Comparison of the relative bioavailability of different coenzyme Q\textsubscript{10} formulations with a novel solubilize (Solu\textsuperscript{TM} Q10)

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Abstract
The relative bioavailability of coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) is markedly influenced by its delivery systems. The aim of this study was to compare four standard CoQ\textsubscript{10} supplements available on the market with a novel solubilize formulation of CoQ\textsubscript{10} (Solu\textsuperscript{TM} Q10). Pharmacokinetic parameters were assessed in 54 healthy volunteers after single and multiple intakes of 60 mg CoQ\textsubscript{10} over a time period of 14 days. Solubilizes showed earlier flooding compared with oily dispersions and crystalline CoQ\textsubscript{10}, resulting in significantly elevated area under the curve between 0 and 4 h (\(P < 0.01\) solubilizes versus crystalline). The difference in the pharmacokinetic parameters of maximum plasma concentration, time to reach the peak plasma concentration and area under the curve between 0 and 12 h was not statistically significant between formulations. Long-term supplementation resulted in significantly higher plasma levels (\(P < 0.01\)) for all formulations, with Solu\textsuperscript{TM} Q10 performing best. Intracellular CoQ\textsubscript{10} levels measured in buccal mucosa cells were increased (\(P < 0.05\)) in response to supplementation when starting within the physiological range. In summary, solubilizes were clearly superior to oily dispersions and crystalline CoQ\textsubscript{10} in their overall bioavailability, with the best absorption characteristics seen for the novel Solu\textsuperscript{TM} Q10 solubilize.

Keywords: Coenzyme Q10, bioavailability, solubilize, oily dispersion, buccal mucosa cells

Introduction
Coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) is a ubiquinone present in both animal and plant cellular membranes. It is ubiquitously found in the diet with daily intakes ranging from 3 to 5 mg/day (Weber et al. 1997). Besides uptake with food, CoQ\textsubscript{10} is endogenously synthesized via the mevalonate pathway. CoQ\textsubscript{10} plays a key role in mitochondrial cell physiology as part of the electron transfer chain. In addition, CoQ\textsubscript{10} is an important antioxidant. The cellular CoQ\textsubscript{10} content is homeostatically regulated; however, it is altered in a number of disease states and during statin therapy. Ghirlanda et al. (1993) showed a 40% decrease in plasma CoQ\textsubscript{10} levels after treatment of hypercholesterolemia. During statin therapy, co-treatment with CoQ\textsubscript{10} has been suggested (Silver

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et al. 2003). Moreover, as part of a physiological process, CoQ10 levels in blood and tissues decline during aging (Crane 2001). Oral supplementation with CoQ10 has been shown to improve the health status in several diseases (Shults et al. 2002; Beal and Shults 2003; Cooper and Schapira 2003; Roffe et al. 2004).

Since the bioavailability of CoQ10 in humans is relatively low due to its lipophilic nature and large molecular weight, several formulation technologies exist to improve its bioavailability from dietary supplements (and therapeutics). Different galenic preparations are available: crystalline CoQ10 powder in hard gelatin capsules, as well as oily dispersions and solubilizates of CoQ10 in soft gel capsules. These formulations largely differ in their bioavailability of CoQ10. The main objective of the present study was to compare CoQ10 supplements (market standard formulations) with a novel solubilize formulation of CoQ10 (Solu™ Q10).

Since CoQ10 underlies biologic regulation and metabolism, we focused on early absorption parameters (0–4 h after ingestion). Additionally, long-term accumulation in blood and tissue was examined after consecutive dosing during 2 weeks.

The effect of CoQ10 supplementation on tissue levels was analyzed using buccal mucosa cells as a marker tissue.

Materials and methods

Study design

The study was approved by the ethics committee in Stuttgart, Germany and informed consent was obtained from each participant prior to enrolment to the trial. It was performed as an open, comparative mono-center design. Fifty-four male healthy volunteers were randomly assigned to one of five study arms. The test substances were either commercially available CoQ10 supplements, the novel Solu™ Q10 formulation filled in soft gel capsules (5% CoQ10) or pure, crystalline CoQ10 filled in hard gel capsules.

