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CoQ10 bioaccessibility and Caco-2 cell uptake improved with novel medium chain triglyceride encapsulation†

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Coenzyme Q10 (CoQ10) serves as a key component of the electron transport chain. Although it can be produced endogenously, genetic mutations and drugs (*e.g.,* statins) limit the amount absorbed, thus dietary sources provide a supplement. The hydrophobicity of CoQ10 limits its absorption during digestion. Encapsulation with medium chain triglycerides (MCTs) + phospholipid improves the water solubility of CoQ10, but the effect on bioaccessibility and Caco-2 cell uptake is understudied. This study compared the bioaccessibility and Caco-2 cell uptake of a powdered CoQ10 (control), as compared to equivalent doses of CoQ10 (2 mg) provided as ubiquinone encapsulated with MCTs + phospholipid in a VitaDry® and VitaSperse® product. Following sample hydration (for the control and VitaDry®) *in vitro* digestion was conducted. Samples were extracted and CoQ10 quantitated using high performance liquid chromatography-diode array detection (HPLC-DAD). The Vita encapsulated CoQ10 was 1.4× more bioaccessible as compared to the control, with no difference between the VitaDry® and VitaSperse® products. The VitaDry® and VitaSperse® encapsulated CoQ10 was 6.0× and 5.5× better taken up by Caco-2 cells. This study demonstrates that novel MCT and phospholipid based encapsulated CoQ10 is more bioaccessible, and *in vitro* results support future studies to establish if it may provide a more bioavailable alternative to CoQ10 alone.

1. Introduction

Coenzyme Q10 (CoQ10) serves as an important intermediate carrier of electrons in the electron transport chain of mitochondria, transferring electrons through the protonmotive Q cycle.[1] CoQ10 supplementation has been shown to improve symptoms of congestive heart failure,[2] lower blood pressure,[3] and lower high-density lipoprotein cholesterol and triglyceride levels in diabetes patients.[4] CoQ10 may help relieve myopathy caused by statin use,[5] and prevent migraines.[6] Since CoQ10 is involved in bioenergy production, CoQ10 supplements have been hypothesized to improve human athletic performance.[7]

The importance of oral intake of CoQ10 gradually emerges with aging because human CoQ10 concentrations in the lung, heart, spleen, liver, and kidney gradually decrease after the age of twenty.[8] Crystalline CoQ10 is known to have low and variable bioavailability in humans due to its poor solubility in water.[9] Absorption of CoQ10 alone following a single oral dose (*i.e.,* 10 mg CoQ10 per 100 g body weight) in rats has been reported as 2–3%.[10] These results highlight how approaches to increase bioavailability can help to compensate for the limited absorption observed for this non-polar compound.

Similar to other hydrophobic compounds, the absorption of CoQ10 could be greatly improved by co-consumption with oil, or *via* encapsulation.[11,12] Medium chain triglycerides (MCTs) have better stability as an encapsulation material,[13] and the energy required for spontaneous emulsification of MCTs is also lower as compared to long-chain triglycerides.[14] The bioavailability of MCT-encapsulated CoQ10 was evaluated with a Sprague-Dawley rat model following oral administration. The blood area under curve of CoQ10 following the administration of a 5% MCT-containing nanoemulsion was higher than blood CoQ10 concentrations following administration of a dry-emulsion containing 15% MCTs.[15] In a randomized, controlled study in 13 healthy humans, MCT-encapsulated CoQ10 had a higher bioavailability following a single dose, as compared to non-encapsulated CoQ10.[16]

Although bioaccessibility and cellular uptake of the MCT-encapsulated CoQ10 has been demonstrated to be higher in both pre-clinical,[15,17] and human studies,[16] encapsulation approaches to enhance CoQ10 suspension in aqueous preparations and food products have improved. However, it is not known whether these improvements correspondingly increase bioaccessibility and Caco-2 cell uptake following gastric and intestinal digestion.

This study determined whether 2 mg of CoQ10 encapsulated with phospholipid and MCTs increased bioaccessibility

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and Caco-2 cell uptake, relative to a non-encapsulated control. This study also determined whether various final product forms (*i.e.,* a dry powder and a liquid form) resulted in any difference in bioaccessibility and Caco-2 cell uptake.

