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Assessment of coenzyme Q10 absorption using an *in vitro* digestion-Caco-2 cell model

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Abstract

The feasibility of using a coupled *in vitro* digestion-Caco-2 cell uptake as a model for examining the digestive stability and absorption of coenzyme Q10 (CoQ10) from a variety of commercially available CoQ10 products was examined. The products were first subjected to simulated digestion to mimic their passage through the GI tract to generate micelles containing CoQ10, and the micelle fractions added to monolayers of Caco-2 cells to determine CoQ10 uptake. The data demonstrate enhanced uptake of CoQ10 from formulations containing solubilized forms of CoQ10 and also from a CoQ10–γ-cyclodextrin complex as compared with pure CoQ10 powder or tablets based on CoQ10 powder. The CoQ10 uptake by the cells was correlated with the extent of micellarization of CoQ10 during simulated digestion. Most of CoQ10 taken up by the cells was converted to ubiquinol either during or following uptake. The data also indicate a correlation between *in vitro* dissolution of CoQ10 products and uptake of CoQ10 by Caco-2 cells. Thus, this study demonstrates the utility of coupled *in vitro* digestion-Caco-2 cell model as a cost-effective screening tool that will provide useful information for the optimal design of human trials to assess the bioavailability of CoQ10 and also other bioactive compounds.

Keywords: Coenzyme Q10; Ubiquinone; Caco-2 cells; Intestinal absorption; *In vitro* models; Bioavailability

1. Introduction

Coenzyme Q (CoQ, ubiquinone) belongs to a homologous series of compounds widely distributed in nature. They share a common benzoquinone ring structure with an isoprenoid side chain of varying lengths (Fig. 1). In humans and several other mammalian species, the side chain is comprised of 10 isoprene units, hence it is called coenzyme Q10 (CoQ10). The chemical nomenclature of CoQ10 is 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone in the trans configuration (natural).

Although CoQ10 functions like a vitamin it is not considered one because it is synthesized in the body. CoQ10 is a cofactor in the mitochondrial electron transport chain and is essential for the production of ATP (Ernster and Dallner, 1995). The activities of CoQ10 extend beyond its cofactor role in the mitochondria (Ernster and Dallner, 1995; Crane, 2001). For example, the reduced form of CoQ10 (i.e. the hydroquinone called ubiquinol) is a potent lipophilic antioxidant and is capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate (Ernster and Dallner, 1995; Crane, 2001). Additional functions of CoQ10 are reviewed elsewhere (Crane, 2001).

Potential health benefits of CoQ10 supplementation have been recognized and its therapeutic value, often as an adjunct to standard medical therapy, also has been reported with particular reference to cardiovascular and neurodegenerative diseases (Overvad et al., 1999; Langsjoen and Langsjoen, 1999; Beal, 2002). As such, CoQ10 has become an increasingly popular dietary supplement in recent years. Numerous CoQ10 products are available on the market in the form of both chewable and non-chewable tablets, powder-filled capsules, and soft gelatin

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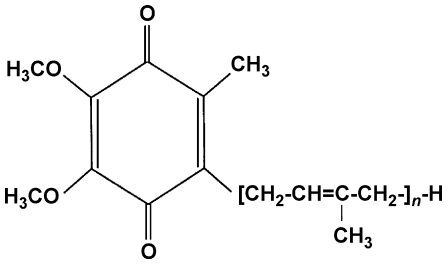

Fig. 1. Structure of coenzyme Q ($n = 10$ for coenzyme Q10).

capsules containing an oil suspension of CoQ10. However, the bioavailability of CoQ10 in most of these products is very low because CoQ10 powder used in these formulations is insoluble in water. We previously demonstrated that solubilized formulations of CoQ10 as either ubiquinone (Q-Gel®) or ubiquinol (Q-Nol®) possess superior bioavailability as shown by animal and human studies (Chopra et al., 1998; Miles et al., 2002; Zaghloul et al., 2002), and this was consistent with their excellent dissolution characteristics in the *in vitro* dissolution test.

