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Antioxidative Efficacy of Parallel and Combined Supplementation with Coenzyme Q10 and d-α-Tocopherol in Mildly Hypercholesterolemic Subjects: a Randomized Placebo-Controlled Clinical Study

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It has been claimed that coenzyme Q10 (Q10) would be an effective plasma antioxidant since it can regenerate plasma vitamin E. To test separate effects and interaction between Q10 and vitamin E in the change of plasma concentrations and in the antioxidative efficiency, we carried out a double-masked, double-blind clinical trial in 40 subjects with mild hypercholesterolemia undergoing statin treatment. Subjects were randomly allocated to parallel groups to receive either Q10 (200 mg daily), d-α-tocopherol (700 mg daily), both antioxidants or placebo for 3 months. In addition we investigated the pharmacokinetics of Q10 in a separate one-week substudy. In the group that received both antioxidants, the increase in plasma Q10 concentration was attenuated. Only vitamin E supplementation increased significantly the oxidation resistance of isolated LDL. Simultaneous Q10 supplementation did not increase this antioxidative effect of vitamin E. Q10 supplementation increased and vitamin E decreased significantly the proportion of ubiquinol of total Q10, an indication of plasma redox status *in vivo*. The supplementations used did not affect the redox status of plasma ascorbic acid. In conclusion, only vitamin E has antioxidative efficiency at high radical flux *ex vivo*. Attenuation of the proportion of plasma ubiquinol of total Q10 in the vitamin E group may represent *in vivo* evidence of the Q10-based regeneration of the tocopheryl radicals. In addition, Q10 might attenuate plasma lipid peroxidation *in vivo*, since there was an increased proportion of plasma ubiquinol of total Q10.

Keywords: α-Tocopherol, antioxidant, coenzyme Q10, interaction, lipid peroxidation, oxidation resistance

INTRODUCTION

Vitamin E and coenzyme Q10 (Q10) are two of the most widely studied lipid soluble antioxidants in human plasma. Several *in vitro*[1–4] and some uncontrolled supplementation studies[5,6]

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have suggested that Q10 might be an important plasma antioxidant. Our previous controlled finding[7] with a battery of lipid peroxidation measurements, suggested that supplementation with 90 mg of Q10 daily for two months did not increase the oxidation resistance of isolated lipoproteins in healthy smoking men. This finding was based predominantly on *in vitro* measurements of oxidation resistance in high radical flux conditions. In addition to the inconsistency of those findings, no reliable *in vivo* lipid peroxidation measurements have been carried out in previous Q10 studies.

The antioxidative efficiency of orally supplemented vitamin E has been established in isolated lipoproteins exposed to high radical flux *in vitro*.[8–10] In addition to vitamin C,[11] also Q10 may recycle tocopherols, although evidence for this comes from *in vitro* studies (partly based on *ex vivo* measurements).[12] This implies that the lipid peroxidation inhibiting effect of vitamin E supplementation would be expected to be greater if subjects were supplemented simultaneously with Q10, and furthermore, additional vitamin E should enhance these effects of Q10. However, the plasma concentration of Q10 is very low. As a consequence, the regenerative efficacy of Q10 is highly dependent on the reduction rate of oxidized Q10. At a tissue level, several enzymes have been found to have Q10 regenerating activities.[13] It is unclear how Q10 is reduced in plasma, even though the reduction seems to occur readily based on the observation that Q10 is mainly in its reduced form in plasma.[5] Nevertheless, there are no previous placebo-controlled clinical trials evaluating the possible interaction between plasma Q10 and vitamin E in parallel study groups.

The purpose of the present study was to assess in a double-blind and placebo-controlled clinical oral supplementation trial (1) whether there is an interaction between Q10 and vitamin E in the antioxidative efficiency and in the change of plasma concentrations and (2) whether a high dose (200 mg) of Q10 daily would increase the antioxidative capacity of plasma and the oxidation resistance of atherogenic plasma lipoproteins. This randomized and double-masked study was conducted in 40 mildly hypercholesterolemic subjects. The proportion of plasma ubiquinol of total Q10, the plasma ascorbate/total ascorbic acid ratio and, as a *post-hoc* analysis, the plasma ascorbyl radical concentration were used to assess the *in vivo* interactions between the antioxidants.

In addition, we studied the pharmacokinetics of 90 mg of Q10 daily in a separate one-week substudy and further assessed the change in the proportion of plasma ubiquinol (reduced form, $Q10H_2$) of total Q10 (Q10 ratio) during that time in 20 healthy subjects. A shortcoming of previous pharmacokinetic studies[14–18] has been that plasma Q10 concentrations have not been corrected with respect to cholesterol in lipoproteins, which are the carriers of Q10 in plasma.[19,20] Neither of these studies were placebo-controlled nor included measurement of the proportion of plasma ubiquinol of total Q10.

SUBJECTS AND METHODS

Three-Month Supplementation Study

This study was a randomized double-masked trial with a two by two factorial design, and was carried out in the spring 1997. Forty subjects, 11 men and 29 postmenopausal women, aged 60.7 ± 5.7 years, with body mass index $26.9 \pm 3.6\,kg/m^2$ (mean $\pm$ SD), mild hypercholesterolemia (serum cholesterol 5.90 ± 0.96 mmol/l, mean $\pm$ SD) and a regular HMG-CoA reductase inhibitor treatment, were recruited by newspaper advertisements from Eastern Finland. Exclusion criteria included regular intake of antioxidants, any drug with antioxidative properties, acetylsalicylic acid or other investigational products within the last month. Also malabsorption, treatment with oral estrogen, use of anticoagulants, manifest insulin-dependent diabetes, cancer or

other severe diseases which would cause difficulties in the participation were exclusion criteria. The subjects were randomly allocated to receive either oil-based Q10 (2×100 mg daily), oil-based d-α-tocopherol (2×350 mg daily), both antioxidants or placebo for 3 months (10 subjects in each group). All the capsules contained soybean oil. The subjects were advised to take supplements within meals, two capsules in the morning and in the evening. Two smokers, randomized to parallel groups were included in the study. The subjects were advised to maintain their statin treatment, smoking and normal exercise and dietary habits during the study.