2. Materials and methods

2.1 Materials

Unencapsulated CoQ10 (Control), powdered CoQ10 (VitaDry®), and an aqueous dispersion of CoQ10 (VitaSperse®) were kindly provided by 3i Solutions, containing 97.8%, 12.3%, and 10.4% (*w/w*), respectively of ubiquinone. VitaSperse® was mainly composed of medium chain triglycerides (MCTs), decaglycerol monomyristate, and phospholipid which suspend CoQ10 in the aqueous solution. VitaDry® was obtained by spray drying after mixing VitaSperse® with modified starch (Table 1). Soybean oil was purchased from a local grocery (Columbus, OH). Pepsin (P7000), pancreatin (P7545), lipase (L3126), porcine bile extract (B8631), and non-essential amino acid (NEAA), HPLC-grade methanol, hexane, methyl tertiary-butyl ether (MTBE), Optima grade LC/MS formic acid, ACS grade NaCl, $CaCl_2$, $NaHCO_3$, butylated hydroxytoluene, *trans*-β-apo-8′-carotenal and ubiquinone standard were purchased from Sigma-Aldrich (St Louis, MO). Ubiquinol standard was purchased from Toronto Research Chemicals (North York, ON, CA). ACS grade KCl, 1 N HCl and Pierce™ BCA Protein Assay Kits were purchased from Fisher Scientific (Pittsburgh, PA). Type I water was produced by a Milli-Q® reference water purification system (Millipore, Sigma, Burlington, MA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and antibiotic antimycotic solution (Anti–anti) were purchased from Gibco (Grand Island, NY).

2.2 *In vitro* digestion of CoQ10

Three formulations of CoQ10 (containing 2 mg ubiquinone) were tested (n = 6 per product type). The *in vitro* digestion model employed uses volumes and doses scaled-down 10–30× as compared to what might be consumed by a human. The dose of ubiquinone selected reflects the high end of what might be consumed in an American meal (*e.g.,* the consumption of two double bacon cheeseburgers and large serving of French Fries),[18–21] and the low end of what might be consumed from a CoQ10 supplement, which is commercially dispensed as capsules containing anywhere from 30 to 600 mg CoQ10.[22] Before digestion, control samples (purity 98%) were bath sonicated for 30 s in type I water (70 mL, 0.21 mg control product per mL). VitaDry® (purity 12.3%) was diluted to 1.63 mg mL^{-1} and VitaSperse® (purity 10.4%) was diluted to 1.93 mg mL^{-1}, with type I water. All samples were vortexed for 30 s using a VWR analog vortex mixer (Radnor, PA). The digestion procedure was modified from a previous study as follows.[23] Prepared CoQ10 suspensions (10 mL) were added to digestion tube, followed by soybean oil containing α-tocopherol (109 μL, 0.023 μmol α-tocopherol per μL soybean oil), and an ascorbic acid solution (89 μL, 49 μg of ascorbic acid per μL distilled water). Simulated gastric fluid (20 mL) containing 120 mM NaCl and 5 mM KCl was added. The pH was adjusted to 2.5 with 1 M HCl and then a pepsin solution (2 mL of a solution containing 550 mg pepsin with 575 U mg^{-1} dissolved in 45 mL 0.1 M HCl) was added to achieve 400 U mL^{-1} activity in the final gastric phase volume (35 mL). The sample was incubated in a VWR shaking bath (model 89032-226, Radnor, PA) held at 37 °C, at 250 rpm, for 1 h as previously described. Before the end of gastric digestion (~15 min), a bile salt solution was prepared by dissolving bile salts (2800 mg) in $NaHCO_3$ solution (70 mL, 0.1 M), and a pancreatin-lipase solution was prepared by dissolving pancreatin (234 mg, 32 USP U mg^{-1}) and lipase (1098 mg, 13 U mg^{-1}) together in $NaHCO_3$ solution (45 mL). After the gastric phase was complete, the pH was increased to 6 with 1 M $NaHCO_3$, and the bile salt solution (3 mL) and the pancreatin-lipase solution (2 mL) were added. A simulated intestinal fluid containing 120 mM NaCl, 5 mM KCl, and 6 mM $CaCl_2$ was added to bring the volume to 50 mL. Thus, the final enzyme activity of pancreatin was 6.7 USP U mL^{-1} and lipase was 12.5 U mL^{-1}. The final concentration of bile salts was 2.4 mg mL^{-1}, α-tocopherol was 50 μM, and ascorbic acid was 500 μM. Argon gas was used to evacuate the air in the headspace, and the mixture digested for a further 2 h. An aliquot of chyme (10 mL) was centrifuged at 12 000*g* for 45 min with a Beckman Coulter centrifuge (Avanti J-E, Brea, CA) using an JA 20.1 rotor, and filtered through 0.22 μm PVDF membrane to obtain the micelle fraction.

