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Original Contribution

EFFECT OF ORAL COENZYME Q10 SUPPLEMENTATION ON THE OXIDATION RESISTANCE OF HUMAN VLDL+LDL FRACTION: ABSORPTION AND ANTIOXIDATIVE PROPERTIES OF OIL AND GRANULE-BASED PREPARATIONS

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Abstract—Coenzyme Q10 (Q10) is supposed to be an important endogenous lipid-soluble antioxidant. We studied 60 healthy 46 ± 7 (mean ± SD)-year-old smoking men. They were randomized into three groups to receive oil-based or granular Q10 (90 mg/d) or placebo for 2 months. Oil-based capsule elevated Q10 in plasma by 178% and in VLDL+LDL fraction by 160%. The granular preparation increased Q10 in plasma by 168% and in VLDL+LDL by 127%. However, the 2-month Q10 supplementation did not increase the oxidation resistance of VLDL+LDL fraction, as assessed by copper induced VLDL+LDL oxidation, haemin+H_2O_2-induced VLDL+LDL oxidation, total antioxidative capacity of LDL, and plasma malondialdehyde measurements. The first and the last dose was used to carry out a 12 h pharmacokinetic study (five subjects per group), which indicated that only a small part of supplemented Q10 was absorbed to the circulation in 12 h and that the absorption varied extensively between subjects. Our results suggest that at least among smoking men, 90 mg of orally supplemented Q10 daily does not increase the oxidation resistance of VLDL+LDL. Bioavailability of both the granular and the oil-based Q10 preparation was similar during the long-term supplementation, but one dose of 30 mg had only a marginal effect on the plasma levels of Q10. © 1997 Elsevier Science Inc.

Keywords—Antioxidant, Clinical trials, Coenzyme Q10, Oxidation resistance, Pharmacokinetics, Plasma lipoproteins

INTRODUCTION

Lipid peroxidation has probably an important role in the etiology of many pathologic conditions including atherosclerosis.[1] Coenzyme Q is supposed to be an effective lipid-soluble antioxidant increasing oxidation resistance, tested in different model systems including mitochondria, reconstituted membrane systems, and submitochondrial particles.[2] There are also some experiments suggesting that coenzyme Q10 (Q10) is an important antioxidant in human plasma.[3–8] However, compared with other antioxidants or other organs, the Q10 concentration is very low in plasma.[9–11] There are only a couple of small uncontrolled studies[7,8] concerning the antioxidative efficiency of orally supplemented Q10 in human plasma. Our aim was to investigate the effect of 2-month oral Q10 supplementation (90 mg/d) on the oxidation resistance of VLDL+LDL fraction in smoking men, using two different Q10 preparations. To our knowledge, the present study is the first placebo-controlled clinical trial concerning the antioxidative effect of orally supplemented Q10 in blood lipoproteins. We also carried out a one dose (30 mg) pharmacokinetic study to investigate the absorption of the same Q10 preparations. Previous pharmacokinetic studies are few and restricted to few persons and mainly a single dose,[12–14] whereas we investigated both single-dose and steady-state kinetics after a pharmacological dose similar to that recommended by Q10 producers.

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MATERIALS AND METHODS

Description of study population

This study is a part of a large block randomized, single-blind, and placebo-controlled MASI study (Multiple Antioxidant Supplementation Intervention study). Healthy smoking male subjects were recruited by newspaper advertisements from eastern Finland. Exclusion criteria included regular intake of any drug with antioxidative properties or acetylsalicylic acid, intake of other investigational products within the last 3 months, severe obesity (body mass index > 31 kg/m^2), insulin-dependent (type 1) diabetes, and other severe diseases. A total amount of 142 men were randomized into the trial. To test the effect of 2-month Q10 supplementation on oxidation resistance, 60 men (23 ± 9 cigarettes/d, aged 46 ± 7 years, mean $\pm$ SD) were randomly allocated either to Q10 supplementation (30 mg granule or oil-based Q10 preparations three times a day) or placebo (two times a day), 20 men in each group. The investigators were blinded with regard to the identity of the granular or oil-based Q10 preparations given to subjects. Five volunteers from the each group were chosen at random to the 12 h pharmacokinetic study, which was carried out at baseline and repeated in an identical way at the end of 2-month supplementation. All subjects gave a written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