One-Week Pharmacokinetic Study

In the placebo-controlled single-blind pharmacokinetic study, a total of 20 healthy male ($n = 8$) or female ($n = 12$) 29.8 ± 5.8 (mean ± SD) years old subjects (body mass index $22.8 \pm 2.9\,kg/m^2$, mean ± SD) were recruited from Kuopio, Eastern Finland. Exclusion criteria included any kind of malabsorption which could affect the results, diarrhea, intake of Q10 preparations, other lipid or water soluble vitamins or other investigational products within one month. Subjects were randomly allocated to parallel groups to receive either soybean oil-based, gelatin coated Q10 ($3 * 30$ mg daily, Pharma Nord, Denmark) or placebo for 7 days. To confirm the homogeneity in gender between the study groups, male and female subjects were randomized separately, resulting in 4 male and 6 female subjects in each group. Blood samples were drawn after a fast of 12 h at baseline and after 2- and 7-day supplementation. One smoker was included in the study.

In both studies, all of the subjects provided a written informed consent and the study protocols were approved by the Research Ethics Committee of the University of Kuopio.

Analytical Procedures

Plasma total Q10 and the proportion of ubiquinol of total Q10 (Q10 ratio) were determined by a chromatographic method, modified from that presented by Finckh *et al.* (1995).[21] Coenzyme Q7 (Sigma, St. Louis, MO) was used as an internal standard and a frozen EDTA plasma pool as a secondary Q10 standard. The total Q10 concentration of the plasma pool was determined with a method described previously.[7] EDTA plasma samples were thawed, extracted (at +4°C) and analyzed one at a time to ensure as minimal sample oxidation as possible during the sample preparation. Q10 concentrations were measured using a Coulochem 5200 A electrochemical detector (ESA, Bedford, MA) with a model 5020 guard cell after the column for reducing quinones to quinols, and a model 5011 analytical cell for oxidizing quinols back to quinones. Ubiquinol (reduced form) and ubiquinone (oxidized form) were separated by an HPLC system consisting of an HPLC Pump 420 (Kontron Instruments, Milan, Italy), a Model 7125 sample injector (Rheodyne, Cotati, CA) with a 20 µl loop, the Personal Chromatograph software (System Gold, Beckman, San Ramon, CA) and a column (LiChroCART, 125×4 mm, Merck, Darmstadt, Germany). The eluent was 13.4 mM lithium perchlorate in methanol : ethanol : 2-propanol (880/240/100, v/v) at a flow rate of 1.2 ml/min. The within-batch CV of the frozen non-supplemented plasma pool was 3.2% for plasma total Q10 and 2.2% for the proportion of ubiquinol of total Q10 ($n = 9$). The between-batch CV was 3.0% for plasma total Q10 and 1.4% for the proportion of ubiquinol of total Q10 ($n = 7$).

Heparin plasma for α-tocopherol, β-carotene, retinol and lycopene measurements was extracted with ethanol and hexane. After centrifugation, the top layer was evaporated to dryness under nitrogen and the residue was dissolved in the mobile phase, acetonitrile/methanol/chloroform (47 : 47 : 6, v/v). Samples were injected in a column (LiChroCART, 125×4 mm, Merck, Darmstadt, Germany) and detected as previously described.[22,23] Plasma ascorbate and total ascorbic acid levels were determined by chromatographic methods.[24,25]

For copper induced LDL oxidation, LDL was separated from frozen EDTA plasma by a short single-step ultracentrifugation. Plasma was adjusted to a density of 1.24 g/ml by potassium bromide and layered underneath a solution with density of 1.006 g/ml. The tube was centrifuged for 2.5 h at 417 000*g*. EDTA and gradient salts were removed chromatographically using PD-10 columns (Pharmacia, Uppsala, Sweden). LDL was diluted with oxygen-saturated PBS to a protein concentration of 0.05 mg/ml. The formation of conjugated dienes was started by adding 33.5 μl of 100 μM copper chloride to 2 ml of diluted LDL fraction and the reaction was assessed spectrophotometrically at 234 nm. The final Cu^{2+} concentration was 1.65 μM and the ratio of copper to protein (nmol/mg) was 33.0. Lagtime to the maximum oxidation rate (lagtime), the maximum oxidation rate (maximum slope) and a overall measure of oxidation susceptibility (maximum slope/lagtime) were determined. The between batch CV for the lagtime was 5.1% and for the maximum slope 10.8% ($n = 35$).

Serum cholesterol and urate (Kone Instruments, Espoo, Finland) and triglycerides (Boehringer Mannheim, Mannheim, Germany) were measured with enzymatic colorimetric tests in an autoanalyzer (Kone Specific, Kone Ltd, Espoo, Finland). Serum high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were determined with precipitation methods.[26]

Plasma ascorbyl radicals were measured by Electron Spin Resonance spectroscopy (ESR) in unthawed EDTA plasma samples, stored for two years at −80°C. The samples were thawed and immediately inserted into the ESR spectrometer cavity using a small volume flat ESR cell. The spectrometer (X-band Bruker ESP 300, Bruker Instruments, Karlsruhe, Germany) settings were: modulation frequency 100 kHz, modulation amplitude 0.991 G, microwave power 10 mW, receiver gain 8×10^5, response time 163.9 ms, acquisition by signal averaging two scans. Radical concentrations were determined by double integration of the first derivative signal, using an aqueous solution of 2,2,6,6-tetramethylpiperidine-1-oxyl free radical as a standard. All spectra were filtered and base corrected before integration.

Statistical Analysis

Non-parametrical Kruskal–Wallis one-way ANOVA test and Wilcoxon rank sum test were used to compare the heterogeneity in measured parameters between the groups and Wilcoxon matched-pairs signed rank test within the groups. Ninety-five percent confidence intervals and standard errors were computed based on *t*-distribution.