The coupled simulated gastric/small intestinal digestion with Caco-2 human intestinal cell model has been tested and applied successfully to study the digestive stability and absorption/bioavailability of several essential nutrients and other dietary bioactive compounds (Garrett et al., 1999a,b; Etcheverry et al., 2004; Chitchumroonchokchai et al., 2004; Failla and Chitchumroonchokchai, 2005). This approach has consistently provided results that are qualitatively comparable to bioavailability data from human studies (Lau et al., 2004; Failla and Chitchumroonchokchai, 2005). We now present data on the relative absorption of several marketed CoQ10 products using the coupled *in vitro* digestion-Caco-2 cell model. The products tested included several powder-based and liquid formulations of CoQ10 along with a new product form based on CoQ10–γ-cyclodextrin complex. A preliminary report on this study was presented in part recently (Craft et al., 2005).

2. Materials and methods

2.1. Test products

The CoQ10 products tested included currently marketed chewable tablets, a softgel capsule containing solubilized CoQ10, a CoQ10–γ-cyclodextrin complex as a powder and a liquid formulation (Table 1).

CoQ10 content was first analyzed in the products by dissolving them in stabilized tetrahydrofuran, diluting in ethanol and determining their concentration by reversed-phase HPLC with UV detection at 275 and 290 nm (described in detail under *CoQ10 analysis*). The dissolution testing of the products was also carried out according to the USP dissolution test (USP 27/NF 22) using six tablets or capsules per product.

2.2. In vitro digestion

The materials for the *in vitro* digestion were prepared as follows. In the case of tablets, it was necessary to grind them first (three to four tablets for each product) using an ice cold mortar and pestle to a fine homogenous powder. For softgel capsules, contents of three to four capsules were squeezed into a glass test tube and mixed. Aliquots containing about 2 mg CoQ10 were mixed with 4 g plain fat-free yogurt and 100 μL olive oil to prepare "test meals" (the exact amount of CoQ10 added determined by HPLC). The procedure was repeated using fresh test products (identical lots) for second series of digestion thus increasing total N to six to eight independent digestions per test sample. Final data were normalized to 2.0 mg CoQ10 added to each test meal.

Table 1
Products tested using the *in vitro* digestion/Caco-2 cell model

Product #	Product name	Strength
1	CoQ10 powder (USP) (reference product)	99.8%
2	CoQ10 Tablet A	60 mg
3	CoQ10 Tablet B	100 mg
4	CoQ10 Tablet C	300 mg
5	Chew-Q®[a] tablet (γ-cyclodextrin complex)	100 mg
6	Q-Gel®[a] softgel capsule (solubilized CoQ10)	30 mg
7	Liquid-Q®[a] (Li-Q-Sorb®[a]) (liposomal solution)	10%
8	Hydro-Q-Sorb®[a] powder (γ-cyclodextrin complex)	20%

[a] Tishcon Corporation.

Each meal was subjected to simulated gastric and small intestinal digestion by a slight modification of procedure of Garrett et al. (1999a,b). Briefly, test sample was diluted with saline before adjusting pH to 3.0 with 1N HCl. Porcine pepsin (final concentration of 2 mg/mL) was added and the volume was adjusted to 40 mL with saline. The samples were blanketed with nitrogen, sealed, and incubated in a shaking water bath (90 rpm) at 37 °C for 60 min. The gastric phase was terminated by adding 1N sodium bicarbonate to increase the pH to 6, and adding porcine pancreatin, lipase and bile extract (final concentrations of 0.4, 0.2 and 2.4 mg/mL, respectively). The pH was adjusted to 6.9 with 1N NaOH and final volume increased to 50 mL. Samples were blanketed with nitrogen, sealed, and returned to shaking bath at 37 °C for 120 min. The resultant mixture is referred to as the digesta. Tubes were placed in an ice bath and aliquots of digesta were placed in polyallomar tubes, sealed and centrifuged at 167,000 × *g* at 4 °C for 35 min. This separated the aqueous fraction from residual oil droplets (generally absent) and the non-digested particulate material. The aqueous fraction was filter-sterilized (0.22 μm pores). Aliquots of the predigested "meal", digesta and filtered aqueous fraction (referred to as micelle fraction) were stored at −80 °C under nitrogen. The digesta and aqueous fractions were analyzed by HPLC to determine the oxidized, reduced and total CoQ10 for each test product.

