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Plasma Coenzyme Q_{10} Concentrations are not Decreased in Male Patients with Coronary Atherosclerosis*

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Coenzyme Q_{10} (CoQ_{10}) is an important mitochondrial electron transfer component and has been postulated to function as a powerful antioxidant protecting LDL from oxidative damage. It could thus reduce the risk of cardiovascular disease. Thus far, beneficial effects of supplementation with CoQ_{10} have been reported. To study the relation between unsupplemented concentrations of plasma CoQ_{10} and coronary atherosclerosis, we performed a case–control study among 71 male cases with angiographically documented severe coronary atherosclerosis and 69 healthy male controls free from symptomatic cardiovascular disease and without atherosclerotic plaques in the carotid artery.

Plasma CoQ_{10} concentrations (mean ± SE) were 0.86 ± 0.04 vs. 0.83 ± 0.04 μmol/l for cases and controls, respectively. The CoQ_{10}/LDL-cholesterol ratio (μmol/mmol) was slightly lower in cases than in controls (0.22 ± 0.01 vs. 0.26 ± 0.03). Differences in CoQ_{10} concentrations and CoQ_{10}/LDL-cholesterol ratio did not reach significance. The odds ratios (95% confidence interval) for the risk of coronary atherosclerosis calculated per μmol/l increase of CoQ_{10} was 1.12 (0.28–4.43) after adjustment for age, smoking habits, total cholesterol and diastolic blood pressure.

We conclude that an unsupplemented plasma CoQ_{10} concentration is not related to risk of coronary atherosclerosis.

Keywords: Plasma, coenzyme Q_{10}, ubiquinol, coronary atherosclerosis, antioxidants, LDL oxidation

INTRODUCTION

Antioxidants have been implicated to play a protective role in the atherogenic process. They are believed to delay atherogenesis by protecting lipid fractions within the low-density lipoprotein (LDL) particle against oxidation by free radicals.

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Oxidative modification of LDL has been implicated as an important step in the development of atherosclerosis. Oxidative modification accelerates the uptake of LDL by macrophages which is the beginning of formation of fatty streak.[1,2]

Coenzyme Q_{10} (CoQ_{10}) is an important carrier for two-electron transfer within the mitochondrial membrane and has been shown to function as an endogenous lipid-soluble antioxidant in blood and tissues. Previous studies have shown that reduced CoQ_{10} (ubiquinol) exerts a protective effect on LDL oxidation[4–8] and that reduced CoQ_{10} is depleted before tocopherols during lipid oxidation[4,9,10] consistent with the notion that reduced CoQ_{10} acts as a scavenger of the tocopheroxyl radical produced during lipid oxidation.[11–13] Ubiquinol is also believed to function as a chain breaking antioxidant in the lipid peroxidation process.[3,9,14]

CoQ_{10} supplementation has been suggested to have beneficial effects in treatment of coronary heart disease (CHD) patients[15,16] and to result in decreased thiobarbituric acid-reactive substances (TBARS) concentrations[17] and conjugated diene[7] production after oxidative stress. Some authors also have reported decreased concentrations of CoQ_{10} in cardiomyopathy patients compared to normal controls,[18] and the LDL/ubiquinone ratio has been suggested to be a coronary risk factor.[19] However, little information is available on plasma CoQ_{10} concentrations in CHD patients and healthy subjects under unsupplemented conditions.

To study the association between plasma CoQ_{10} concentrations and the risk of coronary atherosclerosis, we performed a case–control study among cases with severe coronary atherosclerosis and healthy controls.

METHODS

Study Population

The study was conducted in several hospitals and clinical centres in Rotterdam and Dordrecht, The Netherlands, in the period 1993–1995.[20] The study was approved by an ethical committee on human research and all participants gave informed consent. We selected a group of patients with coronary atherosclerosis and a group of population controls without symptomatic cardiovascular disease. All were men between 45 and 80 years of age. Enrolment procedures allowed for similar distributions of age (in 5-year categories) and smoking status (smoking, non-smoking).