RESULTS

Three-Month Supplementation Study

Baseline characteristics of the subjects are presented in Table I. Due to differences in serum cholesterol concentration both within and between the groups, the serum cholesterol corrected plasma values of the supplemented vitamins were calculated (Tables I and II). Neither baseline plasma Q10 nor vitamin E concentrations differed significantly between the groups. Three-month supplementation with 200 mg of Q10 daily increased the plasma Q10/cholesterol ratio by 522% (95% CI, 412–632%, $P = 0.005$) and the daily dose of 700 mg of d-α-tocopherol elevated the plasma vitamin E/cholesterol ratio by 127% (95% CI, 99–155%, $P = 0.005$) (Table II). In the group that received both antioxidants, the increase in plasma Q10/cholesterol ratio was 221% (95% CI, 168–274%, $P = 0.005$) and that in plasma vitamin E was increased by 127% (95% CI, 102–152%, $P = 0.005$). The elevation of plasma Q10 concentration was significantly attenuated in the group which received both supplements, compared to the Q10 group ($P < 0.001$ for cholesterol corrected values, Figure 1). Plasma concentrations

TABLE I Baseline characteristics of the subjects in the 3-month study (mean ± SE)

	α-Tocopherol ($n=10$)	Q10 ($n=10$)	Q10 + α-tocopherol ($n=10$)	Placebo ($n=10$)	P for difference*
Serum cholesterol (mmol/l)	5.82 ± 0.25	5.41 ± 0.35	5.97 ± 0.31	6.40 ± 0.26	0.083
Serum LDL cholesterol (mmol/l)	3.67 ± 0.21	3.38 ± 0.37	3.74 ± 0.32	4.23 ± 0.23	0.068
Serum HDL cholesterol (mmol/l)	1.44 ± 0.12	1.33 ± 0.11	1.32 ± 0.10	1.33 ± 0.10	0.856
Serum triglycerides (mmol/l)	1.62 ± 0.36	1.73 ± 0.20	2.16 ± 0.54	2.20 ± 0.38	0.504
Copper induced LDL oxidation					
Lagtime (min)	74 ± 2	78 ± 2	77 ± 2	74 ± 2	0.676
Maximum slope (mabs/min)	12.02 ± 0.23	11.02 ± 0.50	11.04 ± 0.49	11.34 ± 0.31	0.138
Oxidation susceptibility (μabs/min^2)	164 ± 7	143 ± 7	146 ± 10	156 ± 8	0.235
Antioxidants					
Plasma Q10 (μmol/l)	0.99 ± 0.11	0.83 ± 0.04	0.97 ± 0.07	1.07 ± 0.10	0.182
Plasma Q10 (mmol/mol chol.)	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.891
Plasma Q10H_2/total Q10 (%)	87.8 ± 0.7	87.9 ± 0.6	87.7 ± 0.2	87.2 ± 0.8	0.917
Plasma α-tocopherol (μmol/l)	34.4 ± 1.3	33.5 ± 1.6	35.3 ± 3.0	37.1 ± 2.9	0.863
Plasma α-tocopherol (mmol/ mol chol.)	5.93 ± 0.14	6.32 ± 0.36	6.05 ± 0.58	5.75 ± 0.27	0.629
Plasma β-carotene (μmol/l)	0.47 ± 0.09	0.35 ± 0.07	0.30 ± 0.06	0.31 ± 0.05	0.420
Plasma retinol (μmol/l)	1.95 ± 0.12	1.86 ± 0.10	1.99 ± 0.14	2.11 ± 0.15	0.646
Plasma lycopene (μmol/l)	0.26 ± 0.07	0.16 ± 0.04	0.14 ± 0.04	0.11 ± 0.01	0.477
Serum urate (μmol/l)	253 ± 21	267 ± 14	264 ± 17	296 ± 29	0.441
Plasma ascorbate (μmol/l)	73.4 ± 11.3	72.1 ± 7.4	85.9 ± 6.9	85.4 ± 11.6	0.481
Plasma ascorbate/total ascorbic acid (%)	94.2 ± 1.8	88.3 ± 3.1	92.0 ± 2.1	93.8 ± 1.7	0.328
Plasma ascorbyl radical (nmol/l)	100 ± 34 ($n=7$)	88 ± 15	119 ± 21	83 ± 18	0.585

*Heterogeneity in baseline values between groups was tested with Kruskal–Wallis one-way ANOVA test for independent samples. Oxidation susceptibility = maximum slope/lagtime.

TABLE II Three-month changes in the measured variables (mean ± SE)

