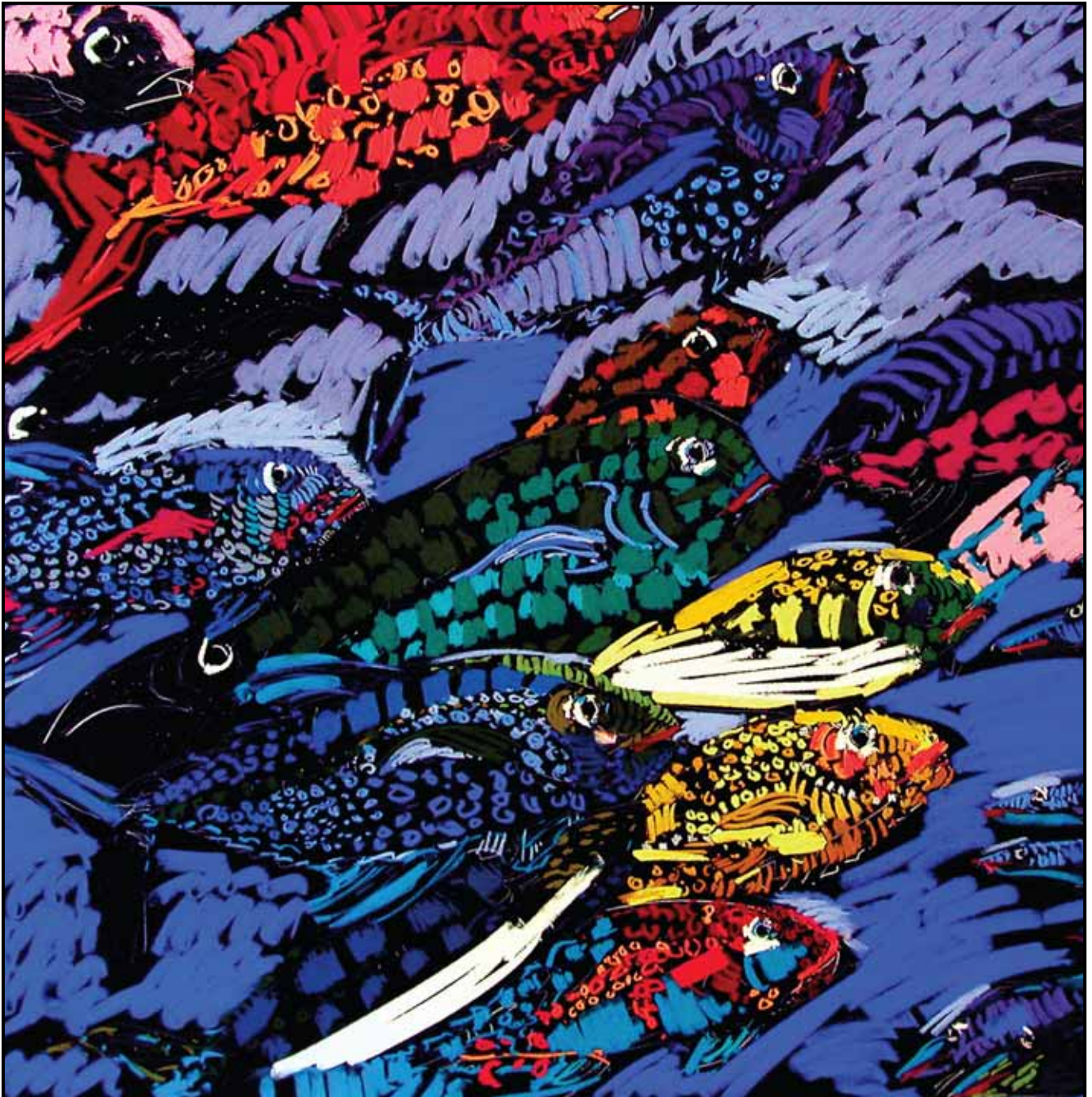


ALTERNATIVE THERAPIES

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MUTANS · HEALTHCARE REFORM AND INTEGRATIVE MEDICINE · EFFICACY OF
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ORIGINAL RESEARCH