Pharmacokinetic study

Subjects for the pharmacokinetic study fasted overnight. After the first sample was taken in the next morning the breakfast (containing bread, margarine, salami, cheese, coffee, tea, water, and milk) was served and the single dose of 30 mg Q10 was administered. After this, blood samples for Q10 measurements were taken every 2 h. Lunch (as breakfast plus white rice and meat stew) was served after the second sample and dinner (equal to lunch) after the fifth sample was taken. Last blood sample was taken 12 h from the start. Coffee was available and smoking was allowed during the kinetic study.

Analytical procedures

VLDL+LDL Q10 determination was done by isolating VLDL+LDL fraction from fasted EDTA plasma by ultracentrifugation (in a Beckman XL-90 ultracentrifuge) for 23 h at 4°C (32,000 rpm, Beckman 50.4 Ti rotor, Palo Alto, CA) using potassium bromide gradient. The top layer from the tube (<1.063 g/ml) was collected with a pipette and suspended into 0.9% NaCl. After this, Q10 samples were prehandled as Grossi et al.[15] using hexane extraction and solid-phase extraction cartridges. SDS (Sigma, St. Louis, MO) was used to improve the gain of hexane extraction. Coenzyme Q9 (Sigma) was used as an internal standard. Total Q10 (including reduced and oxidized form of coenzyme Q10) was separated by a HPLC-system consisting of an Autosampler 507 (System Gold, Beckman, San Ramon, CA), HPLC Pump 420 (Kontron Instruments, Milan, Italy) and a column (SuperPac, Pep-S, 5 μm, 4 $\times$ 250 mm, Pharmacia, Uppsala, Sweden). Q10 concentrations were measured by Coulochem 5200 A electrochemical detector (ESA, Bedford, MA) with a model 5020 guard cell before the column for reducing quinones to quinols, and a Model 5011 analytical cell with a plotter. The eluent was ethanol:metanol:acetonitrile: 70% perchloric acid (880:660:660:2.2, v/v) containing 0.05 M sodiumperchlorate (Merck, Darmstadt, Germany). Plasma total Q10 was measured using the modified HPLC method of Lang et al.[16] at the University of Tampere. Samples and standards were first reduced by Na-dithionite (Riedel, Seelze, Germany) and then extracted into hexane phase, which was dried in nitrogen flow. The residue was suspended into chloroform:methanol (1:1, v/v) for HPLC measurement, which was done by an amperometric detector in the oxidative mode. The HPLC system consisted of a Pharmacia LKB model 2248 pump, Model 2157 Autosampler, Spherisorb C18 column with 3 μm particles (Phenomenex, Torrance, CA), amperometric LC-4B detector (Bioanalytical Systems Inc., West Lafayette, IN) and Hewlett Packard 3396 Integrator (Avondale, PA). The eluent was methanol:ethanol:2-propanol (100:855:45, v/v) containing 20 mM lithium perchlorate (Aldrich, Milwaukee, WI).

All measurements of resistance to oxidation were done from EDTA plasma samples frozen (−80°C) immediately after plasma separation and determined within a week after blood drawing. Copper and hemin+H_2O_2 mediated VLDL+LDL oxidation was assessed in isolated VLDL+LDL fraction using sample handling and methods presented previously.[17,18] Briefly, VLDL+LDL fraction was isolated from EDTA plasma using ultracentrifugation. For copper-induced oxidation, EDTA was removed chromatographically using PD-10 columns (Pharmacia, Uppsala, Sweden). VLDL+LDL was diluted with oxygen-saturated PBS to a protein concentration of 0.05 mg/ml. Formation of conjugated dienes was started by adding 33.5 μl of 100 μM copper chloride (final concentration 1.65 μM) to 2 ml of diluted VLDL+LDL fraction and the reaction was assessed spectrophotometrically at 234 nm. The ratio of copper to lipoprotein was 33.0 nmol of copper to 1 mg of protein. Lag time to the maximal oxidation rate (lag time), maximal oxidation velocity (V_{max}), the