	α-Tocopherol ($n=10$)	Q10 ($n=10$)	Q10 + α-tocopherol ($n=10$)	Placebo ($n=10$)	P for difference*
Serum cholesterol (mmol/l)	0.41 ± 0.16	0.33 ± 0.14	−0.08 ± 0.31	−0.43 ± 0.19	0.013
Serum LDL cholesterol (mmol/l)	0.18 ± 0.17	0.12 ± 0.12	−0.07 ± 0.30	−0.45 ± 0.15	0.045
Serum HDL cholesterol (mmol/l)	0.18 ± 0.04	0.21 ± 0.04	0.12 ± 0.04	0.09 ± 0.06	0.169
Serum triglycerides (mmol/l)	-0.07 ± 0.16	−0.30 ± 0.14	−0.36 ± 0.28	−0.54 ± 0.22	0.365
Copper induced LDL oxidation					
Lagtime (min)	33 ± 5	3 ± 2	36 ± 4	0 ± 3	< 0.0001
Maximum slope (mabs/min)	−2.31 ± 0.35	0.73 ± 0.43	−1.97 ± 0.29	0.48 ± 0.41	< 0.0001
Oxidation susceptibility (μabs/min^2)	−71 ± 8	3 ± 7	−63 ± 6	6 ± 6	< 0.0001
Antioxidants					
Plasma Q10 (μmol/l)	0.14 ± 0.06	4.58 ± 0.35	2.18 ± 0.27	−0.11 ± 0.05	< 0.0001
Plasma Q10 (mmol/mol chol.)	0.01 ± 0.01	0.81 ± 0.08	0.37 ± 0.04	−0.01 ± 0.01	< 0.0001
Plasma Q10H_2/total Q10 (%)	−3.2 ± 1.2	3.0 ± 0.7	1.1 ± 0.2	0.1 ± 0.8	< 0.001
Plasma α-tocopherol (μmol/l)	49.6 ± 5.6	2.6 ± 0.9	45.5 ± 5.9	−4.4 ± 1.4	< 0.0001
Plasma α-tocopherol (mmol/ mol chol.)	7.53 ± 0.74	0.04 ± 0.14	7.69 ± 0.68	−0.26 ± 0.12	< 0.0001
Plasma β-carotene (μmol/l)	−0.10 ± 0.05	−0.11 ± 0.05	−0.02 ± 0.03	0.03 ± 0.02	0.022
Plasma retinol (μmol/l)	−0.23 ± 0.11	0.06 ± 0.11	−0.09 ± 0.11	−0.23 ± 0.11	0.238
Plasma lycopene (μmol/l)	−0.01 ± 0.04	−0.04 ± 0.03	0.05 ± 0.04	0.06 ± 0.04	0.214
Serum urate (μmol/l)	−16 ± 10	−1 ± 15	3 ± 16	−19 ± 19	0.814
Plasma ascorbate (μmol/l)	−4.7 ± 4.6	7.4 ± 9.3	4.1 ± 8.1	−7.4 ± 6.1	0.597
Plasma ascorbate/total ascorbic acid (%)	4.7 ± 2.1	8.1 ± 3.4	6.9 ± 2.2	6.0 ± 1.6	0.857
Plasma ascorbyl radical (nmol/l)	−3 ± 16 ($n=6$)	−1 ± 13	−17 ± 19	−4 ± 24	0.964

*Differences inchange between groups were tested by Kruskal–Wallis one-way ANOVA test for independent samples. Oxidation susceptibility maximum slope/lagtime.

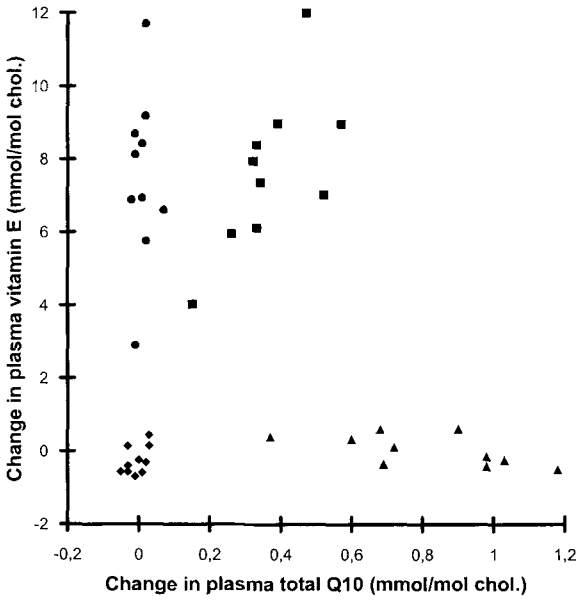

FIGURE 1 Effect of 3-month Q10 (200 mg daily) and vitamin E (700 mg daily) supplementation on the plasma total Q10 and vitamin E levels in placebo (◆), Q10 (▲), vitamin E (●) and Q10 + vitamin E (■) groups.

of the supplemented vitamins did not change to any major extent in the placebo group.

Vitamin E supplementation increased significantly the oxidation resistance of isolated LDL (Table II). The lagtime to copper induced oxidation was prolonged by 43.9% (95% CI, 29.5–58.4%, $P = 0.005$) and the maximal oxidation rate was decreased by 19.2% (95% CI, 12.6–25.8%, $P = 0.005$). In the group receiving both antioxidants, the lagtime to oxidation was lengthened by 46.1% (95% CI, 33.1–59.1%, $P = 0.005$) and the maximal oxidation rate was attenuated by 17.8% (95% CI, 12.0–23.7%, $P = 0.005$) indicating that the simultaneous Q10 supplementation did not increase the antioxidative efficiency of vitamin E. Also the overall index of oxidation susceptibility is presented in Table II. Q10 supplementation alone did not attenuate the oxidation susceptibility of isolated LDL. No change in this index of oxidation susceptibility was observed in the placebo group, either.

Proportion of plasma $Q10H_2$ of total Q10, an indication of plasma redox status *in vivo*, was increased by 3.4% (95% CI, 2.5–4.3%, $P = 0.007$) in the Q10 group, decreased by 3.6% (95% CI, 2.2–5.0%, $P = 0.028$) in the vitamin E group and elevated by 1.3% (95% CI, 0.7–1.8%, $P = 0.005$) in the group of both antioxidants (Table II). All of these changes were statistically significant ($P < 0.05$), compared with the placebo group. The Q10 ratio did not change in the placebo group.

Supplementation with either Q10 or vitamin E was unable to change plasma water-soluble antioxidants, urate and ascorbate. Furthermore, no effects on the proportion of plasma ascorbate of total ascorbic acid or on the ascorbyl radical concentration, used as indicators of vitamin C redox status *in vivo*, were noted following the treatments. No difference in the plasma ascorbyl radical/total ascorbic acid ratio was observed between the groups, either (not presented).

All of these findings were confirmed by using covariance analysis, adjusted for the baseline levels of serum cholesterol and serum triglycerides and for their change during the study. Changes in lipid peroxidation variables were further adjusted for their baseline levels. These adjustments did not change the results presented in Table II.