Selection of the cases was based on angiographic reports. To reduce the impact of disease on dietary and life-style patterns, we selected only those patients who underwent their first angiography and who had not experienced a myocardial infarction in the year prior to the study. For the same reason, blood was collected within 2 months after angiography. Subjects using HMG-CoA reductase inhibitors were excluded because of the possible inhibiting effect of this drug on ubiquinone production by interfering in the mevalonate pathway[21] and its possible influence on LDL oxidation.

In the study period 2830 patients underwent coronary angiography for suspected CHD, including 1966 male subjects. Subjects were not eligible if they met one of the following exclusion criteria: under 45 or over 80 years of age ($n = 144$), not the first coronary angiography ($n = 389$), MI in the 12 months prior to the study ($n = 180$), diabetes mellitus ($n = 84$), liver, kidney or thyroid disease ($n = 15$), alcohol or drug abuse ($n = 4$), use of HMG-CoA reductase inhibitors ($n = 82$), vegetarian diet ($n = 3$), psychiatric complaints ($n = 2$). For 88 subjects more than 2 months had elapsed between angiography and case selection and 12 patients had died in the meantime, leaving a population of 963 eligible subjects. Of this group 124 refused to participate and 50 could not be contacted or were otherwise indisposed. From the remaining 789 men, 71 cases with at least 85% stenosis in one and at least 50% stenosis in a second of the three major coronary vessels were selected for assessment of plasma CoQ_{10}. Of these subjects, 57% had a narrowing of at least 50% in all three major coronary vessels. The percentage

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of stenosis was scored by the cardiologist performing the angiography.

Population controls were selected from participants in the Rotterdam Study. The rationale and design of this population-based prospective cohort study have been described previously.[22] We selected subjects without any plaques in the carotid artery as assessed by ultrasound. Further, these subjects reported not to have a history of cardiac treatment, not to have diabetes mellitus, or liver, kidney or thyroid disease, not to use HMG-CoA reductase inhibitors nor to be vegetarian. As the participants in the Rotterdam Study were all 55 years and over at baseline, we additionally recruited men between 45 and 55 years of age through an advertisement in a local newspaper. Recruitment took place in the area the other population controls originated from. A questionnaire was used to obtain information on medical history; when candidates fulfilled the inclusion criteria they were invited to the research centre. Enrolment in the study took place after it had been echographically ascertained that subjects' carotid artery was free of plaques. A total of 69 population controls were included in which plasma CoQ_{10} was assessed.

Data Collection

For the cases information on medical history was obtained from hospital records and through a questionnaire within 2 months after angiography. Information on dietary patterns, smoking and drinking patterns, medicine use, use of vitamin supplements, occupation and family history of CVD was obtained. Weight, height and blood pressure were measured. A fasting venous blood sample was collected in EDTA Vacutainer tubes and immediately placed on ice and cooled to 4°C. Plasma was prepared within 1 h after blood collection by centrifugation at 1750× *g* for 15 min, frozen in methanol of −80°C or liquid nitrogen. Samples were stored at −80°C. At this temperature it has been shown that the reduced form of CoQ_{10} is stable for at least 13 months.[23]

Analytical Measurements

Procedures for analysis of total CoQ_{10} and tocopherols in plasma were as follows: 100 µl plasma was extracted after addition of 25 µl 1 mg/ml BHT in EtOH, and 900 µl 0.1 M SDS and 2 ml hexane. 1.5 ml of the hexane layer was taken to dryness under nitrogen and redissolved in 200 µl ethanol. Ten µl was used for HPLC analysis (method adapted from Lang *et al.*[24]). The HPLC analysis was performed on a Waters system with Waters 610 pumps, a Beckman Ultrasphere ODS C-18 column, 4.6 mm i.d., 25 cm, 5 µm particle size, a Waters Wisp 717 autosampler, and Millenium software and using a Coulochem 5100A electrochemical detector (Environmental Sciences Assoc., Bedford, MA, USA), equipped with a Model 5020 Conditioning cell set at −750 mV, and a Model 5011 Analytical cell with two electrodes in series, the first set at −750 mV, and the second set at +500 mV. The eluent was ethanol/methanol/isopropanol 715 : 245 : 40 containing 0.1% w/v lithium perchlorate at 1.2 ml/min[6] (slightly modified). Measurements were performed in duplicate and quantification was carried out by comparing peak areas to the area of standard curves obtained with authentic compounds.