maximal absorbance (A_{max}) and time to the maximal absorbance ($A_{maxtime}$) were determined. Lag time was defined as the time from the start of the reaction to the beginning of the steepest slope and was computed by means of least squares regression (LSR) equation. V_{max} was computed also by using LSR method from the slope of the absorbance curve during the propagation phase. A_{max} was defined as the absorbance from the decomposition state of curve when the slope decreases below 10^{-3} abs/min. $A_{maxtime}$ was defined as the time needed to reach the maximal oxidation (maximal absorbance). The within-batch CV of a frozen serum pool was 3.3% for the lag time and 2.9% for the V_{max}, 1.0% for the Amax and 8.5% for the Amaxtime ($n = 6$). The between-batch CV was 11.2% for the lag time, 10.9% for the V_{max}, 7.8% for the A_{max} and 10.3% for the $A_{maxtime}$ ($n = 9$).

VLDL+LDL fraction resistance to oxidation was assayed also by using H_2O_2-induced degradation of haemin, which was assessed spectrophotometrically at 405 nm. Lag time and maximal velocity of haemin degradation were determined, as previously described.[17]

Plasma malondialdehyde (MDA) was measured from frozen EDTA plasma after thiobarbituric acid (TBA) reaction using a slight modification of the liquid chromatographic method described by Rabl et al.[19] The plasma samples were thawed up immediately before the assay and a volume of 100 μl was mixed with 100 μl water, 300 μl 0.15 M phosphoric acid, 10 μl butylated hydroxytoluene (0.2% methanolic solution) plus 100 μl 0.6% TBA and incubated at 95°C for 60 min. The chromogen was extracted with 1.25 ml butanol-1 and analyzed by HPLC with fluorimetric detection (exitation wavelength 525 nm, emission wavelength 550 nm). The MDA-TBA adduct was calibrated with tetramethoxypropane standard solutions, processed as the plasma samples.

Total antioxidative capacity of LDL (LDL TRAP) was measured with a modified method of Metsä-Ketelä et al.[20] LDL was precipitated from 1 ml of EDTA plasma with 7.5 ml of heparin, diluted to a concentration of 50 IU/ml in 64 mM sodium citrate, pH 5.04. The precipitate was extracted with chloroform:methanol (1:1, v/v). One milliliter of the extract was evaporated and the remainder was dissolved with 500 μl of hexane:isopropanol (19:1, v/v) and divided for determinations of cholesterol and for chemiluminescent assay of chain-breaking antioxidants. Cholesterol was measured with gas chromatography. The other aliquot was exposed to peroxyl radicals produced by the thermal decomposition of 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN). The reaction mixture contained 600 μl of chloroform:methanol (6:4, v/v), 50 μl of 10 mM luminol (Sigma) in borate buffer and methanol (1:1, v/v), 50 μl of 25 mM AMVN in benzene and 50 μl of sample in hexane-isopropanol. Peroxyl radical reactions were followed by the luminol-enhanced chemiluminescence at 32°C (Bio-Orbit 1251 Luminometer, Turku, Finland). D-α-tocopherol was used as a standard, but its stoichiometric trapping factor was not taken into account, when calculating the results.[21] LDL TRAP results were expressed as pmol/nmol cholesterol in LDL.

Plasma ascorbate and α-tocopherol were determined using liquid chromatographic methods.[17] Baseline and 2-month measurements of different antioxidants were carried out after the study in the same batch using frozen samples (−80°C).

