically significant in LDL_2 and LDL_3 subfractions ($P < 0.05$; Fig. 2). The basal levels of hydroperoxide showed a different trend in the three LDL subfractions. In LDL_1 and LDL_2 subfractions the basal hydroperoxide levels were unchanged. LDL_3 subfraction showed a significant decrease in hydroperoxide levels when compared with the levels before CoQ_{10} supplementation (Fig. 3). A

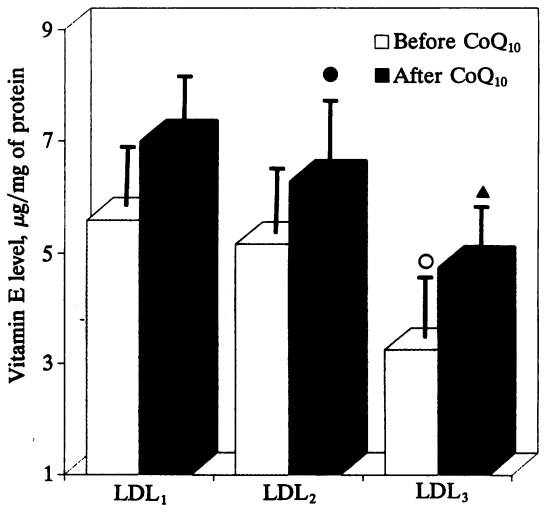

FIG. 2. Vitamin E levels. ○, LDL_3 vs. LDL_1 and LDL_2 before CoQ_{10}, $P < 0.001$; ●, LDL_2 before CoQ_{10} supplementation, $P < 0.001$; ▲, LDL_3 vs. LDL_2 and LDL_3 before CoQ_{10} supplementation, $P < 0.05$.

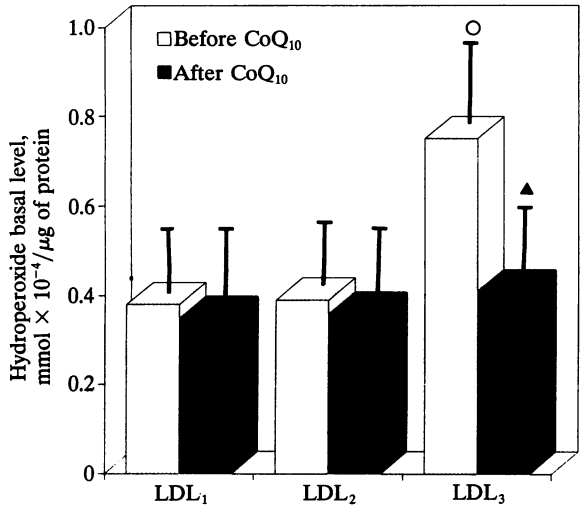

FIG. 3. Hydroperoxide basal levels. ○, LDL_3 vs. LDL_1 and LDL_2 before CoQ_{10} supplementation, $P < 0.001$; ▲, LDL_3 basal levels before vs. after CoQ_{10} supplementation, $P < 0.001$.

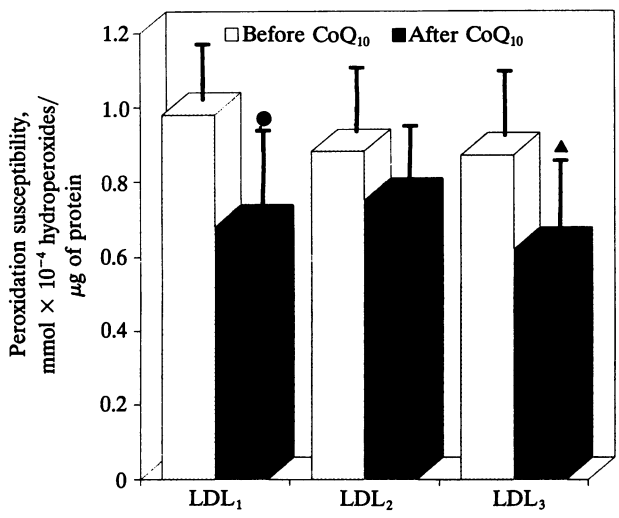

FIG. 5. Peroxidation susceptibility. ●, LDL_1 before vs. LDL_1 after CoQ_{10}, $P < 0.05$; ▲, LDL_3 before vs. LDL_3 after CoQ_{10}, $P < 0.01$.

decrease of the hydroperoxide levels (after AAPH-induced oxidation) was seen in all three subfractions, but there was statistical significance in the difference for only LDL_3. The decrease of susceptibility to oxidation was significant for LDL_1 and LDL_3 (Figs. 4 and 5).