2.3. Cellular uptake of CoQ10

A pilot experiment preceded the main study in order to characterize the concentration and time-dependent uptake of micellarized CoQ10 by Caco-2 cells. Highly differentiated cultures of Caco-2 cells were prepared as described by Garrett et al. (1999a). Synthetic micelles containing CoQ10 were prepared as

described elsewhere for delivery of carotenoids to Caco-2 cells (Chitchumroonchokchai et al., 2004; Chitchumroonchokchai and Failla, 2006). A bioenhanced formulation containing solubilized CoQ10 (Q-Gel®, Product #6) was used as the source of CoQ10 in the pilot study. Previous data have shown that exposure of Caco-2 cells to natural and synthetic micelles for as long as 6 h does not compromise cellular morphology or metabolic integrity (Garrett et al., 1999a,b). The mid-concentration of the linear range for uptake of CoQ10 after 4 h was used to evaluate the effect of length of exposure on accumulation. Cellular accumulation was examined after exposure to medium with micellarized CoQ10 for 4 h (total concentration of micelles in medium was constant) and time-dependent cellular uptake from medium with a fixed concentration of CoQ10. The stability of micellarized CoQ10 in medium placed in culture dishes without cells and incubated identically to cultures with Caco-2 cells also was determined in this trial.

In the main study, filtered aqueous fraction generated during simulated digestion of meals containing CoQ10 from the different formulations was diluted (1:4) with DMEM medium containing 15 mM HEPES, 2 mM glutamine and 1% non-essential amino acid mixture. The prepared medium was added to triplicate wells containing differentiated monolayers of Caco-2 human intestinal cells (HTB 37 at passage 27–29; 11 days after monolayer was confluent). Following incubation (5% CO_2:95% air, 37 °C, 100% humidity) for 4 h, spent medium was removed and the monolayers washed twice with cold PBS containing 5 mmol/L taurocholate followed by two washes with cold PBS. Cellular CoQ10 content was determined by HPLC. Cell protein content was determined by BCA assay (Pierce Chemical Co.) for replicate sets of cells exposed to diluted aqueous fraction.

Data are expressed as pmol CoQ10 per mg cell protein. Each test formulation was digested independently in two to three separate experiments with three replicate cultures per experiment ($n = 6$–9). Replicate cultures of cells not treated with CoQ10 were used to establish baseline values of CoQ10 in the Caco-2 cells.

2.4. CoQ10 analysis

CoQ10 analyses of the various fractions were carried out according to the HPLC methods of Rousseau and Varin (1998) and Tang et al. (2001) using a ThermoSeparation Products liquid chromatograph with the following components: P4000 solvent delivery system, vacuum degasser, AS3000 autosampler, UV1000 or UV2000 detector, and PC1000 computer-controlled data system (Fremont, CA). An electrochemical detector (ESA CouloChem II, Chelmsford, MA) was also used. For direct injection, samples were prepared by precipitating proteins and extracting CoQ10 with nine parts cold 1-propanol. After 1 min of homogenization, samples were centrifuged for 5 min at 10,000 rpm. The supernatant was transferred to vials and placed in an autosampler maintained at 15 °C. For samples requiring concentration, samples were precipitated with ethanol and extracted twice with hexane. The combined hexane extract was dried in a SpeedVac, then redissolved in ethyl acetate and diluted with ethanol.