Statistical analysis

Nonparametrical Kruskal-Wallis one-way ANOVA test and Wilcoxon rank sum test were used to compare the heterogeneity of area under plasma Q10 concentration-time (0–12 h) curve (AUC) between the groups and Wilcoxon matched-pairs signed rank test within the groups in the pharmacokinetic study. AUC values were calculated by the linear trapezoidal method[22] after the endogenous Q10 values (baseline values before the first dose) were subtracted from the values obtained after supplementation. One-way analysis of variance (ANOVA) and covariance (MANOVA) adjusted for age, smoking, change of smoking, and baseline plasma Q10 concentration were used in addition to nonparametrical tests to compare 2-month changes between the treatment groups. Post hoc comparisons between groups were done using Bonferroni and Duncan Multiple Range tests with significance level .05. Ninety-five percent confidence intervals were computed based on t-distribution.

RESULTS

Q10 and oxidation resistance

Two-month supplementation increased significantly Q10 levels in plasma and in VLDL+LDL fraction, as compared with placebo (Table 1). Bioavailability of both supplements was similar. However, according to Duncan test the relative bioavailability of the oil-based preparation was significantly better than for the granule in VLDL+LDL fraction. The oil-based capsule elevated Q10 in plasma in 2 months by 178% (95% CI, 135 to 221%) and in VLDL+LDL fraction by 160% (95% CI, 126 to 193%). Correspondingly the granular preparation increased Q10 in plasma by 168% (95% CI, 132 to 203%) and in VLDL+LDL by 127% (95% CI, 110 to 145%). Neither plasma nor VLDL+LDL Q10 levels changed in the placebo group.

Table 1. Baseline Values and Changes of Plasma and VLDL+LDL Q10 Concentrations and Indicators Describing Plasma and Lipoprotein Antioxidative Capacity (Mean ± SD)

	Group						
	Oil (n = 20)		Granule (n = 20)		Placebo (n = 20)		p for
Variable	Baseline Value	Change	Baseline Value	Change	Baseline Value	Change	Difference
Plasma Q10 (μmol/l)	1.07 ± 0.34	1.90 ± 0.97	1.08 ± 0.31	1.81 ± 0.82	0.89 ± 0.33	−0.01 ± 0.26	<0.0001
VLDL+LDL Q10 (μmol/l)	1.04 ± 0.29	1.67 ± 0.74	1.02 ± 0.30	1.29 ± 0.38	1.02 ± 0.32	−0.03 ± 0.28	<0.0001
Copper induced VLDL+LDL oxidation							
Lag time (min)	79 ± 7 (19)	1 ± 8 (19)	74 ± 9	5 ± 11	74 ± 11 (19)	1 ± 8 (19)	0.306
Maximal velocity (mabs/min)	9.4 ± 1.9 (19)	0.9 ± 1.4 (19)	9.8 ± 1.6	0.4 ± 1.3	10.6 ± 2.4 (19)	0.0 ± 2.0 (19)	0.216
Maximal absorbance (abs)	0.58 ± 0.09 (19)	0.03 ± 0.10 (19)	0.58 ± 0.07	0.02 ± 0.10	0.60 ± 0.06 (19)	0.00 ± 0.09 (19)	0.783
Time to the maximal absorbance (min)	138 ± 22 (19)	− 1 ± 13 (19)	129 ± 13	9 ± 22	134 ± 28 (19)	−2 ± 28 (19)	0.244
Hemin + H_2O_2 induced VLDL + LDL oxidation							
Maximal velocity (mabs/min)	0.75 ± 0.36	−0.05 ± 0.25	0.72 ± 0.27	0.02 ± 0.18	0.84 ± 0.22	0.01 ± 0.20	0.505
Lag time (min)	126 ± 50	2 ± 8	125 ± 53	2 ± 8	113 ± 40	0 ± 5	0.761
Other measurements							
LDL TRAP (pmol/nmol chol)	12.5 ± 3.5	−0.5 ± 3.1	11.6 ± 3.3	−0.0 ± 4.5	12.3 ± 3.4	−0.2 ± 3.3	0.930
Plasma MDA (μmol/l)	0.44 ± 0.15	−0.02 ± 0.19	0.39 ± 0.16	−0.02 ± 0.16	0.40 ± 0.13	−0.05 ± 0.12	0.830
Plasma ascorbate (μmol/l)	65.6 ± 25.4 (19)	−7.4 ± 20.6 (19)	59.4 ± 22.1 (18)	−2.4 ± 19.9 (18)	59.3 ± 18.3 (18)	−4.5 ± 23.6 (18)	0.777
Plasma α-tocopherol (μmol/l)	30.9 ± 11.2	−1.1 ± 5.2	29.7 ± 5.0	−0.9 ± 3.6	29.3 ± 5.2	−1.3 ± 3.7	0.960

