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Relative Bioavailability and Antioxidant Potential of Two Coenzyme Q10 Preparations

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Key Words
Coenzyme Q10 · Bioavailability · Antioxidant · Yeast · Fermentation

Abstract
Coenzyme Q10 (CoQ10) is synthesized by the human body and found in certain foods. Daily supplementation of CoQ10 could protect against heart disease but the bioavailability of CoQ10 supplements depends on the formulation taken. We compared the bioavailability and antioxidant properties of two commercial CoQ10 formulations, a commercial grade CoQ10 powder (commercial grade CoQ) and a new BT-CoQ10 BIO-TRANSFORMED® (BT-CoQ10) obtained by fermentation of a soy-based, CoQ10-rich media with baker's yeast. Eleven healthy individuals participated in a randomized two-way crossover trial, with a 3-week washout period. Capsules containing 300 mg of either BT-CoQ10 or commercial grade CoQ10 were given daily for 1 week and multiple blood samples were taken for CoQ10, glutathione and glutathione peroxidase (GPx) determination. In 3 subjects, baseline plasma CoQ10 levels were lower prior to BT than prior to commercial grade CoQ treatment. In the remaining participants, ingestion of BT vs. commercial grade CoQ significantly increased maximum plasma CoQ10 concentration (+126%, $p = 0.04$) and tended to increase CoQ10 area under the curve from 0 to 24 h (+160%, $p = 0.07$). One week of treatment with each formulation increased plasma CoQ10 but did not alter plasma glutathione or GPx activity. The enhanced bioavailability of the BT product might be due to its predominantly reduced, hydrophilic membrane-complex form.

Introduction

Coenzyme Q10 (CoQ10) is an obligatory redox component of the mitochondrial respiratory chain, which is also postulated to protect LDL from the oxidative damage. Several studies suggest that CoQ10 plays an important role in cardiovascular health [1]. Low myocardial tissue CoQ10 levels have been found in patients with cardiovascular disease [2] and low ratios between the reduced and oxidized form of CoQ10 were reported in patients with angiographically confirmed coronary artery disease [3]. Plasma CoQ10 concentrations were also found to be reduced by treatment with statins [4].

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CoQ10 is produced in the body as well as obtained from dietary sources. It has been postulated that daily CoQ10 supplementation could benefit individuals at risk of or suffering from heart disease. Previous experimental and human studies showed that dietary CoQ10 supplements are bioavailable but their uptake into the bloodstream is dependent on the formulation [5, 6]. The antioxidant properties of CoQ10 preparations may be relative to their bioavailability since beneficial responses were found in animals [7] but not in humans [6, 8]. The present study was designed to compare the bioavailability of two commercial CoQ10 products, an oxidated commercial grade formulation and a newly developed reduced form obtained by yeast fermentation with a soy-rich media. An additional objective was to compare the antioxidant potential of both products after a 1-week period of supplementation, by measuring changes in selected indices of antioxidant protection, plasma concentrations of glutathione and activity of glutathione peroxidase. Previous studies demonstrated that in animals and humans subjected to oxidative stress and given supplement of CoQ10, increases in total and reduced plasma CoQ concentration were associated with increased whole blood glutathione levels [9, 10]. Other reports also suggested that CoQ10 administration may prevent oxidative stress-induced increases in activity of glutathione peroxidase [11, 12].

Subjects and Methods

Formulations

Two different CoQ10 preparations, the commercial grade CoQ10 powder and the BT-CoQ10 BIO-TRANSFORMED® (BT-CoQ10), were used in this study. The commercial grade preparation was crystalline CoQ10 (distributed by Aceto Corp.) and the new BT-CoQ10 was developed by fermentation of a soy-based, CoQ10-rich media with baker's yeast *(Saccharomyces cerevisiae)*. The CoQ10 content of the commercial grade product was 98.7% while for the BT product it was 7.61%, as determined by the modified method of Vadhanavikit et al. [13] (1984) described below. The in vitro antioxidant potential of both formulations was assessed prior to the study. This was done by sodium hydrosulfite reduction of CoQ [14] followed by copper-induced lipoprotein oxidation assay [15] with fluorometric detection of lipid peroxidation products [16]. The results showed that substantially lower concentrations of BT product than commercial grade CoQ product were needed to inhibit lipoprotein oxidation by 50% (7.5 vs. 30.0 μM/l for BT vs. commercial grade CoQ, respectively), suggesting a superior antioxidant potential of BT formulation [unpubl. data]. Both formulations were mixed with powdered ω–3 fatty acids (1.155 mg/mg CoQ10) and encapsulated into hard gelatin capsules. The daily dosages were designed to contain 300 mg of CoQ10 and 346.5 mg of ω–3 fatty acids.