Study preparations

The study arms were as follows: Solubilize 1, Solu™ Q10 (patented and produced by AQUANOVA® German Solubilize Technologies (AGT) GmbH, Darmstadt, Germany) consisting of 30 mg CoQ10 per soft gel capsule in medium-chain triglycerides and polysorbate 80; Solubilize 2, Swanson Ultra™ Q-Get® soft gel capsules (Tishcon Corp., Westbury, NY, USA) consisting of 30 mg CoQ10 and 6 IU vitamin E in gelatin, purified water, glycerin, water, titanium dioxide, annatto seed extract, polysorbate 80, medium-chain triglycerides, sorbitol and sorbitan monooleate; Oily dispersion 1, CoQsol® soft gel capsules (Soft Gel Technologies, Inc., Los Angeles, CA, USA) consisting of 30 mg CoQ10, 1295 IU Vitamin A (100% as β-carotene) and 30 IU vitamin E in rice bran oil, yellow beeswax, gelatin, glycerin, water and annatto extract; Oily dispersion 2, Nature Made® CoQ10 soft gel capsules (Pharmavite®, Northridge, CA, USA) containing 30 mg CoQ10 and 1500 IU vitamin A (100% as β-carotene) in soybean oil, gelatin, glycerin and water; and Crystalline CoQ10, 30 mg crystalline CoQ10 (ubidecarenone) and cornstarch filled in hard gel capsules.

Each study arm comprised 12 volunteers except for the arm receiving crystalline CoQ10, which consisted of six volunteers (this arm served as a control group only).
confirm the known poor absorption characteristics of crystalline CoQ$_{10}$ under the study conditions. All supplements were given at a dosage of 60 mg/day.

Screening and sampling

Each subject was determined to be healthy by medical history, physiologic examination, electrocardiograph examination and blood routine profile, including hematology, fat status, liver and kidney parameters. Further criteria were met: age 18–30 years, non-smoker, body mass index 19–30 Kg/m$^2$, and total cholesterol 110–250 mg/dl. Intake of CoQ$_{10}$ supplements during the trial and 4 weeks prior to the trial start was not allowed.

Following an overnight fast, blood samples for determination of baseline CoQ$_{10}$ levels were taken. After consumption of a single dose of 60 mg CoQ$_{10}$ venous blood samples were collected in ethylenediamine tetracetic acid tubes via a central venous blood catheter at 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h after dosing. The capsules were taken with a standardized breakfast including 15 g butter, 200 ml apple juice and two bread rolls. Further standardized meals were served during the kinetic day. Twenty-four hours after this first dosing, a second fasting blood sample was taken. Dosing continued for further 15 days in the morning with a standardized breakfast. On days 7 and 15 blood samples were taken 24 h after the last morning dose, again after overnight fast. Plasma was separated and stored at −80°C until analysis.

Besides plasma concentrations, CoQ$_{10}$ was determined in buccal mucosa cells (BMC) on day 1 (prior to CoQ$_{10}$ intake) and on day 15 at 24 h after the last morning dose. Collection of buccal mucosa cells was performed as described previously (Peng et al. 1995).

Methods of analysis

Total CoQ$_{10}$ was analyzed in blood and tissue samples after oxidation with benzoquinone. CoQ$_{10}$ was extracted by the modified method of Mosca et al. (2002). In brief, CoQ$_{10}$ in plasma was incubated with benzoquinone. After extraction with isopropanol, the supernatant was used for analysis with reversed-phase high-performance liquid chromatography using an Alliance 2690 System (Waters, Eschborn, Germany). CoQ$_{10}$ was separated with methanol:ethanol (35:65) as the mobile phase and detected with an ultraviolet-visible detector ($\lambda = 275$ nm) (UV-Detector 2487; Waters). Peak areas were analyzed and the quantification was performed by calibration with external standards (Coenzyme Q$_{10}$; Sigma-Aldrich, Munich, Germany).