Table 2
Dissolution testing of CoQ10 products

Product #	Product name	Dissolution (%)
1	CoQ10 powder (USP) (reference product)	0–3
2	CoQ10 Tablet A	0–5
3	CoQ10 Tablet B	0–5
4	CoQ10 Tablet C	0–5
5	Chew-Q®[a] Tablet (γ-cyclodextrin complex)	75–80
6	Q-Gel®[a] softgel capsule (solubilized CoQ10)	90–100
7	Liquid-Q®[a] (Li-Q-Sorb®[a]) (liposomal solution)	100
8	Hydro-Q-Sorb®[a] powder (γ-cyclodextrin complex)	75–100

[a] Tishcon Corporation.

A 20 μL aliquot was used for injection. The mobile phase consisted of methanol/hexane/isopropanol/acetic acid/water (82:15:1.5:1.5:0.1) containing 100 mM sodium acetate and 15 mM sodium perchlorate at a flow rate of 1.0 mL/min. The separation was performed at 35 °C on a Spherisorb ODS2, 3 μm, 150 mm × 4.6 mm column protected by a Phenomenex guard column. A porous carbon in-line filter was placed between the pump and autosampler, the eluent exiting the column and passing though a dual wavelength programmable UV detector (275 nm for ubiquinone and 290 nm for ubiquinol). Following passage through the UV detector, all of the CoQ10 in the separated sample was reduced at the first analytical electrode (−0.7 V), then oxidized at the second electrode (+0.5 V) for quantitation. Total CoQ10 was determined by adding up the oxidized and reduced forms. The system was calibrated using graded quantities of pure CoQ10 (USP). To determine the retention time of ubiquinol, ubiquinone was reduced with sodium borohydride prior to injection. The concentrations of both ubiquinone and ubiquinol standards were determined spectrophotometrically using Beer's Law (oxidized CoQ10 $E^{1\%}_{cm} = 165$, reduced CoQ10 $E^{1\%}_{cm} = 46.4$). The detection limit was ~5 ng/mL with both direct injection and organic solvent extraction. The direct injection is preferred because it minimizes sample handling. Solvent extraction is necessary for samples with very low concentrations of CoQ10.

2.5. Statistical analysis

Data for micellarization and cell uptake were analyzed by one-way ANOVA and expressed as mean ± S.E.M. Significant differences at $p < 0.05$ level are noted by a different letter above the error bars.

3. Results

3.1. Test products

The test products met their stated label claims and their CoQ10 contents ranged from 97 to 106% of the claim. CoQ10 was present entirely in the oxidized form as ubiquinone in all the products.

Dissolution testing data of the products are presented in Table 2. Data show very poor dissolution (ranging from 0 to

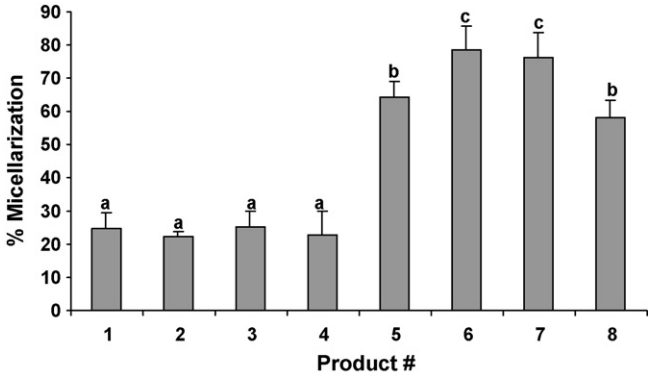

Fig. 2. Efficiency of micellarization of CoQ10 during simulated digestion of test products.

5%) for pure CoQ10 powder and also for the three tablet formulations based on CoQ10 powder (products #1–4).

On the other hand, the CoQ10–γ-cyclodextrin complex-based tablet and the powder (products #5 and 8) yielded values ranging from 75 to 100% whereas the softgel formulation containing solubilized form of CoQ10 (product #6) and the liposomal solution (product #7) showed values of 90–100 and 100%, respectively. The poor dissolution of pure CoQ10 powder (product #1, the reference product) and the tablet formulations (products #2–4) was indicative of their rather limited potential for micellarization and eventual uptake by the Caco-2 cells.