Cholesterol and triglyceride concentrations were determined enzymatically using commercially available reagents (CHOD-PAP kit 236.691 and Triglyceride kit 701.904, Boehringer-Mannheim, Mannheim, Germany). High-density lipoprotein (HDL)-cholesterol was measured after precipitation of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL using the precipitation method with sodium phosphotungstate-Mg^{2+}.[25] LDL-cholesterol concentrations were calculated by the formula of Friedewald *et al.*[26]

Preparation and Oxidation of LDL

The procedure for preparation and lipid peroxidation of LDL was adapted from Esterbauer *et al.*[26] with some major modifications as

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described previously in detail.[27–30] The kinetics of LDL oxidation were followed by continuously monitoring the change of absorbance at 234 nm.[27–29] Absorbance curves of LDL preparations obtained from an equal number (3) of subjects from each study group were determined in parallel. Each LDL preparation was oxidized in three consecutive oxidation runs on the same day. Means were calculated on the basis of these three observations.

Statistical Analysis

Characteristics of the case group and the control group were compared with Student's t-test for unpaired samples. Age-adjusted means were compared by analysis of covariance. Pearson's correlations were calculated between CoQ_{10} concentrations and risk factors for CHD. Odds ratios (ORs) were calculated by means of multiple linear resgression analysis to quantify the association between plasma CoQ_{10} concentrations and coronary atherosclerosis. ORs were calculated per μmol/l increase of CoQ_{10}. Confounding factors taken into account were: age, smoking habits, body mass index, total, HDL- and LDL-cholesterol, diastolic and systolic blood pressure. Data were analysed with the statistical package BMDP.[31]

RESULTS

Table I lists the characteristics of cases and controls. Groups were comparable regarding the prestratification factors age and smoking status. Total cholesterol, LDL-cholesterol and triglycerides were lower in controls, and HDL-cholesterol and diastolic blood pressure were higher in controls. The frequency of reported prescribed diet use was similar in both groups (17.6% in cases, 13.0% in controls). Cases more frequently reported use of antihypertensive medication (93.0% vs. 10.1%) and aspirin and coumarin derivatives (93.0% vs. 4.3%), while 37% of the cases reported a history of MI.

The mean concentrations of plasma antioxidants for the two groups are listed in Table II. No differences in CoQ_{10} concentrations between patients and controls were found. A slightly lower CoQ_{10}/LDL ratio was seen in the case group. However, a significant age-adjusted difference was found for α-tocopherol only, with higher tocopherol concentrations in cases. After adjustment for cholesterol levels, the α-tocopherol/cholesterol ratio was not statistically different between cases and controls (5.1 ± 1.2 vs. 4.8 ± 1.1), whereas the β-carotene/cholesterol ratio was significantly higher in controls ($p < 0.01$).

In the control group we calculated correlation coefficients for the association between

TABLE I Characteristics of the study population (mean ± SD)

	Cases ($n = 71$)	Controls ($n = 69$)
Age (years)	61.9 ± 9.3	61.4 ± 8.8
Smokers (%)[a]	33.8	21.7
Ex-smokers (%)[a]	9.9	18.8
Body mass index (kg/m^2)	26.3 ± 2.4	26.2 ± 3.3
Total cholesterol (mmol/l)	5.9 ± 1.1	5.4 ± 1.1[b]
Triglycerides (mmol/l)	2.1 ± 0.8	1.6 ± 0.9[b]
HDL (mmol/l)	0.8 ± 0.2	1.0 ± 0.3[b]
LDL (mmol/l)	4.2 ± 1.0	3.6 ± 1.0[b]
Systolic blood pressure (mmHg)	132.9 ± 16.6	136.4 ± 18.5
Diastolic blood pressure (mmHg)	81.4 ± 8.0	85.4 ± 9.9[b]

[a]Ex-smoker stopped smoking more than one year ago, otherwise current smoker.
[b]Significant difference $p < 0.05$.