In case of BMC, the CoQ$_{10}$ content of the cell pellet was calculated and corrected with the DNA content for standardization. Following sample preparation for high-performance liquid chromatography analysis, the remaining insoluble cellular debris was dried under vacuum and the DNA content was determined according to the method of Natarajan et al. (1994). In short, a reaction mixture of acetaldehyde in perchloric acid and diphenylamine in acetic acid was added to the cell pellet, mixed and incubated overnight at 37°C. The following day, the resulting dye was measured colorimetrically using a microtiter plate reader ($\lambda = 595/750$ nm). To quantify the DNA content, a standard curve generated with herring sperm was used.
Statistical analysis

Data were analyzed using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA) and Microsoft Excel software (Microsoft Office 2000 Professional; Microsoft, Unterschleißheim, Germany).

For descriptive purposes, the mean and standard deviations of the mean were calculated. Five volunteers showed no distinctive change in plasma concentration curve after the single dose of 60 mg CoQ_{10} independent of the study arm. These volunteers were excluded from statistical analysis of pharmacokinetic parameters. The areas under the curve (AUC) were calculated according to the trapezoidal method. Individual pre-dose plasma CoQ_{10} concentrations were used as the baseline for the calculation of AUC and all values falling below the individual pre-dose level were neglected (positive AUC). To compare the relative bioavailability of the five formulations the early time of absorption AUC_{0-4 h} was determined. Additionally, the pharmacokinetic parameters AUC_{0-12 h}, the observed maximum plasma concentration (C_{max}) and the observed time to reach the peak plasma concentration (T_{max}) were calculated for each volunteer. Comparisons within groups over the time were made using repeated-measures analysis of variance (ANOVA). The AUC and C_{max} values were compared after log transformation using ANOVA with the post-hoc Newman–Keuls test. T_{max} values were evaluated by non-parametric tests using the Kruskall–Wallis test on the untransformed data.

For the assessment of CoQ_{10} levels in BMC, the data from all study groups were pooled to yield a bigger sample size (n = 54). This seems legitimate since post absorption, once CoQ_{10} enters the systemic circulation, incorporation into tissues is expected to be independent of the composition of the diet or supplement. Results were evaluated within two subgroups with a cut-off point of 12 pmol CoQ_{10}/ug DNA (upper level of physiologic range of intracellular CoQ_{10} content in BMC) according to baseline levels.

A probability level of 0.05 or less was considered to indicate statistical significance.

Results

Volunteers between 18 and 45 years of age were randomly assigned to one of five study groups. Concerning age, body mass index and total cholesterol, no statistically significant differences between the groups were found. Data are summarized in Table I.

A high correlation of both total cholesterol and low-density lipoprotein-cholesterol with baseline CoQ_{10} levels was observed (r = 0.3281, P = 0.0154 and r = 0.2750, P = 0.0441, respectively). No correlation was found between high-density lipoprotein-cholesterol and triglycerides versus baseline levels of CoQ_{10}. Due to these findings, results are presented as values corrected for total cholesterol.

No correlation was seen between baseline levels of CoQ_{10}, ΔC_{max} and AUC, suggesting that there is no interaction between systemic supply and absorption rate when starting with plasma levels in a normal reference range.

Characteristic pharmacokinetic parameters including the AUC_{0-4 h}, AUC_{0-12 h}, C_{max} and T_{max} are summarized in Table II. To emphasize short-term absorption characteristics, not only the time frame 0–12 h but also 0–4 h was integrated. The latter was chosen because the second standardized meal was served 4 h post dosing.
### Table 1. Summary of demographic data and mean plasma CoQ10 baseline values.