Table 1 Mixed formula of CoQ10 encapsulation in this study, percentage%

Ingredients	VitaSperse®	VitaDry®[a]
Water	65 ± 5	—
Medium chain triglycerides	10	12
Decaglycerol monomyristate	3.15 ± 0.65	3.71 ± 0.77
Phospholipid	5 ± 1	5.9 ± 1.2
Citric acid	0.4	0.47
Potassium sorbate	0.1	0.1
Starch	—	~60

[a] Hexametaphosphate is also added in minute quantities to the VitaDry® product.

2.3 Caco-2 cell culture and cellular uptake

The Caco-2 cell line was a gift from Drs. Chitchumroonchokchai and Failla. Cellular uptake experiments largely followed the previous study with minor modifications, testing one of the six digesta replicates per formulation type on an individual well.[24] Under appropriate culture conditions, Caco-2 cells spontaneously differentiate into a polarized monolayer with characteristics typical of enterocytes,[25] and express cholesterol transporter Niemann-Pick C1 Like 1 (*i.e.,* NPC1L1) which has been implicated in the transport of physiological doses of CoQ10.[26] Caco-2 cells cultured under the conditions used herein produce a polarized morphology, which mimics that of small intestine enterocytes, with maximal differentiation 11–14 days post-confluency, as previously evaluated by colleagues *via* assessment of sucrase and

alkaline phosphatase activity.[27] Cells 11 days post-confluence were used for the incubation experiments. A fresh micelle fraction (or phosphate buffered saline, PBS for negative control, 0.5 mL) was diluted with DMEM (1.5 mL) and added to the monolayer for incubation. Cells were incubated for 4 h at 37 °C under 5% CO_2, reflective of average duodenal transit time of a lipid-containing meal previously reported in humans.[28,29] After incubation, monolayers were washed with cold PBS containing albumin (2 g L^{-1}), followed by 2x washing with cold PBS alone, collected, and sealed under argon gas. Cells were stored at −80 °C before analysis. Caco-2 cell protein content was tested using the Pierce™ BCA Protein Assay Kit, following manufacturer instructions.

2.4 Sample extraction and analysis

To the CoQ10 samples of chyme, micelle, and cells, a butylated hydroxytoluene solution (10 μL of a 10 mg BHT/mL water solution) and *trans*-β-apo-8′-carotenal (internal standard) were added prior to extraction. Sample protein was precipitated with equal volumes of methanol and vortexed for 1 min, and then hexane was added (1 : 4, *v*/*v*) followed by probe sonication for 10 s and vortexing for 2 min. The hexane extraction was repeated, and the combined extracts were dried under argon and redissolved in methyl *tert*-butyl ether (MTBE)/methanol (1 : 1, *v*/*v*) just prior to analysis. Samples were separated on a C18 ZORBAX SB column (3.5 μm particle size, 100 mm × 4.6 mm, Agilent Inc., Santa Clara, CA) installed on a high-performance liquid chromatography system equipped with diode-array detection (HPLC-DAD, 1200 series, Agilent Inc., Santa Clara, CA). Mobile phase A = methanol/water (80 : 20, *v*/*v*), containing 0.1% formic acid and mobile phase B = MTBE/methanol/water (78 : 20 : 2, *v*/*v*/*v*) containing 0.1% formic acid. The gradient was as follows: beginning with 0% B; linear increase to 100% B over 8 min, holding at 100% B for 1 min; then a rapid return and holding of 0% B for 2 min. The flow rate was 1.2 mL min^{-1}, with column temperature held at 40 °C. The injection volume of digesta extracts and standard solutions was 10 μL, and that of the cell extracts was 15 μL. Ubiquinone and ubiquinol were quantitated using authentic standards. A series of dilutions of each was produced using the following molar extinction coefficients: ubiquinone (ε = 14 200 M^{-1} cm^{-1} in ethanol at 275 nm),[30] and ubiquinol (ε = 3510 M^{-1} cm^{-1} in isopropanol at 290 nm).[31] External calibration curves were built (peak area *vs.* pmol of compound on column), and recovery adjusted using the internal standard.