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TABLE II Concentrations of plasma antioxidants for patients with coronary atherosclerosis and controls (mean ± SE)

	Cases ($n = 71$)	Controls ($n = 69$)	p-value[a]
Coenzyme Q_{10} (μmol/l)	0.86 ± 0.04	0.83 ± 0.04	0.50
CoQ_{10}/LDL cholesterol (μmol/ml)	0.22 ± 0.01	0.26 ± 0.03	0.21
γ-Tocopherol (μmol/l)	2.44 ± 0.14	2.16 ± 0.11	0.13
α-Tocopherol (μmol/l)	29.8 ± 0.6	25.4 ± 0.7	< 0.01
β-Carotene (μmol/l)	0.22 ± 0.02	0.26 ± 0.02	0.17

[a] Age-adjusted.

TABLE III Odds ratios (and 95% CI) for risk of coronary atherosclerosis per μmol/l increase of CoQ_{10} and per unit increase in CoQ_{10}/LDL cholesterol

	Age-adjusted	Multivariate[a]
CoQ_{10}	1.50 (0.48–4.67)	1.12 (0.28–4.43)
CoQ_{10}/LDL cholesterol	0.18 (0.01–3.55)	1.01 (0.09–11.2)

[a] Adjusted for age, smoking, total cholesterol and diastolic blood pressure.

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cardiovascular risk factors and the concentration of CoQ_{10}. Positively correlated ($p < 0.05$) to CoQ_{10} concentrations were: total cholesterol ($r = 0.57$), LDL-cholesterol ($r = 0.49$), diastolic blood pressure ($r = 0.25$), γ-tocopherol ($r = 0.40$) and α-tocopherol ($r = 0.53$). No correlations were found with age, body mass index, systolic blood pressure, HDL-cholesterol and triglyceride concentrations.

In Table III ORs (and 95% confidence interval) for the risk of coronary atherosclerosis per μmol/l increase of plasma CoQ_{10} and per unit increase of CoQ_{10}/LDL-cholesterol ratio are presented. No association was found between plasma concentrations of CoQ_{10} and risk of coronary atherosclerosis. Because crude and age-adjusted ORs differed only marginally, only the age-adjusted OR is presented. To ensure that other differences between the cases and controls did not confound the risk estimate, we adjusted the OR for the potential confounding factors by including them as independent variables in the multiple regression analysis. In the final model age, total cholesterol, diastolic blood pressure and smoking habits was controlled for (Table III). The OR was 1.12 which means that every μmol/l increase of CoQ_{10} results in a non-significant 12% increase in risk of coronary atherosclerosis. Additional adjustment for α-tocopherol resulted in an OR of 0.37 (0.08–1.78). The interaction between plasma concentrations of CoQ_{10} and α-tocopherol did not reach significance ($p = 0.38$).

Calculation of the ORs over quartiles of CoQ_{10} concentrations in the control group yielded essentially similar results. ORs for the successive quartiles were 1.0 (reference), 0.90 (0.29–2.73), 0.73 (0.23–2.36) and 1.32 (0.43–4.03) after adjustment for age, total cholesterol, smoking habits and diastolic blood pressure.

Stratified analysis in separate strata of total cholesterol (< 5.5 or ≥ 5.5) or smoking status (smokers or never/ex-smokers) did not essentially change the results.

Associations with Oxidation Parameters

A possible mechanism by which CoQ_{10} and other plasma antioxidants may play a role in the atherogenic process is by inhibition of LDL oxidation. Resistance time, as a measure of LDL

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TABLE IV Oxidation characteristics for patients with coronary atherosclerosis and controls (mean ± SE)

	Cases ($n = 71$)	Controls ($n = 68$)[a]	p-value[b]
Resistance time (min)	87 ± 1	90 ± 1	0.20
Maximum rate of oxidation (nmol diene/min per mg protein)	10.4 ± 0.1	10.3 ± 0.1	0.54

[a]One control with unreliable data for oxidation parameters was left out for the analyses.
[b]Age-adjusted.

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resistance to oxidation *ex vivo*, and maximum rate of oxidation did not differ between cases and controls (Table IV) and were not correlated to plasma CoQ_{10} concentrations. The OR (95% confidence interval) for coronary atherosclerosis per μmol/l increase of CoQ_{10} was 0.99 (0.24–4.06), after further adjustment for resistance time and maximum rate of oxidation.

DISCUSSION

We investigated the relationship between plasma total CoQ_{10} concentrations and the risk of coronary atherosclerosis in unsupplemented individuals in a case–control study. No association could be detected.