Study Design

The study was conducted as a randomized two-period crossover trial. Eleven healthy volunteers (6 males and 5 females, with a mean (± SD) age of 30.9 ± 10.8 years, average body weight of 70.7 ± 11.7 kg and average body mass index 24.3 ± 4.7 kg/m^2) were assigned to receive capsules containing either the BT product or the commercial grade CoQ10 product for a period of 1 week. A single daily dose of the BT product was 9 capsules and a single daily dose of the commercial grade product was 3 capsules (4.4 ± 1.0 mg CoQ10/kg b.w./day). Blood samples were collected in heparinized tubes either by direct venipuncture or via a catheter inserted into the antecubital vein, immediately prior to the first dose, at 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24 h after administration of the first dose, and at the end of the 1-week treatment period. Plasma was separated and stored at –70°C. After the 3-week washout, participants were given the second CoQ10 formulation at the same dosage and the protocol was repeated. The subjects were instructed to fast overnight prior to each study day where the initial CoQ10 dose was administered (at 8 a.m.) and for the first 4 h following the administration of the first dose of each preparation. The subjects consumed a standardized low CoQ lunch at 12 noon and low CoQ dinner (no organ meat) on the day of the initial dose. All participants were instructed to avoid grapefruit and herbal products 1 week before the study and during the study. They were also asked not to consume alcohol and caffeine-containing products 72 h before and 24 h after the first dose of each CoQ10 preparation. The experimental protocol was approved by the Human Ethics Committee of The University of Western Ontario and informed consent was obtained from each subject.

Methods of Analysis

All plasma samples were assayed for concentration of CoQ10. The CoQ10 was extracted by the modified method of Vadhanavikit et al. [13]. Briefly, 0.5-ml plasma samples were mixed with an equal volume of 20% dodecyl sulfate in saline and with CoQ9 (internal standard). Samples were mixed for 5 min with 2 ml of methanol:isopropanol (95:5), and then back extracted into 4 ml of hexane by mixing for 15 min. The aqueous and organic layers were separated by centrifugation. The organic layer was dried and the residue was dissolved in 300 µl of ethanol. The ethanol extract was kept in the refrigerator for 2 h to allow complete oxidation. Twenty microliters of the extract were analyzed by reversed-phase HPLC using the Hewlett-Packard 1100 Series system equipped with a variable wavelength detector and ChemStation. Chromatography was carried out on Phenomenex Prodigy ODS3 C18 column (5 µm, 15 cm × 4.6 mm). The mobile phase was methanol:ethanol (40:60). The column was maintained at a flow rate of 1.5 ml/min and the total oxidized CoQ10 was detected at 275 nm [17]. Serum spiked with purified CoQ10 was used as quality control. The CoQ10 recovery was 98 ± 1%. The reproducibility of the procedure was excellent, with within-run and between-run coefficients of variation determined to be less than 1%.

Plasma samples taken at baseline and at the end of week 1 of each treatment were also tested for glutathione peroxidase activity and for plasma glutathione concentration. Glutathione peroxidase activity was measured with a colorimetric kit from Randox Laboratories Canada Ltd (Mississauga, Ont., Canada). Plasma total glutathione concentrations were determined by the modified method of Jacobsen et al. [18]. Briefly, the plasma (50 µl) was mixed with 2.5 µl of *n*-amyl alcohol and with 18 µl of 1.43 *M* sodium borohydride (made in 0.05 *M* NaOH) and the mixture was incubated for 5 min at 40°C. After neutralization with 20 µl of 1.2 *M* HCl, 25 µl monobromobi-