Differences in change between groups were tested with the one-way variance analysis (ANOVA). ( ), number of subjects if not 20; LDL TRAP, total antioxidative capacity of LDL fraction standardized by α-tocopherol; MDA, malondialdehyde.

Neither of the Q10 supplements increased the oxidation resistance of VLDL+LDL fraction during the 2-month supplementation. Baseline values and changes in measurements describing the susceptibility of VLDL+LDL to oxidation are presented in Table 1. Q10 supplementation had no influence on either plasma ascorbate or on plasma α-tocopherol concentrations. We also used covariance analysis and nonparametrical Kruskal-Wallis test in addition to variance analysis to compare differences between the supplemented groups (not presented). No significant effects were observed in these tests, either. Neither of the Q10 supplements had any effect on either LDL TRAP or plasma MDA concentration (Table 1).

Pharmacokinetic trial comparing different preparations

Baseline levels and absorption of Q10 varied extensively between subjects (Fig. 1). The granular preparation elevated best plasma Q10 at baseline, but the increase did not differ significantly from the oil-based capsule, as assessed by AUC (Table 2). Bioavailability of Q10 varied more between the subjects than between preparations (Fig. 1). Kinetic study was repeated at the end of 2-month supplementation. This indicated that a single dose of 30 mg cannot increase saturated Q10 levels in plasma. However, 2-month supplementation (90 mg/d) caused Q10 to accumulate into the plasma lipid fraction.

DISCUSSION

Our study is the first placebo-controlled clinical trial concerning the effect of orally supplemented Q10 on the oxidation resistance of human lipoproteins. The results indicate that the relative bioavailability of both the oil-based and the granular supplements is similar, but at least among unselected smoking men, 90 mg of orally supplemented Q10 daily for 2 months does not appear to increase the oxidation resistance of the combined VLDL+LDL fraction. The result is in disagreement with small uncontrolled supplementation studies with the daily dose of 90 mg Q10 for 2 weeks[8] or 300 mg for 11 d[7] or in vitro studies concerning oxidation resistance of nonsupplemented LDL or VLDL.[3-6]

Our oxidation resistance measurements were versatile and concerned different phases of the lipid peroxidation process. As lipid peroxidation initiators in the oxidation resistance tests, both transition metal derived radicals and peroxyl radicals (from AMVN) were used. Of the lipid peroxidation products, malondialdehyde, a product of further advanced lipid peroxidation was measured. Plasma MDA measurement with a liquid chromatographic method is more specific than TBARS measurement used in a previous study.[8] However, as a marker of advanced lipid peroxidation, MDA may be an insensitive measurement for assessing effects of supplemented Q10, which probably acts effectively only for a short time at the beginning of the lipid peroxidation process. Our copper induced oxidation resistance measurement was based on the technique de-