RELATIVE BIOAVAILABILITY COMPARISON OF DIFFERENT COENZYME Q₁₀ FORMULATIONS WITH A NOVEL DELIVERY SYSTEM

Zheng-Xian Liu, PhD; Carl Artmann, PhD

Commercial coenzyme Q₁₀ (CoQ₁₀, ubiquinone) formulations are often of poor intestinal absorption. The relative bioavailability of CoQ₁₀ has been shown in National Institutes of Health-funded clinical trials to be increased by its delivery system. We investigated the bioavailability of a new CoQ₁₀ formulation based on a new and patented technology, VESIsorb, with 3 other commercially available CoQ₁₀ products, an oil-based formulation and 2 solubilizates. This new CoQ₁₀ formulation (commercially branded CoQsource) is a lipid-based formulation that naturally self-assembles on contact with an aqueous phase into an association colloid delivery system (hereafter "colloidal-Q₁₀"). Twenty healthy male and female subjects participated in a double-blind, comparative (parallel design), controlled, single-dose (120 mg) bioavailability study. Plasma concentration of CoQ₁₀ was determined at baseline and at various intervals after administration over a 24-hour period. To compare bioavailability, maximum concentration (C_{max}) and area

under curve from 0 to 10 hours (AUC_(0-10h)) were assessed. The kinetic profiles of all CoQ₁₀ preparations revealed a 1-peak plasma concentration-time course. Highest C_{max} values were seen after colloidal-Q₁₀ administration. Colloidal-Q₁₀ not only had the highest plasma concentration levels after 1 hour, but it continued to increase before reaching C_{max} at about 4 hours. The plasma concentration of colloidal-Q₁₀ remained well above the levels of the 3 other products throughout the 24-hour period. The relative bioavailability calculated using the AUC_(0-10h) values was also the highest for colloidal-Q₁₀; the AUC_(0-10h) values were 30.6, 6.1, 4.9, and 10.7 µg/mL·h for colloidal-Q₁₀, solubilizate 1, the oil-based formulation, and solubilizate 2, respectively. Differences in C_{max} and AUC between colloidal-Q₁₀ and the 3 other formulations were statistically significant. In summary, the data presented suggests that colloidal-Q₁₀ improves the enteral absorption and the bioavailability of CoQ₁₀ in humans. (*Altern Ther Health Med.* 2009;15(2):42-46.)

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Disclosure

The work was funded by Vesifact AG, Baar, Switzerland, and performed at Phacos GmbH, Gauting, Germany. Zheng-Xian Liu, PhD, is chief executive officer of GeroNutra and served as a paid consultant to SourceOne Global Partners in the preparation of this manuscript but holds no other financial interest in the products or technologies studied or in either Vesifact or SourceOne. Carl Artmann, PhD, is chief executive officer of Phacos GmbH and served as a paid consultant to Vesifact in monitoring and analyzing this study but holds no other financial interest in the products or technologies studied or in either Vesifact or SourceOne.

Coenzyme Q₁₀ (CoQ₁₀) plays a key role in mitochondrial cell physiology and is a powerful systemic antioxidant. Its chemical structure is shown in Figure 1. In certain conditions, the body's capacity for adequate CoQ₁₀ homeostasis is impaired. In such situations, supple-

mentation with CoQ₁₀ has been shown to be beneficial.

Due to its poor solubility in water and its relatively high molecular weight (M_r=863), the oral bioavailability of CoQ₁₀ when administered as a powder is low.^{1,2} In the past several years, extensive efforts have been made to improve the oral bioavailability of CoQ₁₀. Examples of formulation strategies aimed at improving the enteral absorption of CoQ₁₀ include oil-based formulations, solubilized formulations, and molecular complexes.³⁻¹⁰ Several of these strategies have been shown to improve the bioavailability of CoQ₁₀ as evidenced by their enhanced plasma CoQ₁₀ response.

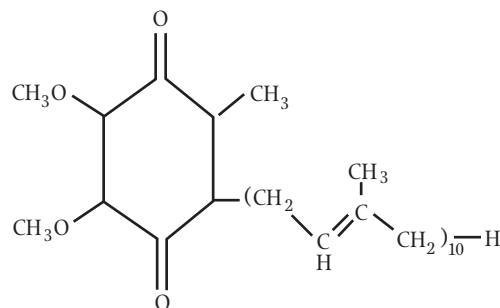


FIGURE 1 Chemical Structure of Coenzyme Q₁₀

It is known that poorly water-soluble supplements (eg, fat-soluble vitamins) are better absorbed when administered after a meal containing fat. One of the reasons for the improved absorption is the enhanced drug solubilization by bile salt-mixed micelles formed from the digestion products of dietary triglycerides (monoglyceride and fatty acids) and bile, a tool developed by nature. The task of naturally formed bile salt-mixed micelles, having a size <10 nm, is to transport the lipophilic molecules through the aqueous environment of the gastrointestinal (GI) tract and across the unstirred water layer to the absorptive epithelium. VESIsorb, a new delivery technology, mimics this natural absorption process to improve bioavailability of poorly water-soluble drugs. The data presented suggest that colloidal-Q₁₀, a CoQ₁₀ formulation based on this delivery system, improves the enteral absorption and the bioavailability of CoQ₁₀ in humans.