Pharmacokinetic Study

Baseline characteristics and comparisons of 2- and 7-day changes in plasma Q10 and in serum cholesterol are presented in Table III. Plasma Q10 concentration increased unequally between the subjects (Figure 2). Ninety mg of Q10 daily elevated plasma Q10 concentration in 2 days by 153% (95% CI, 117–190%, $P = 0.005$) and in 7 days by 231% (95% CI, 193–269%, $P = 0.005$). Correspondingly, the increase in plasma Q10/cholesterol ratio (mmol/mol cholesterol) was 154% (95% CI, 109–199%, $P = 0.005$) in two and 236% (95% CI, 152–321%, $P = 0.005$) in seven days (Figure 2). No significant change in either plasma

TABLE III Baseline characteristics and two and seven day changes in the pharmacokinetic sub-study (mean ± SE)

	Q10 ($n = 10$)			Placebo ($n = 10$)			P for difference*		
	Ba	2-day	7-day	Ba	2-day	7-day	Ba	2-day	7-day
Plasma Q10 (μmol/l)	0.65 ± 0.07	1.00 ± 0.11	1.51 ± 0.11	0.70 ± 0.05	0.11 ± 0.10	−0.00 ± 0.03	0.545	< 0.001	< 0.001
Plasma total Q10 (mmol/mol chol.)	0.15 ± 0.01	0.22 ± 0.03	0.34 ± 0.03	0.15 ± 0.01	0.03 ± 0.03	−0.01 ± 0.01	0.545	0.002	< 0.001
Plasma $Q10H_2$/ total Q10 (%)	88.2 ± 0.9	1.59 ± 1.16	2.56 ± 0.54	87.5 ± 0.9	1.17 ± 1.02	0.40 ± 1.16	0.545	0.762	0.082
Serum cholesterol (mmol/l)	4.43 ± 0.26	0.14 ± 0.12	0.01 ± 0.13	4.57 ± 0.17	0.04 ± 0.13	0.22 ± 0.09	0.406	0.385	0.273
Serum LDL cholesterol (mmol/l)	2.45 ± 0.20	0.13 ± 0.14	0.00 ± 0.07	2.38 ± 0.15	−0.03 ± 0.13	0.21 ± 0.07	0.940	0.940	0.075
Serum HDL cholesterol (mmol/l)	1.34 ± 0.11	−0.02 ± 0.06	0.01 ± 0.06	1.49 ± 0.09	−0.05 ± 0.06	0.06 ± 0.04	0.450	0.880	0.650
Serum triglycerides (mmol/l)	0.92 ± 0.17	−0.07 ± 0.11	−0.09 ± 0.17	0.73 ± 0.05	0.05 ± 0.07	0.04 ± 0.07	0.496	0.412	0.496

*Differences in baseline values and in change between groups were tested with Wilcoxon Rank Sum W Test.

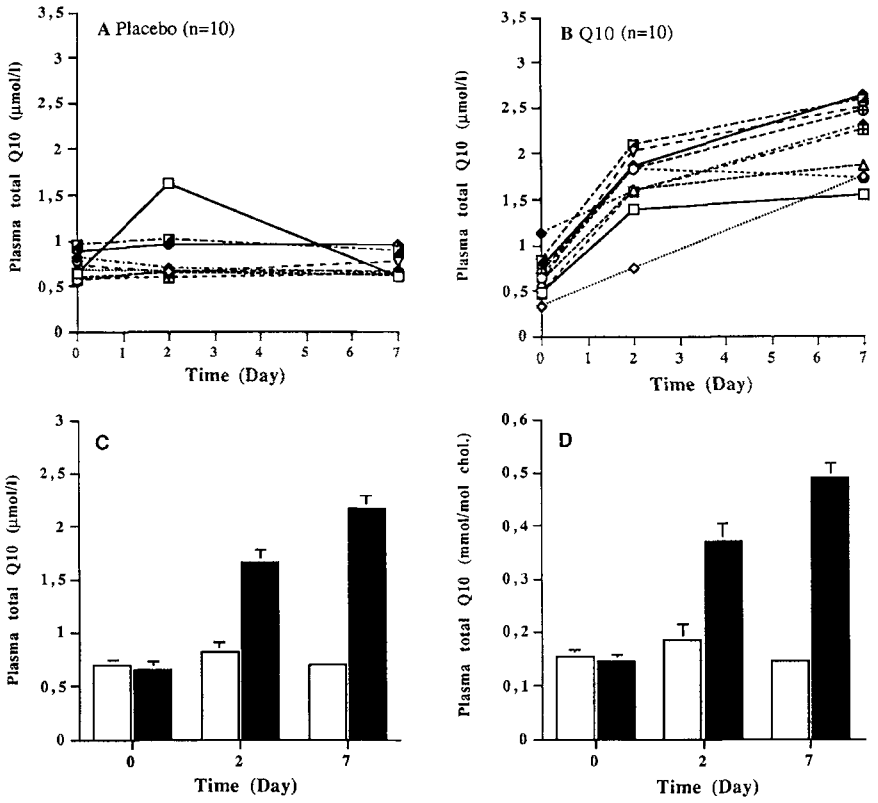

FIGURE 2 Absorption of 90 mg of Q10 daily in a one-week pharmacokinetic study in placebo (A) and in Q10 (B) groups. Means and standard errors are presented both for plasma Q10 (C) and plasma Q10/cholesterol ratio (D) in placebo (white bars) and Q10 (black bars) groups at different time points.

Q10 or in the plasma Q10/cholesterol ratio was observed in the placebo group.

Also in the pharmacokinetic study, the proportion of plasma ubiquinol of total Q10 increased during one-week supplementation by 2.9% (95% CI, 1.5–4.3%, $P = 0.005$) in the Q10 group. However, that change was not statistically significant, compared with the placebo group ($P = 0.082$, Table III).

DISCUSSION

This study was carried out to examine the separate effects and interaction between Q10 and vitamin E in the change of plasma concentrations and antioxidative capacity.

The assessment of oxidative stress and lipid peroxidation in humans is problematic. In addition to the lipid peroxidation method used, the sample preparation and storage may affect the results, especially with respect to Q10. Some loss of reduced Q10 may occur during sample preparation and storage.[27] As a consequence, the lipid peroxidation measurements were carried out as soon as possible after blood collection, or in samples frozen at −80°C, immediately after the samples were thawed. A rapid ultracentrifugation procedure was used to separate LDL.