DISCUSSION

Elevated levels of LDL_3 are commonly found in patients with coronary artery disease or at high risk for coronary artery disease. The increased susceptibility of LDL_3, as found by de Graaf *et al.* (10), might be, in part, responsible for the atherogenic status of this subpopulation. The higher oxidative susceptibility of LDL_3 might also be from the lower endogenous concentrations of antioxidants as compared with LDL_1 and LDL_2 subfractions (8). Our study demonstrates that LDL_3 contains lower levels of CoQ_{10} and vitamin E, which are associated with higher hydroperoxide levels in comparison with LDL_1 and LDL_2 subfractions.

We cannot demonstrate that the hydroperoxide levels represent endogenous levels because they are often regarded as arising from consequences of *in vitro* manipulation of LDL during the isolation. We might reasonably suppose that LDL_3 is more susceptible to hydroperoxide formation during the usual LDL isolation procedures. The relationship between CoQ_{10} level in LDL and oxidative susceptibility is supported by data obtained after oral supplementation with CoQ_{10}. LDL_3 subfraction, which had the greatest increase in CoQ_{10} after supplementation, also showed a significant decrease of the levels of both basal and "AAPH-elicited" hydroperoxide, when compared with LDL_1 and LDL_2. It is important that susceptibility to peroxidation decreased in all three subfractions, and these decreases were statistically significant for LDL_1 and LDL_3 subfractions.

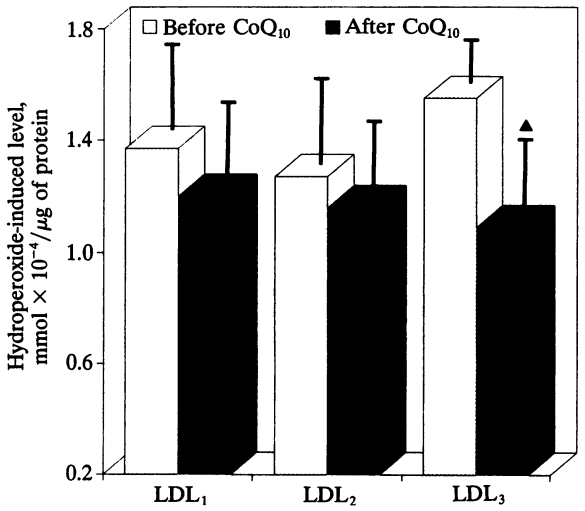

FIG. 4. Hydroperoxide-induced levels. ▲, LDL_3 before vs. LDL_3 after CoQ_{10} supplementation, $P < 0.001$.

Our results confirm that CoQ_{10} is an important antioxidant that can lower the susceptibility of LDL to oxidation. When considering the remarkable increase of CoQ_{10} in LDL_3 subfraction after supplementation, it is possible to hypothesize a different degree of "unsaturation" in CoQ_{10} for different LDL subfractions. LDL_3 subfraction appears to be the one that contains the lowest CoQ_{10} levels with the highest peroxide levels. Even though the administration of CoQ_{10} influenced susceptibility to peroxidation, especially regarding LDL_1 and LDL_3 subfractions, we did not find significant differences in susceptibility among the three subfractions. Besides the antioxidant activity of LDL subfractions, their oxidizability is also affected by the polyunsaturated fatty acids available for oxidation. In particular, the relative abundance of arachidonic and linoleic acid is associated with a higher oxidative susceptibility. As suggested (22), LDL particles depleted of their antioxidant were more resistant to oxidative stress when the subjects consumed a diet with monounsaturated acids compared with polyunsaturated acids. The free fatty acids in our LDL subfractions differ significantly in their unsaturation index. There was a higher content of unsaturated fatty acids in LDL_1 and LDL_2 subfractions than in LDL_3 subfraction. Higher levels of linoleic and arachidonic acid were found in LDL_1 and LDL_2 subfractions than in LDL_3 subfraction. This fatty acid pattern could explain the similar susceptibility exhibited by the different LDL subfractions, even though significant differences in levels of CoQ_{10} and vitamin E were present. The lower levels of CoQ_{10} and vitamin E could be a cause of the higher levels of the hydroperoxides when compared with lower levels after oral supplementation with CoQ_{10}. Susceptibility can be related to the fatty acid composition of LDL_3 subfraction and counterbalance the peroxidative effect of a low level of antioxidants. Parthasarathy *et al.* (23) suggested that a reduction of the polyunsaturated fatty acids in LDL should reduce the "consumption" of antioxidants. Be-

cause the subjects in our study constituted a remarkably homogeneous group, our results are probably not affected by differences in diets and/or life-styles, which may amplify differences in composition and oxidizability of LDL subfractions.

In conclusion, our data support the concept that the CoQ_{10} level of the LDL subfractions is important in minimizing susceptibility of LDL subfractions to oxidation. The chemical composition of the LDL subfractions, especially in fatty acids, affects lipoprotein peroxidation susceptibility. Further investigations to assess LDL oxidizability should evaluate the lipid composition of diets together with the antioxidation status in normal subjects and patients.

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