<table>
<thead>
<tr>
<th>Solubilize 1</th>
<th>Solubilize 2</th>
<th>Oily dispersion 1</th>
<th>Oily dispersion 2</th>
<th>Crystalline CoQ10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.8</td>
<td>27.3</td>
<td>27.7</td>
<td>25.7</td>
</tr>
<tr>
<td>Mean</td>
<td>6.2</td>
<td>7.3</td>
<td>6.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>23.6</td>
<td>24.5</td>
<td>25.1</td>
<td>23.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>1.8</td>
<td>2.6</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>177.5</td>
<td>178.0</td>
<td>184.4</td>
<td>164.3</td>
</tr>
<tr>
<td>Mean</td>
<td>26.6</td>
<td>34.8</td>
<td>30.5</td>
<td>24.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.73</td>
<td>0.38</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>Baseline CoQ10 values (μmol/l)</td>
<td>0.51</td>
<td>0.12</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean</td>
<td>0.17</td>
<td>0.13</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.08</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

As shown in Figure 1, both solubilize formulations showed equal AUC₀₋₄₉ levels, with 0.17 ± 0.13 μmol/mmol*h and 0.17 ± 0.09 μmol/mmol*h, respectively, and both were significantly superior to crystalline CoQ10 (P < 0.01). Both preparations also showed superiority compared with Oily dispersion 1 (0.08 ± 0.07 μmol/mmol*h; P < 0.05) and Oily dispersion 2 (0.10 ± 0.05 μmol/mmol*h; not significant). Differences between formulations in AUC₀₋₁₂₉₀ Cₘ₉₉ and Tₘ₉₉ were not statistically significant (Table II). As expected, crystalline CoQ10 led to the lowest Cₘ₉₉ and highest Tₘ₉₉ values. In general, solubilizes reached higher levels and faster absorption rates compared with oily dispersions and crystalline CoQ10.

After multiple dosing of CoQ10 for 14 consecutive days, a significant increase in plasma CoQ10 concentrations was seen in all groups (P < 0.01). The highest mean

### Table II. Summary of pharmacokinetic parameters Cₘ₉₉, Tₘ₉₉, AUC₀₋₄₉, and AUC₀₋₁₂₉₀ for different formulations after a single oral dose of 60 mg CoQ10.

<table>
<thead>
<tr>
<th>Solubilize 1 (n = 10)</th>
<th>Solubilize 2 (n = 12)</th>
<th>Oily dispersion 1 (n = 10)</th>
<th>Oily dispersion 2 (n = 11)</th>
<th>Crystalline CoQ10 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₘ₉₉ (μmol/mmol)</td>
<td>0.16</td>
<td>0.16</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5.69</td>
<td>5.10</td>
<td>5.39</td>
<td>7.48</td>
</tr>
<tr>
<td>Tₘ₉₉ (h)</td>
<td>2.22</td>
<td>0.79</td>
<td>0.51</td>
<td>2.48</td>
</tr>
<tr>
<td>Median</td>
<td>5.05</td>
<td>5.04</td>
<td>5.04</td>
<td>6.10</td>
</tr>
<tr>
<td>AUC₀₋₄₉ (μmol/mmol*h)</td>
<td>0.17</td>
<td>0.17</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean</td>
<td>0.13</td>
<td>0.09</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.93</td>
<td>1.02</td>
<td>0.77</td>
<td>1.08</td>
</tr>
<tr>
<td>AUC₀₋₁₂₉₀ (μmol/mmol*h)</td>
<td>0.45</td>
<td>0.46</td>
<td>0.47</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean</td>
<td>0.43</td>
<td>0.46</td>
<td>0.41</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Figure 1. AUC (μmol/min/mol) after a single dose of 60 mg CoQ10. Data are expressed as the mean ± standard deviation. Differences between formulations were tested with ANOVA and post-hoc Student–Newman–Keuls test. *P < 0.05 (both solubilizes versus Oily dispersion 1, and Oily dispersion 2 versus crystalline), **P < 0.01 (both solubilizes versus crystalline).

increase was reached by Solubilize 1. As depicted in Figure 2, after 1 week of supplementation Solubilize 1 seemed to have already reached a plateau level in plasma, whereas for the other preparations a further slight increase could be observed in the second week of supplementation. Looking at AUCs–14 days (Figure 3), the relative bioavailability of Solubilize 1 was 142% compared with crystalline CoQ10, followed by Oily dispersion 2 (131%), Solubilize 2 (107%) and Oily dispersion 1 (89%).