3.2. Digestive stability and efficiency of micellarization of the CoQ10 products

CoQ10 added to yogurt with oil was stable during simulated gastric and small intestinal phases of digestion. Mean recovery of CoQ10 following the digestive process was 98.6 ± 9.8%. Fig. 2 shows the amount of CoQ10 transferred to micelles during the small intestinal phase of simulated digestion as assessed by the concentration of CoQ10 in the filtered aqueous fraction for the eight products.

The percentages of micellarized CoQ10 ranged from 22% for product #1 (CoQ10 powder, the reference product) to 79% for product #6 (Q-Gel®, softgel capsule containing solubilized CoQ10). The two products containing solubilized CoQ10 (#6 and 7) had the highest percent micellarization followed by product #8 (CoQ10–γ-cyclodextrin complex powder) and product #5 (tablet based on CoQ10–γ-cyclodextrin complex). Over 98% of CoQ10 in the micelles of all the eight products was in the oxidized form as ubiquinone similar to their redox state at the start of the study.

3.3. Uptake of micellarized CoQ10 by Caco-2 cells

The uptake of CoQ10 from the synthetic micelles by Caco-2 cells was proportional to CoQ10 concentration in the medium from 0 to 13 μM (Fig. 3A). Cellular CoQ10 content also increased in proportion to incubation time (Fig. 3B).

Furthermore, micellar CoQ10 was very stable in cell-free medium with 95.4 and 93.3% recovered after 4 and 8 h, respectively, and 99% of CoQ10 in the micelles was in the oxidized form as ubiquinone. In contrast, CoQ10 accumulated in the cells was mostly (about 80%) in the reduced form as ubiquinol (Fig. 3B).

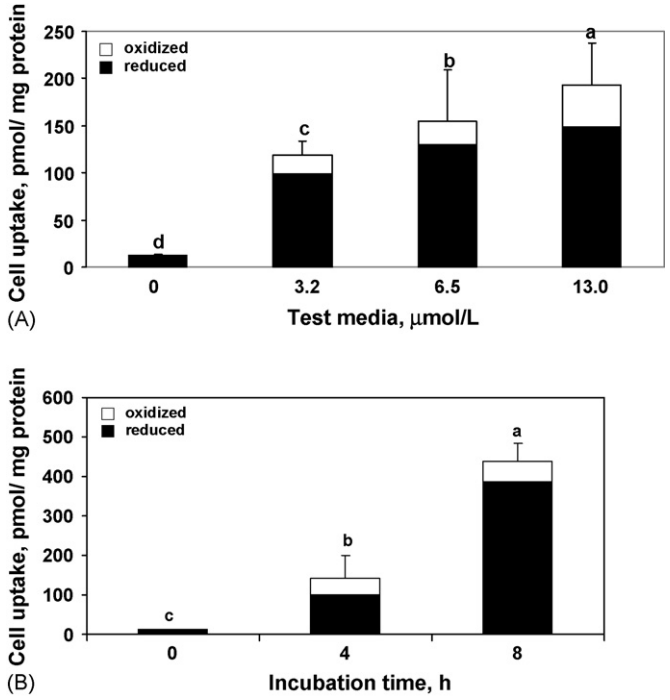

Fig. 3. Uptake of CoQ10 from synthetic micelles by Caco-2 cells.

The amount of CoQ10 accumulated in the cells incubated with micelles generated during simulated digestion of the test products is shown in Table 3.

The amount of CoQ10 taken up by the Caco-2 cells ranged from 30 to 257 pmol/mg protein. In terms of percent uptake relative to the reference product (#1, pure CoQ10 powder), the greatest accumulation occurred with product #8 (Hydro-Q-Sorb®), closely followed by product #7 (Liquid-Q®), and then by product #6 (Q-Gel®) and product #5 (ChewQ®). The uptake was very poor with the other products (Tablets A–C). A good correlation between the efficiency of micellarization and cellular accumulation of CoQ10 from the test products was evident ($R^2 = 0.66$).