2.5 Data analysis and statistics

Bioaccessibility and cellular uptake were calculated as followed equations:

$$\text{Bioaccessibility}\,(\%) = \frac{\text{CoQ10 content of micelle fraction (mg)}}{\text{CoQ10 content before } \textit{invitro} \text{ digestion (mg)}} \times 100\%$$

$$\text{Cellular uptake} = \frac{\text{Total CoQ10 detected in cells (pmol)} - \text{endogenously synthesized CoQ10 (pmol)}}{\text{Cell protein (mg)}}$$

Analysis of variance (ANOVA) was conducted *via* R (**https://www.r-project.org**, version 4.0.3), using package "ggplot2", "ggpubr", "tidyverse", "broom", "AICcmodavg" to determine if there was any difference between bioaccessibility and Caco-2 cellular uptake of any of the 4 products tested, followed by Tukey's *post-hoc* test. A *P*-value of <0.05 was considered statistically significant. Pearson's correlation was performed to evaluate the association between % bioaccessibility and cellular uptake.

3. Results and discussion

3.1 Effect of encapsulation on CoQ10 stability and bioaccessibility

Control CoQ10 (*i.e.,* unencapsulated) poorly dissolved in the simulated gastric and intestinal fluid as compared to the Vita® products, which remained suspended during the full digestion. Recovery of control CoQ10 in the chyme was 64% while recovery of the encapsulated CoQ10 ranged from 98–106%. Similarly, others have reported that CoQ10 encapsulated with MCT-phospholipid was well recovered after incubation with simulated gastric fluid for 1 h and with simulated intestinal fluid for 24 h.[32]

The bioaccessibility of CoQ10 was assessed by calculating the ratio of micellarized CoQ10 after digestion, as compared to the CoQ10 provided in the initial dose. In this study, it was observed that all micellarized CoQ10 was ubiquinone, the oxidized form provided as the ingredient. The bioaccessibility of the control CoQ10 was 12.7%, which was 1.4× lower than the bioaccessibility of VitaDry® (18.0%), and VitaSperse® (17.8%) as shown in Fig. 1. The results herein demonstrate improved bioaccessibility of encapsulated CoQ10, as compared to CoQ10 alone. These results are in line with previous work, where micellarized CoQ10 digested in the absence of any additional macronutrients was shown to be <5%.[33] The bioaccessibility of control CoQ10 digested with soybean oil in this study was lower than that of CoQ10 digested with both protein and lipids,[17] where the addition of 4 g plain yogurt led to 22% bioaccessibility. This hypothesis is further supported by other work where CoQ10 bioaccessibility was dramatically increased from 1.5% to 38% with sodium caseinate or with whey protein concentrate, possibly through the formation of CoQ10-proteins complexes.[34] It is also worth noting that these authors only reported the (*w*/*v*) (*i.e.*, the mass of enzyme provided in a given volume of digesta) used in the *in vitro* digestion experiments rather than enzyme units of activity, thus it is unknown how enzyme activity differences may have contributed to the disparity in the two studies.[17,35]

In the study herein, there was no significant difference between the bioaccessibility of all Vita-encapsulated CoQ10, indicating the delivery efficiency of VitaSperse® and VitaDry® was equally good and was ~1.4× higher than control. The