Intracellular CoQ10 levels in BMC were analyzed independent of formulations. A significant increase (P = 0.0282) in intracellular CoQ10 content was observed in volunteers with baseline levels <12 pmol/μg DNA (Figure 4). Within this group, the correlation between plasma and intracellular CoQ10 status pre and post supplementation was evaluated and found to be significant (r = 0.2659 and P = 0.0164, Pearson

Figure 2. Increase in plasma CoQ10 concentrations (μmol/ml/mol cholesterol) after 1, 7 and 14 days of consecutive supplementation (60 mg/day). Means are plotted after baseline correction and correction for total cholesterol.
correlation coefficient). The increase of CoQ₁₀ levels in plasma over time was more prominent compared with BMC. Volunteers starting with very high intracellular concentrations of CoQ₁₀ leveled off normal values within 2 weeks of supplementation, indicating that there might be regulatory biologic systems within the physiologic range.

**Discussion**

The aim of this study was to compare the relative bioavailability of five different formulations of CoQ₁₀ including all common formulation techniques: crystalline CoQ₁₀, oily dispersions, and water-soluble solubilizes. Particularly, CoQ₁₀ supplements (market standard formulations) were compared with a novel solubilize formulation of CoQ₁₀ (Solu™ Q10).

CoQ₁₀ plasma baseline levels were found to be within a range of 0.34–1.35 µmol/l, equivalent to 0.08–0.33 µmol/mmol cholesterol. The mean value of CoQ₁₀ was

![Figure 3. AUC_{0-15 days} (µmol/mmol*³h), after multiple dosing of 60 mg CoQ₁₀. Data are expressed as the mean±standard deviation.](image)

![Figure 4. Intracellular CoQ₁₀ content in BMC pre and post supplementation (14 days). Values are expressed as the mean±standard deviation. Volunteers with baseline levels <12 pmol/µg DNA were pooled (n=4). *P<0.05, paired t-test.](image)
0.64 ± 0.19 μmol/l or 0.14 ± 0.04 μmol/mmol. These data are similar to the literature indicating a well-nourished population group (Crane 2001). Since total cholesterol and low-density lipoprotein-cholesterol are highly correlated with CoQ₁₀ baseline levels, it was suggested that changes in CoQ₁₀ levels over time are correlated with total cholesterol (transport capacity) as reported previously (Molyneux et al. 2004). However, in the present study, no correlation between Cₘₐₓ levels and total cholesterol was found.

Much effort has been made to improve the poor bioavailability of crystalline CoQ₁₀ in humans, which is due to its high molecular weight and lipophilic nature. Surprisingly, in our trial the crystalline CoQ₁₀ showed a rather high absorption rate compared with literature data (Tang et al. 2002). Lipophilic agents are naturally absorbed after micellizing with bile acid in coherence with lipid digestion. In the present study, CoQ₁₀ intake took place under standardized conditions including 15 g fat to have same starting conditions for all preparations and to prevent bias. Our results strengthen the importance of fat and meal standardization for bioavailability of CoQ₁₀. For vitamin E, another lipophilic substance, systematic examinations about the importance of fat have been reported. Leonard et al. (2004) and Jeanes et al. (2004) concluded that both the amount of fat and the physical properties of a meal influence the absorption of supplemental vitamin E to a great extent.