Table 3
CoQ10 uptake (%) by Caco-2 cells

Product #	CoQ10 uptake* (pmol/mg protein)	% Uptake[a]
1	35 ± 6 f	100
2	49 ± 4 e	141
3	34 ± 9 f	98
4	30 ± 7 f	86
5	119 ± 33 d	342
6	126 ± 26 c	362
7	241 ± 10 b	693
8	257 ± 5 a	739

[a] Product #1 (pure CoQ10 powder, the reference product) was assigned a value of 100%.

* Values are mean ± S.E.M., $n = 6$. Means with a different letter (superscript) differ significantly ($p < 0.05$).

4. Discussion

The absorption efficiency of orally ingested CoQ10 is poor because of its insolubility in water and limited solubility in lipids. It has been reported that in rats only 2–3% of orally administered CoQ10 is absorbed (Zhang et al., 1995). Being a lipophilic substance, the process of absorption of CoQ10 in the intestine is similar to that of dietary lipids. Digestion facilitates the release of CoQ10 from the food matrix. It is not clear whether the gastric phase of digestion contributes to the enhancement of CoQ10 absorption from supplemental CoQ10 products that are based on pure CoQ10. Secretions from the pancreas and gall bladder facilitate emulsification and micelle formation in the small intestine. Micelles transfer lipophilic compounds to the apical surface of absorptive epithelial cells. The absorption of CoQ10 requires its incorporation into chylomicrons for transport via the lymph to peripheral blood (Katayama and Fujita, 1972), and this is similar to the absorption of Vitamin E and other lipophilic compounds (Kayden and Traber, 1993).

While human studies are desirable for assessing the bioavailability of bioactive compounds, use of human Caco-2 intestinal cells has become well accepted as a surrogate for assessing the uptake and transepithelial transport of various drugs, nutrients and other dietary bioactive components (Delie and Rubas, 1997; Failla and Chitchumroonchokchai, 2005). In the present study, we employed this *in vitro* model to compare the efficiency of micellarization of CoQ10 from several marketed CoQ10 products during simulated digestion of a standardized meal, and the bioaccessibility of CoQ10 in the micellar fraction was determined by measuring its uptake by Caco-2 cells. In a pilot study using a solubilized formulation of CoQ10 (product #6), the cellular uptake of CoQ10 from the micelles was found to be both time and concentration dependent (Fig. 3A and B). This study also showed that the cellular accumulation of CoQ10 was dependent on the efficiency of micellarization during *in vitro* digestion.

Simulated digestion revealed marked differences in the extent of micellarization of CoQ10 from the products tested (Fig. 2). As expected, cellular accumulation of CoQ10 was correlated with the efficiency of micellarization of CoQ10 from the various products. Micellarization and cellular uptake of CoQ10 from solubilized formulations of CoQ10 were markedly greater than those from tablet formulations based on plain CoQ10 powder (Fig. 2; Table 3). This observation is consistent with bioavailability data from previous studies with humans (Chopra et al., 1998; Miles et al., 2002) and also from a dog study (Zaghloul et al., 2002).

Product #8 is a unique product form that is a molecular microencapsulate based upon complexation of CoQ10 with γ-cyclodextrin. This is a powder that showed the highest accumulation of CoQ10 by Caco-2 cells. There are numerous examples of improved bioavailability of bioactive compounds complexed with cyclodextrins, particularly in the pharmaceutical area and this is attributable to the increased aqueous solubility and stability of such complexes (Davis and Brewster, 2004; Shimpi et al., 2005). The present study demonstrates that the bioaccessibility of CoQ10, as assessed by uptake by Caco-2 cells, is also likewise greatly enhanced by complexation with γ-cyclodextrin. The uptake of CoQ10 by product #8 was closely followed by that of product #7 which is a solubilized form as a CoQ10 nanodispersion (liposomal solution).