A protective effect against CHD has been ascribed to CoQ_{10}, possibly due to the antioxidant function of the reduced form of CoQ_{10}, ubiquinol. In this study we measured the total CoQ_{10} concentration, but data from other studies indicate that at least 75–80% of the total CoQ_{10} can be found in reduced form,[5,7,17] and therefore differences in total CoQ_{10} concentrations is also likely to reflect differences in the concentrations of reduced CoQ_{10}. However, we cannot exclude the possibility that the ubiquinol/ubiquinone ratio differs between cases and controls.

It seems unlikely that the finding of no difference between the groups is a result of flaws in the study design. As a result of the disease status changes in life-style and nutritional patterns could have occurred in the patient group. CoQ_{10} is present in a wide variety of foods, but is mainly high in organ meats (e.g. heart, liver, kidney), beef, vegetable oils (e.g. soy oil), fish (e.g. sardines, mackerel), and peanuts. A change in dietary patterns towards these products is not very likely, as organ meats are not very popular in The Netherlands, and diets prescribed to CHD patients commonly are energy- and/or fat-restricted. In addition, recent studies have shown that the average CoQ_{10} intake of an average Danish person is 3–5 mg per day, an amount that is not likely to affect the plasma concentration dramatically.[17]

Another important difference between our two groups is drug use. As many as 93% of our patients used antihypertensive medication, aspirins or coumarin derivatives. From the literature the only drugs which may have an inhibitory effect on ubiquinone production are HMG-CoA reductase inhibitors.[21] In our study subjects who used these drugs were excluded.

Thus far few studies have reported on plasma CoQ_{10} concentrations in coronary artery disease patients. Langedijk *et al.*[23] reported no difference between ubiquinol and ubiquinone concentrations in male patients compared to healthy controls. Only a significant lower ubiquinol/ubiquinone ratio was observed in the patients. Also comparable to our study were findings of Cleary *et al.*,[32] who reported similar plasma total CoQ_{10} concentrations in patients with atherosclerosis and age-matched controls. In these studies also a higher concentrations of α-tocopherol in patients with coronary artery disease were reported, which may be explained by the lower oxidation potential of ubiquinol accompanied by a sparing of α-tocopherol.[4] In the study of Hanaki *et al.*[19,21] a higher LDL/ubiquinone

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ratio was found in patients with coronary artery disease. In our study the ratio CoQ_{10} over LDL ratio was calculated but showed no significant difference between patients and controls. Because of the large age difference between the study populations in Hanaki's study, comparison with these findings is complicated.

Several studies have reported on decreased susceptibility to oxidation after supplementation with CoQ_{10}. Weber *et al.* reported a significant decrease in TBARS production after a 1 μmol/l increase of plasma Q_{10} concentration.[17] Kontush *et al.* reported that incorporation of ubiquinol-10 in LDL, which led to an increase in LDL ubiquinol-10 from 0.10–0.20 to 0.55–1.48 mol/mol LDL, resulted in a decreased conjugated diene production.[7] Further, a decrease in hydroperoxide concentrations was detected after supplementation of LDL with CoQ_{10}.[8] In contrast, Kaikkonen *et al.* did not find an effect of 90 mg orally supplemented CoQ_{10} on oxidation resistance in smoking men.[33] To our knowledge no study has reported on significantly reduced susceptibility to oxidation at physiological plasma concentrations of CoQ_{10} in unsupplemented subjects. Kontush *et al.* described only a small effect of ubiquinone-10 and α-tocopherol incorporated in LDL at physiological concentrations, whereas at high concentrations of incorporation (more than eight-fold increase) an antioxidant effect of ubiquinone-10 was seen.[7] Also in a study by Frei *et al.* no effect of physiological LDL-ubiquinol concentrations on LDL oxidation was found.[34] This is in accordance with the lack of association between CoQ_{10} concentrations and LDL oxidizability in our study in unsupplemented subjects.

In this study we did not detect differences in plasma concentrations of CoQ_{10} between cases with coronary atherosclerosis and controls, nor did we find a decreased risk of coronary atherosclerosis at higher CoQ_{10} concentrations. We conclude that no relation between physiological concentrations of plasma CoQ_{10} and the risk of coronary atherosclerosis was detected.

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