MATERIALS AND METHODS

Design

A double-blind, comparative, controlled (parallel design), single-dose pharmacokinetic study with random assignment of subjects of both sexes was planned. The protocol was approved by the Grosshadern Hospital of Munich ethics commission, and informed consent was obtained from all subjects.

Subjects

Four groups (n=5, n=5, n=5, n=5) of clinically healthy men and women between the ages of 18 and 60 years were recruited. Subjects were selected in accordance with the inclusion and exclusion criteria from among the group at Grosshadern Hospital and its facilities. The subjects were informed at the beginning about the nature of the study, its aims, and its execution. The data were acquired and stored in anonymous form.

Inclusion Criteria

- Men and women aged 18 to 60 years
- Clinically healthy, normal body mass index (18.5-25)
- No abnormalities in internal medical history
- No abnormalities in laboratory status
- Subject's agreement to participation in the study

Exclusion Criteria

- Men and women aged under 18 or over 60 years
- Previous history of hematological diseases (eg, known susceptibility to thrombosis)
- Pathological laboratory status (blood count, thrombocytes)
- Medication with vasoactive substances
- Medication affecting coagulation (eg, acetyl salicylic acid, aspirin)
- Medication affecting cholesterol (eg, statins)
- Diabetes
- Skin diseases (acute, chronic, allergic)
- Malignant tumors
- Disorders of heart, kidney, lung, or liver function
- Feverous or infectious diseases
- Alcohol or drug abuse

- Pregnancy or lactation
- Participation in power sports activities or sport activities during the study
- Failure to submit a statement of consent
- Participation in another clinical study within 4 weeks preceding this study or during this study
- Probable noncompliance of the subject; insufficient reliability

Study Preparations

- Product A (colloidal-Q₁₀): 30 mg CoQ₁₀ per capsule
- Product B (solubilize 1): 60 mg CoQ₁₀ per capsule
- Product C (oil-based formulation): 30 mg CoQ₁₀ per capsule
- Product D (solubilize 2): 30 mg CoQ₁₀ per capsule

Product A was provided by Vesifact AG, Baar, Switzerland. Products B, C, and D are commercially available CoQ₁₀ products.

Intervention

Subjects (12 females, 8 males) qualifying for the study on the basis of the inclusion and exclusion criteria were randomized to consume a single oral dose of 120 mg CoQ₁₀ in the form of one of the following study preparations:

- 4 capsules of product A (colloidal-Q₁₀),
- 2 capsules of product B (solubilize 1),
- 4 capsules of product C (oil-based formulation), or
- 4 capsules of product D (solubilize 2).

The study preparations were given in the morning before breakfast on an empty stomach. The taking of blood samples and mealtimes occurred at predetermined regular time intervals (Table 1). For a controlled diet, the same food was eaten among

TABLE 1 Blood Sampling and Mealtimes

Day	Time	Action	Time Elapsed (after CoQ ₁₀ intake)
1	07:30-08:00	Blood sample, zero value, empty stomach	
		Administration of 120 mg CoQ ₁₀	
	08:00-08:30	Breakfast	
	08:30-09:00	Blood sample	1 h
	09:30-10:00	Blood sample	2 h
	10:30-11:00	Blood sample	3 h
	11:30-12:00	Blood sample	4 h
	12:00-12:30	Lunch	
	12:30-13:00	Blood sample	5 h
	13:30-14:00	Blood sample	6 h
	15:30-16:00	Blood sample	8 h
2	17:30-18:00	Blood sample	10 h
	18:00-18:30	Dinner	
	08:30-09:00	Blood sample, empty stomach	24 h

groups. No other food was eaten (control of compliance).