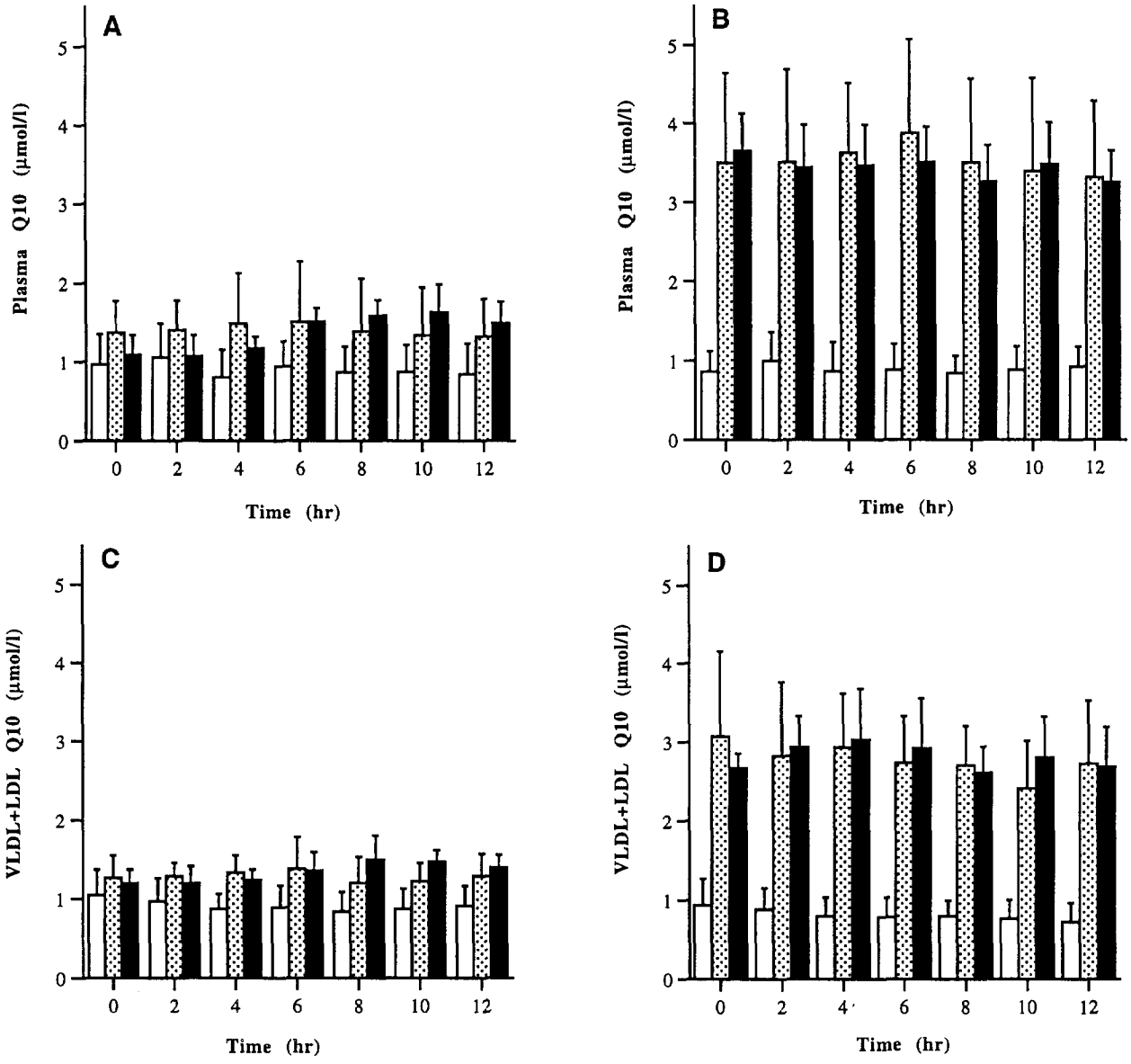

Fig. 1. Changes in Q10 concentration in plasma (A) and in VLDL+LDL (C) after the first 30 mg Q10 dose at baseline and on the steady state in plasma (B) and in VLDL+LDL (D) at the end of 2-month supplementation (five subjects per group). Bars describe mean ± SD concentrations in placebo (white bars), oil (dotted bars), and granule (black bars) groups at different time points.

scribed by Esterbauer et al.[23] Previous studies[17,18] have indicated that this method is valid for the assessment of elevated oxidation resistance of plasma lipid fraction. Kontush et al.[6] used this method (final cholesterol and copper sulfate concentrations were 0.12 mg/ml and 2 μmol/l, respectively) in their cross sectional study in which they observed a positive correlation between plasma initial Q10 concentration and oxidation resistance of LDL. A significant correlation was found only at the beginning of the lag phase (the lag phase was distributed into four time intervals). Thomas and coworkers[24] used a mixture of 5 vol of Ham's F-10 medium (containing copper and iron ions 0.01 and 3 μmol/l, respectively) plus 1 vol of LDL (0.5 to 0.8 mg protein/ml) to initiate LDL peroxidation. Their finding was that Q10 as a cosupplement prevented the prooxidant effect of α-tocopherol and also increased the oxidation resistance of LDL, against transition metal-dependent oxidation. They determined accumulation of cholesteryl ester hydroperoxides as a product of lipid peroxidation, which was, as a chemiluminescence method, probably more sensitive for assessing early events of lipoprotein oxidation compared with our conjugated diene measurement. Tribble et al.[5] used copper