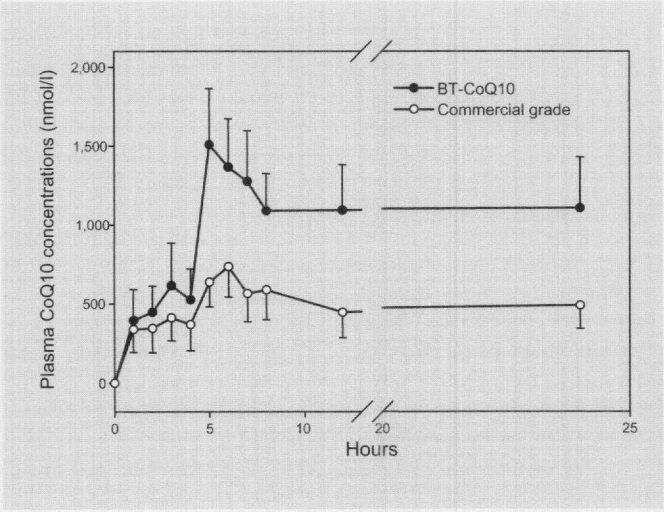

Fig. 1. Mean plasma CoQ10 concentration-time profile (all subjects). Values ± SEM.

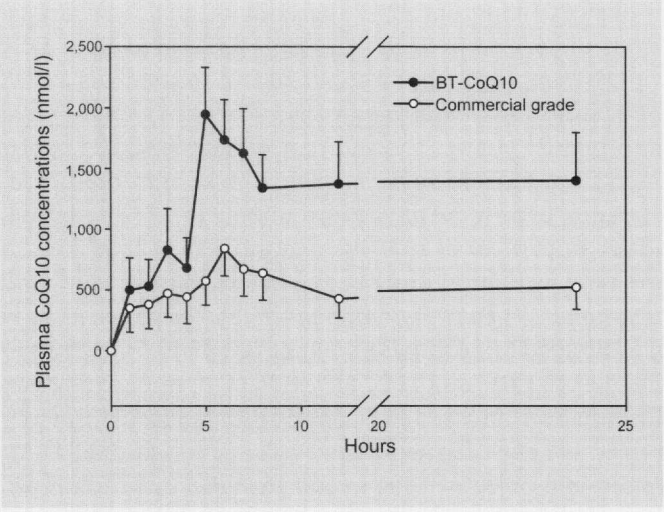

Fig. 2. Mean plasma CoQ10 concentration-time profile (three outliers excluded). Values ± SEM.

mane (12.5 m*M* solution in 4 m*M* EDTA, pH = 7.0) was added and the mixture was incubated at 40°C for 15 min. After cooling the sample to room temperature, 25 µl of 1.5 *M* perchloric acid was added. The mixture was incubated at room temperature for 10 min and centrifuged at 11,500 rpm for 3 min. The supernatant was neutralized with 20 µl of 2 *M* citrate (in 10 *M* NaOH) and re-centrifuged under the same conditions. Ten microliters of the supernatant were analyzed by reversed-phase HPLC Hewlett-Packard 1090 system equipped with a Shimadzu fluorescence detector. Chromatography was carried out on a Spherisorb C8 column (5 µm, 10 cm × 3.2 mm; packed in-house). A step gradient was developed across the column with an initial phase of 4% acetonitrile in 25 m*M* ammonium formate buffer (pH 3.8) and a second wash phase of 70% acetonitrile in 10 m*M* KH_2PO_4 buffer containing 400 µl triethylamine/l (pH 3.0) after 12 min. The column was maintained at a flow rate of 0.5 ml/min and peaks were detected using an excitation wavelength of 390 nm and emission wavelength of 480 nm.

Data Analysis

To compare the oral bioavailability of the two preparations, the AUC from 0 to 24 h (nmol/l × h), the maximum plasma concentration, C_{max} (nmol/l), and the time to maximum concentration, T_{max} (h), were determined for each formulation. Plasma CoQ10 concentration-time curves were plotted and the $AUC_{0-24\ h}$ was calculated using the linear trapezoid rule with exclusion of basic AUC. Basic AUC was calculated as an integral from time 0 to 24 h of the area between the time axis and the CoQ10 concentration at baseline. Individual C_{max} and T_{max} values were determined by visual inspection of the plasma concentration data. Statistical comparisons of $AUC_{0-24\ h}$, C_{max} and T_{max} between the two groups were performed using the nonparametric test for matched pairs (Wilcoxon sign rank test).