Analysis of Plasma Samples

Plasma concentration of CoQ₁₀ were determined by high-performance liquid chromatography (HPLC) using a Merck/Hitachi HPLC system equipped with an auto sampler (Spectra Physics, Newport Corp, Mountain View, California), a UV detector, and an analytical column (Nucleosil RP 18, 5µm, 150 mm x 4 mm, Merck, Whitehouse Station, New Jersey). CoQ₁₀ was eluted with acetonitrile and detected at 275 nm.

Statistical Analysis

Data were analysed using GraphPad Prism 3.0 software (GraphPad Software Inc, San Diego, California). For descriptive purposes, the mean and standard deviations of the mean were calculated. The homogeneity of the CoQ₁₀ baseline levels at the beginning of the study was statistically evaluated using analysis of variance (ANOVA) and Tukey's multiple comparison test (post hoc test). To assess pharmacokinetic parameters, the area under the observed concentration-time curve above baseline (AUC_(0-10h)) and the observed maximum plasma concentration above baseline (Delta C_{max}) were calculated individually for each volunteer. The AUC and Delta C_{max} were compared after log transformation using ANOVA with the post-hoc Dunnett's multiple comparison test.

A probability level of $P < .05$ was considered to indicate statistical significance.

RESULTS

The pharmacokinetic characteristics of the 4 CoQ₁₀ study preparations after a single oral intake of 120 mg CoQ₁₀ are summarized in Table 2 and Figure 2. The data show that the mean plasma CoQ₁₀ values at baseline were similar in the 4 groups, ranging from 0.75 to 0.90 µg/mL. There was no statistically sig-

nificant difference between groups A to D ($P = .1402$). There was a significant increase in CoQ₁₀ plasma levels following supplementation in all 4 groups. The kinetic profiles of all 4 preparations revealed a 1-peak plasma concentration-time course. Maximum plasma level was reached between 3 and 5 hours after oral administration. The highest C_{max} values were seen after colloidal-Q₁₀ application. Colloidal-Q₁₀ had the highest plasma concentration level after 1 hour, and it continued to increase before reaching C_{max} at about 4 hours. The plasma concentration level of colloidal-Q₁₀ remained well above the levels associated with the 3 other products throughout the 24-hour period. The relative bioavailability calculated using the AUC_(0-10h) values was also the highest for colloidal-Q₁₀; the AUC_(0-10h) values were 30.6, 6.1, 4.9 and 10.7 µg/mL*h for product A (colloidal-Q₁₀), product B (solubilize 1), product C (oil-based formulation) and product D (solubilize 2), respectively. Differences in Delta C_{max} and AUC_(0-10h) between colloidal-Q₁₀ and the 3 other formulations were statistically significant. Looking at the AUC_(0-10h), the relative bioavailability of product A was 622% compared to product C, 499% compared to product B, and 286% compared to product D.

DISCUSSION

The absorption of most drugs depends on 2 processes: (1) the dissolution of the drug in physiological fluids and (2) the absorption process itself (ie, the process by which a drug in solution enters the cells at the absorption site and finally enters general blood circulation). Many drugs are absorbed by passive diffusion (ie, a spontaneous migration of drug molecules from a region of high concentration to a region of low concentration). Other drugs are absorbed by facilitated or active transport, which involves the expenditure of energy by the body. In either event, the dissolution of the drug is the first step in the absorption process unless the drug is administered as a solution. On the

TABLE 2 Pharmacokinetic Parameters of the 4 Study Preparations Determined After a Single Oral Intake of 120 mg CoQ₁₀

	Product A (Colloidal-Q10)	Product B (Solubilize 1)	Product C (Oil-based formulation)	Product D (Solubilize 2)
Baseline (µg/mL)				
Mean	0.90	0.76	0.82	0.75
SD	0.12	0.11	0.10	0.09
Delta C_{max} (µg/mL)				
Mean	5.99	1.68	1.42	2.98
SD	0.41	0.33	0.39	0.55
C_{max} (µg/mL)				
Mean	6.89	2.44	2.24	3.73
SD	0.51	0.31	0.30	0.49
T_{max} (h)				
Mean	4.20	3.40	5.00	4.20
SD	0.45	0.55	0.00	0.45
AUC_(0-10h) (µg/mL*h)				
Mean	30.62	6.14	4.92	10.71
SD	4.24	0.16	1.96	2.35