Table 2. Area Under the Plasma Q10 Concentration Curve (AUC)

AUC 0-12 h (μmol × h/l)	Mean			Kruskal–Wallis Test	Wilcoxon Test					
					p for Difference Between Groups			p for Change Within Groups		
	Oil	Gran	Plac	p for Difference Between Groups	Oil vs. Gran	Oil vs. Plac	Gran vs. Plac	Oil	Gran	Plac
Plasma (baseline)	0.45	3.44	−0.77	0.101	NS	NS	0.028			
Plasma (after 2 months)	26.20	28.14	−0.94	0.009	NS	0.009	0.009			
Plasma (baseline vs. 2 months)								0.043	0.043	NS
LDL+VLDL (baseline)	0.10	1.77	−1.72	0.027	NS	NS	0.009			
LDL+VLDL (after 2 months)	17.69	19.56	−2.91	0.009	NS	0.009	0.009			
LDL+VLDL (baseline vs. 2 months)								0.043	0.043	NS

Each value of AUC is mean of five subjects.

sulfate (1.66 μM to 0.1 mg protein/ml) to start and parinaric acid to monitor the progression of in vitro lipid peroxidation. In our reaction mixture, the final ratio of copper to protein (nmol/mg) was 33.0, in the study of Kontush et al. 26.7 nmol (approximation), in the study of Tribble et al. 16.6 and in the trial of Thomas et al. 0.08 nmol of copper (mean) and 23.1 nmol of iron (mean) per 1 mg of protein. High copper (transition metal) to lipoprotein ratio may decrease the sensitivity of the oxidation resistance assay. Varying ratios of transition metal to lipoprotein may be useful in the measurement of the oxidation resistance of different antioxidants. As compared to previous studies, the copper to lipoprotein ratio was slightly higher in our reaction mixture, because of the lower lipoprotein concentration. However, there are no meaningful differences between these studies. Of the factors affecting sensitivity, also the concentration of the reaction mixture may be important. To investigate the effects of the Q10 supplementation on oxidation resistance, the copper oxidation with conjugated diene assessment has not been previously used. Possibly, the method is not sensitive enough to detect a very small effect of oral Q10 supplementation on the oxidation resistance of lipoproteins.

Water-soluble AAPH or lipid soluble AMVN, as a generator of peroxyl radicals have been used in several in vitro studies[3,4,7] describing antioxidative efficiency of Q10 during early stages of lipid peroxidation. In these studies, the products of lipid peroxidation (lipid hydroperoxides) were assessed by HPLC with postcolumn chemiluminescence detection. We did not observe similar results concerning antioxidative efficiency of supplemented Q10 using AMVN as a radical source, and chemiluminescence based system as a measure of the inhibition of radical reactions. Use of lipid soluble AMVN as a lipid peroxidation initiator, is a logical measurement for assessing oxidation resistance particularly in lipoproteins. As noted above, the range of different oxidation resistance measurements is wide. This makes the comparison of our results with previous findings difficult.

It is known that Q10 is unstable and can get oxidized during the sample preparation and storage.[10] We made the oxidation resistance measurements from frozen EDTA plasma samples, whereas previous studies have been made using only fresh samples. For this reason, we carried out a 3-week Q10 supplementation study in two subjects to compare results of oxidation resistance measurements (copper and haemin+H_2O_2-induced oxidation) determined from fresh and frozen (baseline and supplemented samples both kept for 2 weeks at −80°C) EDTA plasma samples. We observed no difference in the effect of Q10 supplementation on oxidation resistance between fresh and frozen samples (data not presented). However, some loss of reduced Q10 possibly occur during sample preparation.