Statistical comparisons between the two preparations with respect to their effect on plasma CoQ10 after the 1-week treatment and with respect to their antioxidant potential (glutathione peroxidase activity, glutathione concentration) were performed using the unpaired t test.

Results

All subjects participated in both phases of the study. The mean plasma concentration-time profile for both CoQ10 formulations are depicted in figure 1. The parameters characterizing bioavailability of CoQ10 were not significantly different between the two formulations, as determined by the Wilcoxon sign rank test. However, the mean $AUC_{0-24\ h}$ tended to be greater for the BT than for commercial grade CoQ preparation (24,212 ± 21,074 vs. 11,130 ± 11,474 nmol/l × h, respectively), representing 118% increase over the commercial grade product. The C_{max} also tended to be higher after a dose of the BT (1,683 ± 1,155 nmol/l) than after a dose of the commercial grade CoQ capsules (938 ± 606 nmol/l), representing 79% increase while T_{max} remained similar between the treatments (6.0 ± 1.3 vs. 5.9 ± 3.0 h for BT and commercial grade CoQ, respectively). With both formulations, some subjects appeared to better absorb CoQ10 than others, which resulted in the large inter-individual variation in the $AUC_{0-24\ h}$ and in the C_{max}. The post-hoc analysis revealed that due to the wide variability, the statistical power in the trial was 34%. Therefore, to detect a difference in AUC at $\alpha = 0.05$, the study would need 30 participants.

Three out of 11 subjects participating in the study had substantially higher baseline plasma CoQ10 concentration prior to the commercial grade dose than prior to the BT dose. As a result, the mean baseline plasma CoQ10 concentrations were substantially lower before the BT

Table 1. Comparison of plasma CoQ10 concentrations and the AUC following oral administration of two CoQ formulations (baseline subtracted, three outliers excluded)

	BT-CoQ10	Commercial grade	p value
$AUC_{0-24\,h}$, nmol/l × h	30,517 ± 21,509	11,754 ± 11,289	0.0700
C_{max}, nmol/l	2,118 ± 1,050	936 ± 645	0.0391*
T_{max}, h	5.625 ± 0.916	6.143 ± 1.676	0.9375

Values are means ± SD. * Significantly different by Wilcoxon sign rank test.

Table 2. Effect of treatment with two CoQ preparations (300 mg/day, 1 week) on plasma CoQ concentrations (nmol/l), plasma glutathione concentrations (μmol/l) and activity of glutathione peroxidase (U/l) (all subjects) (values are means ± SD)

	Plasma CoQ concentration		Plasma glutathione concentration		Activity of glutathione peroxidase	
	BT-CoQ10	commercial grade	BT-CoQ10	commercial grade	BT-CoQ10	commercial grade
Baseline	2,085 ± 1,008	2,884 ± 1,089	4.6 ± 2.1	5.8 ± 3.2	193.9 ± 44.7	193.6 ± 46.5
One week	8,096 ± 3,602	8,687 ± 3,602	3.2 ± 1.9	4.5 ± 2.8	196.6 ± 46.4	210.5 ± 64.4
Change from baseline	+6,011 ± 3,437	+5,809 ± 2,847	−1.4 ± 1.5	−1.3 ± 2.2	+2.7 ± 22.9	+16.9 ± 50.1
Change, %	+367	+205	−30	−22	+1	+9

period than before the commercial grade CoQ period (2,085 ± 1,008 vs. 2,884 ± 1,089 nmol/l, respectively). Since in healthy individuals not exposed to CoQ10-rich foods or supplements, fasting plasma CoQ10 levels were not expected to substantially fluctuate, baseline plasma CoQ10 concentrations and bioavailability parameters were compared between treatments after exclusion of three outliers. The mean $AUC_{0-24\,h}$, C_{max} and T_{max} values for 8 subjects are shown in table 1. The corresponding mean plasma concentration-time profiles are also presented in figure 2.

After exclusion of outliers, the baseline plasma CoQ10 levels became similar between the two treatments (2,409 ± 1,054 and 2,479 ± 996 nmol/l for BT and commercial grade CoQ, respectively). C_{max} was significantly higher for the BT than for the commercial grade CoQ (2.26:1, $p = 0.0391$) and $AUC_{0-24\,h}$ also showed a strong tendency to be higher for BT than for commercial grade CoQ formulation (2.6:1, $p = 0.07$).