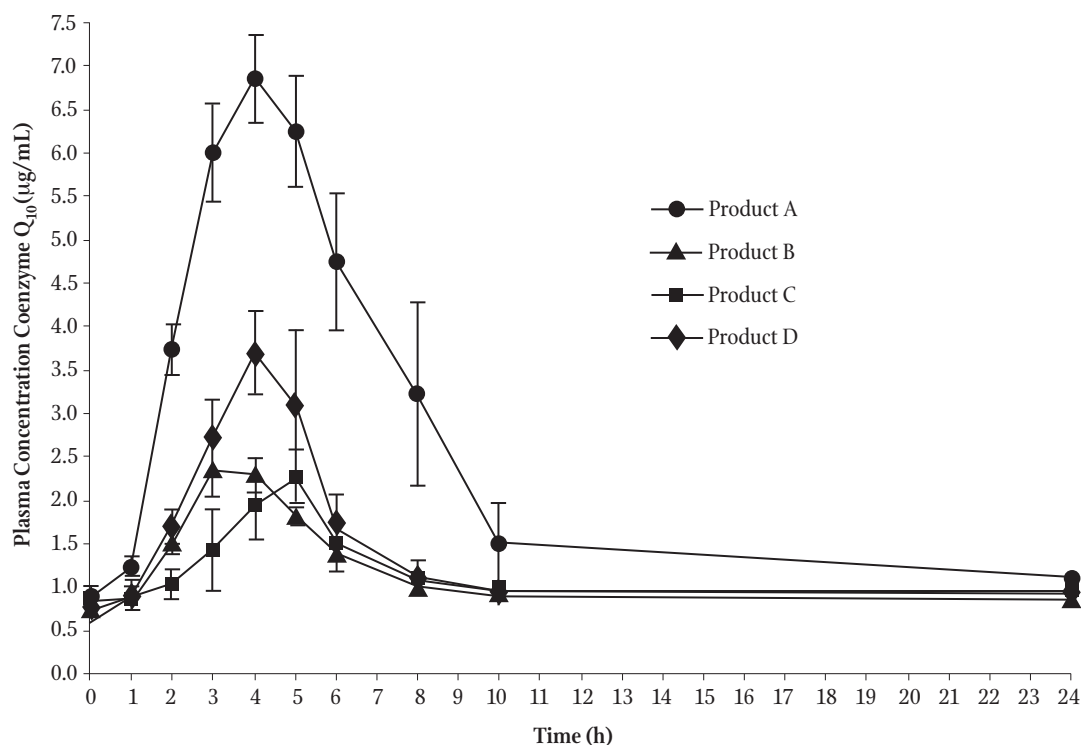


FIGURE 2 Changes in Plasma CoQ₁₀ Concentrations After a Single Oral Intake of 120 mg CoQ₁₀ (n=20)*

*Product A: colloidal-Q₁₀; product B: solubilizate 1; product C: oil-based formulation; product D: solubilizate 2.

other hand, some drugs are absorbed by the process of pinocytosis or endocytosis, which involves the engulfing of solid particles and the incorporation of such particles into the cellular contents.

To compensate for the poor absorption displayed by many drugs, a formulation may use one or more mechanisms to increase the extent to which the administered drug is absorbed. There are vast numbers of such techniques, which can be grouped into the following broad categories: (1) enhancement of the rate and extent of dissolution and (2) facilitation of an absorption process. Formulating a drug with an oil for the purpose of involving the lymphatic system in the absorption of the drug is an example of the second technique. VESIsorb, the delivery system of colloidal-Q₁₀, is an example of the first technique.

VESIsorb was designed to address the poor bioavailability of drugs and natural bioactives like CoQ₁₀, exhibiting poor water solubility but high membrane permeability (Biopharmaceutical Classification System: Class II compounds). This delivery system is a lipid-based formulation that self-assembles on contact with an aqueous phase into a colloidal delivery system. The co-administered drug and/or natural bioactive will be solubilized by the in situ formed colloidal system with a mean diameter of <100 nm and a very narrow size distribution as assessed by dynamic laser light scattering using a Zetasizer Nano (Malvern, Worcestershire, United Kingdom). This colloidal solubilization improves the transport of the drug through the aqueous phase of the GI-lumen to the absorptive epithelium, hence its bioavailability. The improvement of oral drug or natural bioactive bioavailabil-

ity by this technology is broken down into 3 steps: (1) formation of the colloidal delivery system, (2) diffusion across the unstirred water layer, and (3) transfer to the absorption epithelium.