The Tₘₐₓ levels were found to be within 2 and 10 h with an overall mean value of 6.3 ± 2.2 h, which is consistent with previously reported trials (Tomotoro et al. 1986; Kurowska et al. 2003). Differences between Tₘₐₓ values between the groups were not statistically significant. However, concentration-time curves indicate that CoQ₁₀ from solubilizes is absorbed earlier compared with oily dispersions and crystalline CoQ₁₀.

The superiority in early uptake is also reflected in calculating AUC₀₋₄₈h. Both solubilizes indicate clear superiority over oily dispersions and crystalline CoQ₁₀ (P < 0.01) in this time frame. This might be explained by the structure of the water-soluble solubilizes, small enough to be directly incorporated into the intestinal border. For preparation of a solubilize, polysorbate 80 is used. Nerurkar et al. (1996) showed that permeability of Caco-2 cells is enhanced by polysorbate 80 due to inhibition of an apically polarized efflux system. Additionally, Seeballuck et al. (2004) showed that, in an in vitro model with Caco-2 cells, polysorbate 80 stimulates the secretion of triglyceride-rich lipoproteins (chylomicrons). Chylomicrons are the lipoprotein fraction mainly responsible for transport of lipophilic substances out of the intestines into the lymphatic system.

Differences in the pharmacokinetic parameters Cₘₐₓ and AUC₀₋₁₂₃h were not statistically significant but tended to be increased for both solubilizes and Oily dispersion 2. Our results are supported by the literature. Absorption of crystalline CoQ₁₀ is enhanced by dispersion and solubilization techniques, with statistical significance depending on the magnitude of individual differences and varying study designs (Weis et al. 1994; Miles et al. 2002; Joshi et al. 2003; Kurowska et al. 2003; Ullmann et al. 2005).

Besides looking at pharmacokinetics, the long-term effects of supplementation with CoQ₁₀ over a time period of 14 days were investigated. After 14 consecutive dose rates of 60 mg/day, total CoQ₁₀ levels in blood averaged between 1.30 and 1.78 μmol/l. An average plasma increase of 0.73-1.06 μmol/l was observed. The highest plasma
increase was seen for Solubilize 1 (Solu™ Q10), indicating its superiority over comparable formulations. Polysorbate 80 may contribute to this enhancement.

Furthermore, our results suggest an adaptation of different formulations over time, which is in accordance with results of Lyon et al. (2001). Lu et al. (2003) found no statistical difference between formulations after 1 week of supplementation with 50 mg/day, whereas Chopra et al. (1998) found about a 3.2-fold increase in bioavailability with solubilized CoQ10 compared with oil suspension and tablet after 3 weeks of supplementation with 120 mg/day. This result could not be confirmed with the present study design.

The buccal mucosa is a tissue with a high turnover rate and therefore reflects sensitively the actual concentration status in tissue. During the physiologic turnover, mature BMC are discarded into the mouth cavity. Brushing the inside of mouth cheeks with a special toothbrush can enforce this process. As described in previous studies, the test system 'buccal mucosa' is suitable to show response to supplementation programs (Erhardt et al. 2002; Peng et al. 1995). Summarizing the presented data, BMC analysis can clearly demonstrate the cellular uptake of CoQ10 from supplements in humans. Nikolowitz et al. (2004) previously has demonstrated a cellular uptake of CoQ10 by mitochondria-containing platelets. In our study, volunteers starting with very high intracellular CoQ10 levels could not further increase their cellular levels through taking supplements. This might point to interactions between external systemic and intracellular biosynthesis of coenzyme Q10 within a normal physiologic range.

Conclusion

In summary, clear differences in bioavailability between various types of CoQ10 supplements are visible. This is even more prominent during the early stages of absorption. Our results are representative for the selected study design, which reflects common intake habits of people. Standardized preconditions for all formulations, either fat-soluble or water-soluble, were realized to ensure equal absorption opportunities. Solubilizates were clearly superior to oily dispersions and crystalline CoQ10 with the best overall absorption characteristics seen for the novel Solu™ Q10 solubilizate.

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