In the present study, the proportion of plasma ubiquinol of total Q10 and the plasma ascorbate/total ascorbic acid ratio were used as *in vivo* indicators of lipid peroxidation. Previously, these two measurements have not been carried out in studies with combined Q10 and vitamin E supplementation. The Q10 ratio is an *in vivo* indicator (measurement at low radical flux) and probably a very sensitive index of plasma redox status or lipid peroxidation. According to previous studies, the proportion of ubiquinol of total Q10 is lowered in several pathological conditions including hyperlipidemia[28,29] and coronary artery disease.[30]

The copper induced LDL oxidation was used as a high radical flux measurement *in vitro*. This assay is a widely used lipid peroxidation measurement where the accumulation of conjugated dienes is to be assessed.

Our previous finding suggested that 90 mg of Q10 daily is not enough to increase lipoprotein oxidation resistance.[7] On the other hand, it has been proposed that regular statin treatment can decrease the plasma Q10 concentration.[31,32] Thus, to maximize the effects, we carried out the 3-month study in subjects having elevated serum cholesterol concentrations (accelerated lipid peroxidation was expected) and a regular HMG-CoA-reductase inhibitor treatment (lower baseline concentrations of plasma Q10 were expected).

Changes in Plasma Q10 and Vitamin E Concentrations following Supplementation

In the one-week pharmacokinetic study, supplementation with 90 mg of Q10 daily resulted in a 3.3-fold increase in plasma Q10 whether or not the plasma concentrations were corrected for cholesterol. Correspondingly, in the 3-month study, the Q10 supplementation with 200 mg of Q10 daily resulted in 6.1-fold increase in plasma Q10 and 700 mg of α-tocopherol daily correspondingly 2.3-fold elevation in plasma vitamin E (based on cholesterol corrected values). In the group receiving both antioxidants, the increase in plasma Q10 was only half of that observed in the Q10 group. Simultaneous Q10 supplementation did not affect the plasma concentration of vitamin E. On the basis of these data, we propose that there may be a pharmacokinetic interaction between Q10 and vitamin E. However, confirmation of this mechanism will require further investigation. Also in rabbits, supplementation with ubiquinone-10 has been observed to reduce the plasma concentration of α-tocopherol and vice versa (Kontush, A. *et al.*, University of Hamburg, Germany, personal communication).

LDL is a major lipoprotein containing Q10, and furthermore, most of the supplemented Q10

also accumulates into LDL and to a lesser extent into HDL, VLDL and IDL fractions.[20] We have observed recently that following supplementation, Q10 and vitamin E concentrations are elevated to a similar extent in plasma and in atherogenic plasma lipoproteins (VLDL + LDL).[7] Thus, it can be claimed that the elevation of the concentrations of these antioxidants in atherogenic lipoproteins can be estimated by measuring the corresponding change in their plasma levels.

Antioxidative Efficiency of Plasma Q10 and Vitamin E

Copper-induced Oxidation

Only vitamin E increased significantly the oxidation resistance of LDL. The present finding confirms previous observations[7–10] that vitamin E increases the oxidation resistance at high radical flux *ex vivo*, but Q10 does not have any effect, even when supplemented with as high a dose as 200 mg daily.

The Proportion of Plasma Ubiquinol of Total Q10

On the basis of *in vitro* studies, it has been claimed that not only vitamin C, but also ubiquinol can regenerate α-tocopherol radicals.[11,12,33] The decrease in the proportion of plasma ubiquinol of total Q10 in vitamin E group can be best explained by these *in vitro* studies. The vitamin E radical concentration possibly increases in lipoproteins as a consequence of high dose vitamin E supplementation. If plasma ubiquinol acts as a regenerator of these radicals, it would be consumed when vitamin E is supplemented, leading to a decrease in the proportion of plasma ubiquinol of total Q10. This finding may be the first *in vivo* evidence of the Q10-based regeneration of plasma vitamin E. However, we observed no synergism in the antioxidative efficiency between Q10 and vitamin E *ex vivo*, suggesting that Q10 is not a major regenerator of plasma vitamin E. Another less likely possibility for this finding is that high dose supplementation with vitamin E has harmful prooxidative effects *in vivo*, which are reflected in the decrease in the proportion of ubiquinol of total Q10. Previous studies have shown that this can possibly occur *in vitro*.[34,35] One shortcoming of this study was that the plasma tocopheryl radical concentration was not assessed.

Q10 supplementation increased the proportion of plasma ubiquinol of total Q10, even though no effect was observed at high radical flux-based LDL oxidation. As an *in vivo* assay, the Q10 ratio is perhaps a more sensitive measurement than copper oxidation. On the other hand, vitamin E did not confound this finding, because there was no difference in its levels between Q10 and placebo groups. This finding suggests that Q10 supplementation can possibly decrease plasma lipid peroxidation at a low radical flux *in vivo*. Our present finding that even a one-week supplementation with 90 mg of Q10 daily tended to elevate the Q10 ratio supports that observation.

Plasma Ascorbate/Total Ascorbic Acid Ratio and Plasma Ascorbyl Radicals

Previously, the ascorbate-based regeneration of tocopherol has been described in plasma and in lipoproteins *in vitro*.[36,37] These findings suggest that vitamin C is consumed also in the circulation, for tocopheryl radical regeneration rather than directly by pro-oxidants. However, in contrast to ubiquinol, the increase in plasma vitamin E concentration did not affect plasma ascorbate/total ascorbic acid ratio in the present study, i.e. there was no evidence for consumed vitamin C *in vivo*. This result suggests that, at the molecular level, ubiquinol seems to be a proportionally more important regenerator of α-tocopherol than vitamin C. However, at the body level, vitamin C is considered to be the major regenerator of α-tocopherol due to the high concentration of vitamin C in plasma.[38] It is also possible that

dehydroascorbate molecules are recycled very effectively, which could explain why no increase in their levels were observed.