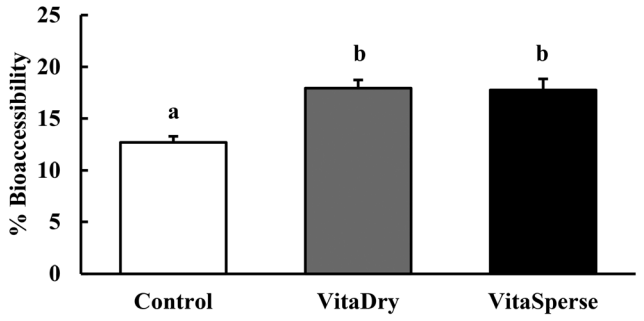

Fig. 1 The influence of encapsulation on CoQ10 bioaccessibility (expressed as % micellarized CoQ10 relative to the CoQ10 dose digested). The isoform of CoQ10 both provided in the digestion and observed the micelles was exclusively ubiquinone. Bars represent average ± SEM, with n = 6 per group. Letters indicate a statistically significant difference between the groups, as assessed *via* ANOVA followed by Tukey's test (P < 0.05 considered significant).

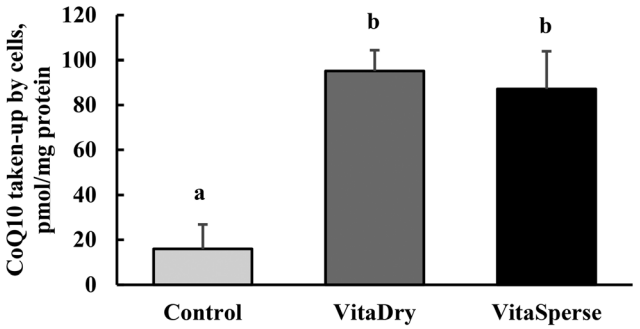

Fig. 2 The influence of encapsulation on CoQ10 Caco-2 cell uptake (calculated as total cellular CoQ10 following incubation with digesta from each treatment, minus endogenously synthesized CoQ10 in cells incubated with DMEM alone). Uptake was assessed following the digestion of the control and Vita products with 100 μL soybean oil. Cells were incubated with the micelle fraction diluted 4× with DMEM for 4 h. Bars represent average ± SEM, with n = 6 per group. Letters indicate a statistically significant difference between groups, as assessed *via* ANOVA followed by Tukey's test (P < 0.05 considered statistically significant).

VitaSperse® equivalent tested in a previous study had ~3× higher bioaccessibility than non-encapsulation CoQ10, and the bioaccessibility of the liquid suspension formulation was significantly higher than previous powdered versions.[17] We also made additional experiments to confirm the *encapsulation* with MCT and phospholipid mixture improved bioaccessibility, as compared to the addition of these excipients alone. Digestion of the CoQ10 ingredient alone *vs.* CoQ10 ingredient + MCT + decaglycerol monomyristate *vs.* CoQ10 ingredient + MCT + decaglycerol monomyristate + phospholipid (n = 6 per group) revealed no significant differences. Thus, the encapsulation was a key factor in enhancing the Vita CoQ10 bioaccessibility.

According to the manufacturer, VitaSperse® diluted to 5% in water provides nanoparticles which measure ~150 nm in diameter, while VitaDry® diluted to 5% in water provides nanoparticles which measure ~180 nm in diameter. These differences in particle size may have offset the slight differences in higher MCT and phospholipid content of the VitaDry® *vs.* VitaSperse® (Table 1), resulting in no observed difference in bioaccessibility.