Effects of the 1-week treatment with BT vs. commercial grade CoQ formulations on plasma CoQ10 concentrations and on selected indices of plasma antioxidant potential are presented in table 2. The results show that exposure to both BT and commercial grade CoQ capsules caused substantial increases in plasma CoQ10 concentrations (3.9- and 3.0-fold for BT and commercial grade CoQ, respectively) but the final plasma CoQ10 concentrations after the 1-week exposure to the BT product were not significantly higher than those found after a similar exposure to commercial grade CoQ. The observed elevation of the final plasma CoQ10 levels was not associated with substantial changes in plasma glutathione or in the activity of plasma glutathione peroxidase, although both products tended to reduce glutathione concentrations (by 30 and 22% for BT and commercial grade CoQ, respectively). Final plasma glutathione concentrations and the final activity of plasma glutathione peroxidase were also not significantly different between the two treatments.

Discussion

The results of our study demonstrated that in healthy adults, the CoQ10 present in the BT formulation was more bioavailable than the CoQ10 present in the commercial grade formulation. The BT product increased C_{max} and tended to increase $AUC_{0-24\,h}$ but did not affect T_{max}. The differences in the pharmacokinetic profiles were obscured by the fact that in spite of all precautions, 3 subjects had much higher baseline CoQ10 concentrations before treatment with commercial grade CoQ than before treatment with the BT capsules. The elevated baseline CoQ10 concentrations were not related to the length of the washout period since one out of the three outliers took

commercial grade CoQ first. The 3 subjects might have ingested high CoQ10 foods and/or substances interacting with CoQ10 absorption during the last few days preceding baseline blood sampling.

The improved bioavailability observed for the BT versus the commercial grade CoQ formulations could be related to different chemical forms of CoQ10 and different intramolecular interactions predominant in commercial grade versus the fermentation-enhanced product. A purified, oxidated form of CoQ10 present in commercial grade formulation has been shown to be poorly absorbable due to its substantial hydrophobicity and tendency to aggregate [19], although its absorption might have been more efficient in the presence of ω–3 fatty acids. In previous studies, the absorption of similar CoQ10 formulations was facilitated by dispersing agents such as emulsifiers, phospholipids and vegetable oils [5]. In contrast, the BT preparation contained CoQ10 predominantly in the reduced, more hydrophilic form. The reduced CoQ10 has been shown to be more stable in lipid bilayers and therefore could be very well dispersed due to its association with the membrane system of the yeast, possibly approximating a molecular arrangement proposed by Quinn [19].

The CoQ10 AUC profiles obtained in the study were comparable to those described in previous pharmacokinetic trials, with plasma concentrations of CoQ10 reaching maximum 6 h after dosing [20]. The efficacy of absorption of both products (defined as percent of initial dose present in the plasma at T_{max} assuming a 2.5 l total plasma volume), calculated to be 1.2 and 0.7% for BT and commercial grade CoQ capsules, respectively, was also within the range reported earlier for similar doses of CoQ10 [21]. The relatively low overall bioavailabilities of both products were likely related to large oral doses, since absorption of nutrients generally tends to diminish with increasing doses, due to saturation of transport system in the intestine. Administration of large doses of CoQ10 could also diminish the difference in pharmacokinetic parameters between the treatments, if their absorption occurred in suboptimal conditions.

The observed increases in plasma CoQ10 levels from baseline to the end of the 1-week treatments were comparable to those found earlier for the most bioavailable formulations of CoQ10 [6, 22]. However, in our study, both baseline and post-treatment CoQ10 concentrations were 3–4 times higher than those reported previously. The apparent discrepancy might be attributed to analytical differences between the laboratories and/or subject group differences. The increases in plasma CoQ10 concentrations from baseline to the end of the 1-week treatment period tended to be more pronounced for the BT than for the commercial grade CoQ product, but the difference did not reach statistical significance, possibly due to the relatively short period of exposure to both formulations. The elevated plasma CoQ10 concentrations observed at the end of each treatment were not associated with the improvement in indices of plasma antioxidant properties, in spite of greater in vitro antioxidant potential observed for BT than for commercial grade CoQ product. The lack of effect might be again related to the short period of exposure and/or to the fact that the young healthy adults participating in this study already had high plasma antioxidant potential and the therapy with CoQ10 could not improve it further.

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