Plasma ascorbyl radical concentration was measured later to determine whether it could be used as an *in vivo* indicator of oxidative stress in long term clinical supplementation trials. Until this study, ascorbyl radicals have been examined only in acute conditions.[39–41] Due to the fact that plasma samples were kept for two years at −80°C before their analysis, the priority of ascorbyl radical measurement is low in our study. Ascorbyl radical levels differed clearly between subjects (range 0–255 nmol/l), but they were not dependent on the plasma total ascorbic acid concentration. It remains unclear whether this finding was real or simply caused by the long-term sample storage. Baseline and three-month ascorbyl radical levels correlated strongly ($r = 0.6$ in Spearman correlation), indicating a good reproducibility of the assay. Vitamin E supplementation did not affect the plasma ascorbyl radical concentration.

SUMMARY

In conclusion, our present results suggest that d-α-tocopherol supplementation attenuates the elevation of plasma Q10 concentration in subjects given that coenzyme. Secondly, under high radical flux *in vitro*, vitamin E supplementation can significantly increase LDL oxidation resistance, whereas Q10 supplementation does not appear to have any effect. However, Q10 supplementation can significantly elevate the proportion of plasma ubiquinol of total Q10, which may indicate that there is decreased plasma lipid peroxidation *in vivo*. Thirdly, vitamin E supplementation decreases this proportion, which could be considered as *in vivo* evidence of the Q10-based regeneration of tocopheryl radicals. In contrast, α-tocopherol supplementation does not increase the oxidation of plasma ascorbate, the recognized major regenerator of vitamin E. On the basis of these data, we suggest that, following vitamin E supplementation, ubiquinol is consumed proportionally more effectively than vitamin C.

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References

[1] D. Mohr and R. Stocker (1994) Radical-mediated oxidation of isolated human very-low-density lipoprotein. *Arteriosclerosis, Thrombosis and Vascular Biology*, **14**, 1186–1192.

[2] R. Stocker, V.W. Bowry and B. Frei (1991) Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol. *Proceedings of the National Academy of Sciences*, **88**, 1646–1650.

[3] D.L. Tribble, J.J. van den Berg, P.A. Motchnik, B.N. Ames, D.M. Lewis, A. Chait and R.M. Krauss (1994) Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and α-tocopherol content. *Proceedings of the National Academy of Sciences*, **91**, 1183–1187.

[4] A. Kontush, C. Hubner, B. Finckh, A. Kohlschutter and U. Beisiegel (1994) Low density lipoprotein oxidizability by copper correlates to its initial ubiquinol-10 and polyunsaturated fatty acid content. *FEBS Letters*, **341**, 69–73.

[5] D. Mohr, V.W. Bowry and R. Stocker (1992) Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initiation of lipid peroxidation. *Biochimica et Biophysica Acta*, **1126**, 247–254.

[6] C. Weber, T. Sejersgard-Jakobsen, S.A. Mortensen, G. Paulsen and G. Holmer (1994) Antioxidative effect of dietary Coenzyme Q10 in human blood plasma. *International Journal for Vitamin and Nutrition Research*, **64**, 311–315.

[7] J. Kaikkonen, K. Nyyssönen, E. Porkkala-Sarataho, H.E. Poulsen, T. Metsä-Ketelä, M. Hayn, R. Salonen and J.T. Salonen (1997) 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. *Free Radical Biology and Medicine*, **22**, 1195–1202.

[8] I. Jialal, C.J. Fuller and B.A. Huet (1995) The effect of α-tocopherol supplementation on LDL oxidation. A dose-response study. *Arteriosclerosis, Thrombosis and Vascular Biology*, **15**, 190–198.

[9] H.M. Princen, W. van Duyvenvoorde, R. Buytenhek, A. van der Laarse, G. van Poppel, J.A. Gevers-Leuven and V.W. van Hinsbergh (1995) Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arteriosclerosis, Thrombosis and Vascular Biology*, **15**, 325–333.

[10] M. Dieber-Rotheneder, H. Puhl, G. Waeg, G. Striegl and H. Esterbauer (1991) Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *Journal of Lipid Research*, **32**, 1325–1332.

[11] J.E. Packer, T.F. Slater and R.L. Wilson (1979) Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature*, **278**, 737–738.

[12] S.R. Thomas, J. Neuzil and R. Stocker (1996) Cosupplementation with coenzyme Q prevents the prooxidant effect of α-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. *Arteriosclerosis, Thrombosis and Vascular Biology*, **16**, 687–696.

[13] E. Cadenas, P. Hochstein and L. Ernster (1992) Pro- and antioxidant functions of quinones and quinone reductases in mammalian cells. *Advances in Enzymology and Related Areas of Molecular Biology*, **65**, 97–146.

[14] K. Folkers, S. Moesgaard and M. Morita (1994) A one year bioavailability study of coenzyme Q10 with 3 months withdrawal period. *Molecular Aspects of Medicine*, **15**, 281–285.

[15] H. Kishi, N. Kanamori, S. Nishii, E. Hiraoka, T. Okamoto and T. Kishi (1984) Metabolism of exogenous coenzyme Q10 *in vivo* and the bioavailability of coenzyme Q10 preparations in Japan. In *Biomedical and Clinical Aspects of Coenzyme Q*, Vol. 4 (Eds. K. Folkers and Y. Yamamura), Elsevier Science Publishers B.V., Amsterdam, pp. 131–142.

[16] P.W. Lucker, N. Wetzelsberger, G. Hennings and D. Rehn (1984) Pharmacokinetics of coenzyme ubidecarenone in healthy volunteers. In *Biomedical and Clinical Aspects of Coenzyme Q*, Vol. 4 (Eds. K. Folkers and Y. Yamamura), Elsevier Science Publishers B.V., Amsterdam, pp. 143–151.

[17] Y. Tomono, J. Hasegawa, T. Seki, K. Motegi and N. Morishita (1986) Pharmacokinetic study of deuterium-labelled coenzyme Q10 in man. *International Journal of Clinical Pharmacology, Therapy and Toxicology*, **24**, 536–541.

[18] M. Weis, S.A. Mortensen, M.R. Rassing, J. Moller-Sonnergaarg, G. Poulsen and S.N. Rasmussen (1994) Bioavailability of four oral coenzyme Q10 formulations in healthy volunteers. *Molecular Aspects of Medicine*, **15**, 273–280.