3.2 Effect of encapsulation on Caco-2 cellular uptake of CoQ10

The utility of the Caco-2 model in predicting differences in CoQ10 bioavailablity has been previously demonstrated. Using the Caco-2 model, Chopra and colleagues observed a 3.6× higher Caco-2 uptake of CoQ10 when incorporated into a Q-Gel® supplement as compared to a CoQ10 control ingredient.[17] Similarly, the team observed 3.2× higher bioavailability of CoQ10 as Q-Gel® *vs.* a control form, when tested in healthy humans.[36]

In the study herein, the amount of CoQ10 taken up by the cells following incubation with the control digesta was 15.9 ± 10.9 pmol CoQ10 per mg protein. Compared to control group, the VitaDry® and VitaSperse® products increased cellular uptake of CoQ10 by 6.0 and 5.5×, respectively (Fig. 2). The increase in Caco-2 cell uptake of CoQ10 from the Vita products demonstrated a more significant rise, compared to CoQ10 nanoparticles prepared with chitosan and dextran sulfate sodium salt, which increased cellular uptake of CoQ10 incorporated into artificial micelles by only 3×.[37] However, the result herein align with a previous study where MCT-encapsulated CoQ10 was 3.4–7.4× better taken up by Caco-2 cells than unencapsulated CoQ10.[17]

The amount of CoQ10 endogenously synthesized by the cultured Caco-2 cells (237 ± 10 pmol CoQ10 per mg protein) was established using a negative control of cells incubated with media alone. Similarly, Failla *et al.,* found that the CoQ10 levels in Caco-2 cells 21 days post-confluency grown on membrane inserts was 220 pmol CoQ10 per mg protein.[38] In contrast, Bhagavan and colleagues reported 53.5 pmol CoQ10 per mg protein (of which 95% was ubiquinol) in cells 11 days post-confluency.[17] These results demonstrate that growth conditions can dramatically influence the CoQ10 levels, and subsequent CoQ10 uptake in Caco-2 cells.

Of the CoQ10 endogenously synthesized within Caco-2 cells, 35% was in the reduced, ubiquinol form. The amount of ubiquinol in the cells incubated with Control digesta was not different from the media-incubated cells, while increases were observed following incubation with digesta containing Vita® products (data not shown). The CoQ10 accumulation in the Caco-2 cells was also well correlated with bioaccessibility, producing an R = 0.64 (Fig. S1†), similar to previous studies.[17] Furthermore, it has previously been shown that cell culture media by itself does not reduce ubiquinone to ubiquinol over an 8 h period.[17] Taken together, these results support the hypothesis that chemical reduction primarily occurs once the CoQ10 (as ubiquinone) is taken up by the enterocyte.

MCT-encapsulated CoQ10 also has advantages in cell delivery efficiency as compared to other emulsifying agents. First, as compared to long-chain triglyceride stabled emulsions, less

lipid droplets and lipoproteins were observed in Caco-2 cells after incubation with an MCTs stabilized-emulsion,[39] suggesting an attenuation of the post-prandial lipemic response which may be of concern in certain individuals (*e.g.,* those predisposed to cardiovascular disease). Second, MCTs can reduce the intestinal inflammatory response, modulate intestinal microbiota, and modulate intestinal permeability.[40] Also, other encapsulation agents like milk proteins, *i.e.,* whey proteins or casein, have a potential allergy risk to ~5% United State general population.[41] Vitasperse® is a starch-free and a safe CoQ10 supplement for individuals avoiding the consumption of wheat or corn.[42]

4. Conclusions

This study evaluated the bioaccessibility and cellular uptake of three dispersions of CoQ10 encapsulated in medium-chain fatty acids and emulsifiers. Compared with the non-encapsulated control, the encapsulated CoQ10 was more stable and had significantly improved bioaccessibility (1.4×) during *in vitro* gastrointestinal digestion. The redox state of CoQ10 did not change during the simulated digestion. The uptake of unencapsulated CoQ10 by Caco-2 cells was very limited, while encapsulated CoQ10 was better delivered, with an uptake increase of up to 6×, demonstrating superior delivery efficiency. This study supports future *in vivo* research to unequivocally elucidate whether improved absorption in humans is observed for these MCT-encapsulated products, as compared to non-encapsulated controls.

Author contributions

Ziqi Li: methodology, formal analysis, investigation, writing – original draft, visualization. Rachel E. Kopec: conceptualization, funding acquisition, validation, writing – review & editing, supervision, project administration.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

There are no conflicts of interest to declare.

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