Similar to vitamin E and other lipophilic substances, CoQ₁₀ is absorbed, at least partially, by the lymphatic route.¹ Lymphatic absorption involves the following steps: (1) incorporation of CoQ₁₀ into lipoproteins/chylomicrons within the enterocyte, (2) secretion of the lipoproteins/chylomicrons from the enterocyte into the lymph vessel, and (3) transport of the lipoproteins/chylomicrons within the lymph vessel to the bloodstream. Adequate stimulation of the lipoprotein/chylomicron production is thus of paramount importance for optimal CoQ₁₀ absorption by the lymphatic route. This can be achieved by administering CoQ₁₀ with or after a meal containing some fat.

CoQ₁₀ exhibits nonlinear pharmacokinetics (ie, the fraction of a single dose absorbed falls as the dose increases).¹¹⁻¹³ For example, it has been shown that divided dosages (2 x 100 mg) of CoQ₁₀ caused a larger increase in plasma levels of CoQ₁₀ than a single dose of 200 mg.¹² Larger daily doses of CoQ₁₀ should therefore be divided into several doses. Dividing the daily CoQ₁₀ dose into several doses will not only maximize the CoQ₁₀ absorption, but also reduce the difference between maximal and minimal steady states plasma levels of CoQ₁₀.

In the current study, the posttreatment CoQ₁₀ plasma levels of all 4 products are relatively high in comparison to those reported previously. It is difficult to compare the results of this study to other studies: interstudy comparisons are difficult to

make, as variables from food intake to dosing strategy to plasma lipoprotein levels to analytic procedures may affect the results. And there is substantial variation in people's ability to absorb CoQ₁₀ in the normal population.^{5,14} Additional clinical studies are indicated to verify that the improved absorption with colloidal-Q₁₀ correlates with clinical response to treatment.

In the course of the last 25 years of clinical research in treating heart failure of diverse etiology with supplemental CoQ₁₀, it became clear that the initial strategy of normalizing plasma CoQ₁₀ status was not effective. Only patients with plasma CoQ₁₀ levels >2.5 µg/mL showed significant clinical improvement in heart failure. In fact, therapeutic plasma CoQ₁₀ levels are now considered to be >3.5 µg/mL.¹⁵ Likewise, the pilot trial of CoQ₁₀ in patients with Parkinson's disease showed that the benefit was greatest in subjects receiving the highest dosage (1200 mg/d).¹⁶ Thus, a CoQ₁₀ formulation exhibiting good CoQ₁₀ bioavailability is of great value.

The safety of CoQ₁₀, even at high dosages, is well documented. In particular, a 52-week study revealed no toxicity at a dose of 1200 mg/kg/day,¹⁷ based on which the acceptable daily intake for adults weighing 50 kg was estimated to be 600 mg/day. It was also reported in clinical studies of patients with early Parkinson's disease (up to 1200 mg/day for 16 months),¹⁵ Huntington's disease (600 mg/day for 30 months),¹⁸ and heart disease (50-150 mg/day for 3 months)¹⁹ that the frequency of side effects was almost equal to that in the control groups, indicating that the dosage levels examined were within the limits of tolerable intake. In a recent study, the safety profile of CoQ₁₀ at high doses for healthy subjects was assessed. CoQ₁₀ in capsule form was taken for 4 weeks at doses of 300, 600, and 900 mg/day by a total of 88 adult volunteers. The findings of the study showed that CoQ₁₀ was well tolerated and safe for healthy adults at an intake of up to 900 mg/day.²⁰ Furthermore, each component of colloidal-CoQ₁₀ is Generally Regarded as Safe per the US Food and Drug Administration's (FDA's) Code of Federal Regulations (CFR 21) and European regulatory standards, which guarantees the wholesomeness and safety of each ingredient for human consumption. Essentially, it is the FDA's assurance that all ingredients used in food products have undergone toxicological and safety testing to guarantee their safe use in foods.

In summary, this study compared the relative bioavailability of colloidal-Q₁₀ with that of 3 commercially available products, 2 CoQ₁₀ solubilizates and an oil-based CoQ₁₀ formulation, after a single oral administration of 120 mg. Our data suggest that the enteral absorption and bioavailability of CoQ₁₀ can be enhanced by colloidal-Q₁₀ that mimics the naturally occurring mixed micellar transport system of the human body. This also increases the likelihood that this technology can be considered as safe for improving the absorption of drugs with low water-solubility. Current research is investigating whether this technology also can be used to improve the absorption of other natural lipophilic actives, such as omega-3, vitamin D, resveratrol, tocotrienols, flavonoids, and gamma-tocopherols.

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