[19] K. Johansen, H. Theorell, J. Karlsson, B. Diamant and K. Folkers (1991) Coenzyme Q10, Alpha-tocopherol and free cholesterol in HDL and LDL fractions. *Annals of Medicine*, **23**, 649–656.

[20] R. Laaksonen, A. Riihimäki, J. Laitila, K. Mårtensson, M.J. Tikkanen and J.J. Himberg (1995) Serum and muscle tissue ubiquinone levels in healthy subjects. *Journal of Laboratory and Clinical Medicine*, **125**, 517–521.

[21] B. Finckh, A. Kontush, J. Commentz, C. Hubner, M. Burdelski and A. Kohlschutter (1995) Monitoring of ubiquinol-10, ubiquinone-10, carotenoids, and tocopherols in neonatal plasma microsamples using high-performance liquid chromatography with coulometric electrochemical detection. *Analytical Biochemistry*, **232**, 210–216.

[22] D.I. Thurnham, E. Smith and P.S. Flora (1988) Concurrent liquid-chromatographic assay of retinol, α-tocopherol, β-carotene, α-carotene, lycopene, and β-cryptoxanthin in plasma, with tocopherol acetate as internal standard. *Clinical Chemistry*, **34**, 377–381.

[23] E. Porkkala-Sarataho, K. Nyyssönen and J.T. Salonen (1996) Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in non-vitamin E supplemented men. *Atherosclerosis*, **124**, 83–94.

[24] K. Nyyssönen, S. Pikkarainen, M.T. Parviainen, K. Heinonen and I. Mononen (1988) Quantitative estimation of ascorbic acid and dehydroascorbic acid by high-performance liquid chromatography. Application to human milk, plasma and leukocytes. *Journal of Liquid Chromatography*, **11**, 1717–1728.

[25] K. Nyyssönen, H.E. Poulsen, M. Hayn, P. Agerbo, E. Porkkala-Sarataho, J. Kaikkonen, R. Salonen and J.T. Salonen (1997) Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation. *European Journal of Clinical Nutrition*, **51**, 154–163.

[26] R. Salonen, K. Nyyssönen, E. Porkkala, J. Rummukainen, R. Belder, J.S. Park and J.T. Salonen (1995) Kuopio atherosclerosis prevention study (KAPS): a population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. *Circulation*, **92**, 1758–1764.

[27] S. Yamashita and Y. Yamamoto (1997) Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Analytical Biochemistry*, **250**, 66–73.

[28] A. Kontush, A. Reich, K. Baum, T. Spranger, B. Finckh, A. Kohlschutter and U. Beisiegel (1997) Plasma ubiquinol-10 is decreased in patients with hypelipidaemia. *Atherosclerosis*, **129**, 119–126.

[29] Y.B. de Rijke, S.J. Bredie, P.N. Demacker, J.M. Vogelaar, H.L. Hak-Lemmers and A.F. Stalenhoef (1997) The redox status of coenzyme Q10 in total LDL as an indicator of *in vivo* oxidative modification. Studies on subjects with familiar combined hyperlipidemia. *Arteriosclerosis, Thrombosis and Vascular Biology*, **17**, 127–133.

[30] J. Lagendijk, J.B. Ubbink, R. Delport, W.J. Vermaak and J.A. Human (1997) Human Ubiquinol/ubiquinone ratio as marker of oxidative stress in coronary artery disease. *Research Communications in Molecular Pathology and Pharmacology*, **95**, 11–20.

[31] K. Folkers, P. Langsjoen, R. Willis, P. Richardson, L.J. Xia, C.Q. Ye and H. Tamagawa (1990) Lovastatin decreases coenzyme Q levels in humans. *Proceedings of the National Academy of Sciences*, **87**, 8931–8934.

[32] G. Ghirlanda, A. Oradei, A. Manto, S. Lippa, L. Uccioli, S. Caputo, A.V. Grego and G.P. Littarru (1993) Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitor: a double-blind, placebo-controlled study. *Journal of Clinical Pharmacology*, **33**, 226–229.

[33] R.E. Beyer (1992) An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. *Biochemistry and Cell Biology*, **70**, 390–403.

[34] A. Kontush, B. Finckh, B. Karten, A. Kohlschutter and U. Beisiegel (1996) Antioxidant and prooxidant activity of alpha-tocopherol in human plasma and low

density lipoprotein. *Journal of Lipid Research*, **37**, 1436–1448.

[35] J. Neuzil, S.R. Thomas and R. Stocker (1997) Requirement for, promotion, or inhibition by α-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. *Free Radical Biology and Medicine*, **22**, 57–71.

[36] M.K. Sharma and G.R. Buettner (1993) Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *Free Radical Biology and Medicine*, **14**, 649–653.

[37] V.E. Kagan, E.A. Serbinova, T. Forte, G. Scita and L. Packer (1992) Recycling of vitamin E in human low density lipoproteins. *Journal of Lipid Research*, **33**, 385–397.

[38] B. Halliwell (1995) Oxidation of low-density lipoproteins: questions of initiation, propagation, and the effect of antioxidants. *American Journal of Clinical Nutrition*, **61**, 670–677.

[39] S. Pietri, J.R. Seguin, P.D. d'Arbigny and M. Culcasi (1994) Ascorbyl free radical: a non-invasive marker of oxidative stress in human open-heart surgery. *Free Radical Biology and Medicine*, **16**, 523–528.

[40] C. Vergely, V. Maupoil, M. Benderitter and L. Rochette (1998) Influence of the severity of myocardial, ischemia on the intensity of ascorbyl free radical release and on postischemic recovery during reperfusion. *Free Radical Biology and Medicine*, **24**, 470–479.

[41] K. Nakagawa, H. Kanno and Y. Miura (1997) Detection and analyses of ascorbyl radical in cerebrospinal fluid and serum of acute lymphoblastic leukemia. *Analytical Biochemistry*, **254**, 31–35.