While both product #8 (CoQ10–γ-cyclodextrin complex powder) and product #5 (tablets based on CoQ10–γ-cyclodextrin complex) showed markedly enhanced accumulation by Caco-2 cells as compared with pure CoQ10 powder, the uptake of CoQ10 from product #5 was somewhat lower. It may be noted that product #8 is a bulk powder form whereas product #5 is a chewable tablet formula that is produced using product #8. This suggests that the processes involved in the production of tablets such as the use of additional inactive ingredients and also compression may have affected the efficiency of micellarization thus resulting in lower accumulation of CoQ10 by Caco-2 cells.

Another interesting observation made in this study is the relationship between *in vitro* dissolution of the products and CoQ10 uptake by Caco-2 cells. As the percent dissolution increased, the uptake by the cells also increased, thus indicating a correlation between percent dissolution of the products and CoQ10 accumulation. This is analogous to the fundamental relationship between *in vitro* dissolution and *in vivo* bioavailability. A positive correlation between the amount of drug substance dissolved *in vitro* and the amount absorbed *in vivo* from the same formulation was demonstrated years ago (Gibaldi and Weintraub, 1970), and the *in vitro* dissolution test is generally considered to be a sensitive and a reliable indicator of *in vivo* bioavailability. In this case, it would also seem to apply to uptake by Caco-2 cells.

The processes of *in vitro* digestion and micellarization did not affect the redox status of CoQ10 which remained in the oxidized state throughout as ubiquinone. However, most of CoQ10 was reduced to ubiquinol either during or following its uptake by the Caco-2 cells thus indicating that the enterocytes have the necessary biochemical systems to reduce CoQ10 *in situ*. It is interesting to note in this context that over 95% of circulating CoQ10 is in the reduced form as ubiquinol. Furthermore, exogenously administered CoQ10 regardless of the dose appears in circulation as ubiquinol (over 95%). This study shows that the reduction of CoQ10 ingested as ubiquinone to ubiquinol takes place in the enterocytes before it enters the lymphatic system. It was shown in an earlier study with rats that orally administered CoQ9 (major homolog in rats) and also CoQ10 were recovered as the corresponding ubiquinols in mesenteric lymph, thus indicating their reduction in the intestine (Mohr et al., 1999). In the present study, there was some indication that the reduction of ubiquinone to ubiquinol by Caco-2 cells in this isolated system might become rate-limiting at high concentrations of micellarized CoQ10 in the medium.

Since CoQ10 is known to be synthesized *de novo* by practically every cell in the body (Ernster and Dallner, 1995), it was of interest to examine the endogenous CoQ10 content of differentiated Caco-2 cells that were not exposed to CoQ10. This was done in a separate experiment where a value of 53.5 pmol CoQ10 per mg protein was obtained for unexposed Caco-2 cells, and CoQ10 was present mostly in the reduced form as ubiquinol. It is interesting to note that in an earlier study on the tissue distribution and redox status of CoQ10 in human tissues, a value of

13.3 nmol/g of intestine (fresh tissue) was reported, with almost all of CoQ10 (95%) present as ubiquinol (Aberg et al., 1992).

In summary, this study demonstrates the feasibility of using coupled *in vitro* digestion-Caco-2 cell model for determining the absorption/bioaccessibility of CoQ10 from a variety of marketed CoQ10 products. The data show enhanced uptake of CoQ10 from solubilized formulations of CoQ10 by Caco-2 cells. Likewise, CoQ10–γ-cyclodextrin complex also shows superior absorption. There is a good correlation between the degree of micellarization and uptake of CoQ10 by Caco-2 cells. This study provides direct evidence for the conversion of CoQ10 (as ubiquinone) to ubiquinol in the enterocytes. The data also indicate a relationship between *in vitro* dissolution and uptake of CoQ10 by Caco-2 cells. The results support the usefulness of the coupled simulated digestion-Caco-2 cell model as a screening tool for assessing the absorption/potential bioavailability of orally ingested bioactive compounds.

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