Q10 concentration is very small in plasma lipoproteins compared to other antioxidants, for example to α-tocopherol (0.1 vs. 6.4 mol/mol LDL).[11] This was also confirmed in the present study. Theoretically, as a molecule containing two hydroxyl groups, one reduced Q10 molecule can probably extinguish only two free radical chain reactions.[25] Despite of more than twofold increase in VLDL+LDL Q10 concentration following the supplementation, the amount of Q10 was still relatively low in lipoprotein fraction compared with concentrations of other defense mechanisms. As Q10 is an endogenous substance, the synthesis of which is downregulated by increased intake, it may be difficult to achieve multiple fold elevations in its lipoprotein content. This can lead to the lack of measurable increase of oxidation resistance or decrease of MDA concentration, even though reduced Q10 may be the first antioxidant consumed during the free radical attacks[4] or even if the trapping capability of Q10 itself or with

other coantioxidants would be quite effective during early stages of lipid peroxidation. Furthermore, the mobility of the large Q10 molecule is probably slow inside or between the lipoprotein particles. Q10 has both antioxidative and prooxidative functions in mitochondria.[26] The balance of these controversial functions could be disturbed by the Q10 supplementation. In addition, reduction mechanism of absorbed Q10 is still unclear in plasma and it is possible that the reduction of supplemented Q10 could attenuate the antioxidative capacity of other antioxidants in plasma. Q10 can probably work as an antioxidant through two different pathways: either directly by taking part to the trapping process of radicals or by regenerating α-tocopherol.[26] Low (nonsupplemented) plasma α-tocopherol concentration could be an antioxidative capacity limiting step, if Q10 acts in plasma mainly by regenerating α-tocopherol. There can also be some other mechanisms of physiological saturation of Q10. The discrepancy between our study and previous trials may be due to, besides reasons discussed above, different preparations and different kind of subjects. In smokers, higher doses of Q10 may be needed to detect an effect on the oxidation resistance of lipoproteins.

In our kinetic study plasma Q10 levels increased only marginally after a single dose, indicating slow and incomplete absorption from the small intestine. Secretion to faeces has been proposed to be at least 62.5% and the main elimination occurs via bile.[12] Our results indicate remarkable differences of absorption properties between the subjects rather than between the used supplements. Furthermore, the suggested enterohepatic cycle of Q10 is causing some variation in the Q10 levels in plasma after supplementation.[12] In previous kinetic studies Q10 doses have been 60–333 mg daily.[12–14] However, it is important to investigate doses that are similar to daily doses of commercial products. The time required to reach the maximal concentration in plasma at baseline was equal to previous studies. This kinetic study was only trend-setting, because of the small dose and the fact that the subjects did not eat Q10 free meals during the kinetic study, but it clearly demonstrates that a regular and long-term intake is needed to elevate Q10 levels in plasma.

The main findings of our study are that 2-month Q10 supplementation (90 mg daily) more than doubles plasma and VLDL+LDL concentrations, that the bioavailability of the oil-based and the granular formulation is very similar, and that we could not detect any effect of the oral 2-month supplementation on the oxidation resistance of lipoproteins in the test conditions used. This observation should be retested in further controlled clinical trials with a variety of lipid peroxidation measurements.

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ABBREVIATIONS

AAPH—2,2-azobis(2-amidinopropane) dihydrochloride
AMVN—2,2-azobis(2,4-dimethylvaleronitrile)
AUC—area under plasma Q10 concentration-time (0–12 h) curve
EDTA—ethylenediaminetetraacetic acid
HPLC—high-performance liquid chromatography
LDL—low-density lipoprotein
LDL—TRAP total antioxidative capacity of LDL
MDA—malondialdehyde
Q10—coenzyme Q10
SDS—sodium dodecyl sulfate
TBA—thiobarbituric acid
TBARS—thiobarbituric acid reactive substances
VLDL—very low-density lipoprotein