

### RESEARCH ARTICLE

## Women have higher levels of CoQ<sub>10</sub> than men when supplemented with a single dose of CoQ<sub>10</sub> with monoglycerides omega-3 or rice oil and followed for 48 h: a crossover randomised triple blind controlled study

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#### Abstract

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), a lipid involved in ATP synthesis, exhibits very limited oral absorption, and its endogenous production decreases with ageing and with the occurrence of oxidative stress. Our group previously showed that monoglycerides omega-3 (MAG-OM3) increase OM3 plasma concentrations. Since CoQ<sub>10</sub> is liposoluble, we hypothesised that its 48 h pharmacokinetics is higher when provided with MAG-OM3 compared to CoQ<sub>10</sub> alone (in powder form) or added to rice oil (a neutral triacylglycerol oil). A randomised triple-blind crossover study was performed with fifteen men and fifteen women consuming the three supplements providing 200 mg of CoQ<sub>10</sub> in a random order. Blood samples were collected before (*t* = 0) and 1, 3, 5, 6, 7, 8, 10, 11, 24 and 48 h after the supplement intake. Plasma total CoQ<sub>10</sub> concentrations were analysed on ultrahigh-performance liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS). Participants were 26.1 ± 4.8 years old. When CoQ<sub>10</sub> was provided with rice or MAG-OM3 oils, the 48 h area under the curve (AUC 0–48 h) was approximately two times higher compared to when provided without an oil. The delta max concentration ( $\Delta C_{\max}$ ) of plasma CoQ<sub>10</sub> was, respectively, 2 (MAG-OM3) and 2.5 (rice oil) times higher compared to CoQ<sub>10</sub> alone. There was a significant sex by treatment interaction (*P* = 0.0250) for the AUC 0–6 h supporting that in postprandial, men and women do not respond the same way to the different supplement. Women had a higher CoQ<sub>10</sub> concentration 48 h after the single-dose intake compared to men. We conclude that CoQ<sub>10</sub> supplements must be provided with lipids, and their kinetics is different between men and women.

**Key words:** CoQ<sub>10</sub>: Crossover study: Monoglyceride omega-3: Pharmacokinetics

#### Introduction

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), also known as ubiquinone, is an endogenously synthesised fat-soluble molecule. It is composed of a benzoquinone ring and 10 isoprene subunits<sup>(1)</sup>. It has two main roles in the body. CoQ<sub>10</sub> plays a major role in ATP synthesis by transporting electrons from complexes I and II to complex III via the Q cycle in the electron transport chain of the mitochondria and it also contributes to the proton gradient<sup>(2,3)</sup>. CoQ<sub>10</sub> is also a scavenger of free radicals, can regenerate

other antioxidants' pools like vitamin E<sup>(4)</sup> and can protect lipoproteins from lipid peroxidation at the initiation and propagation stages<sup>(2)</sup>. A low level of blood CoQ<sub>10</sub> has been reported in those with chronic diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes and cancer<sup>(5,6)</sup>. Moreover, during ageing, CoQ<sub>10</sub>'s blood levels tend to decrease<sup>(7,8)</sup>.

The total amount of CoQ<sub>10</sub> in the body is approximately 2000 mg. Its daily nutritional intake is estimated at 3–6 mg/day but the body needs are estimated to be ~500 mg of

**Abbreviations:** AUC: area under the curve; C 0 h: plasma concentration at baseline; C 48 h: plasma concentration at 48 h post-single-dose intake; C<sub>max</sub>: maximum concentration; CoQ<sub>10</sub>: coenzyme Q<sub>10</sub>; CoQ<sub>9</sub>: coenzyme Q<sub>9</sub>; MAG: monoglyceride; OM3: omega-3 fatty acids; T<sub>max</sub>: time to reach maximum concentration

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CoQ<sub>10</sub> every day<sup>(9)</sup>. Therefore, the consumption of 30–100 mg of CoQ<sub>10</sub> per day by healthy people and of 60–1200 mg of CoQ<sub>10</sub> per day in those with a medical condition as described above is recommended<sup>(9)</sup>. CoQ<sub>10</sub> supplementation is considered safe and well tolerated without any serious adverse effects in human subjects<sup>(10)</sup>. Its acceptable daily intake is 12 mg/kg/d which is about 720 mg/d for a person weighing 60 kg<sup>(10)</sup>. Some studies even used CoQ<sub>10</sub> doses up to 2400 mg/d, which was well tolerated with no safety concerns reported<sup>(11)</sup>. Furthermore, it seems that dietary CoQ<sub>10</sub> intake does not influence its endogenous synthesis and there is no long-term accumulation in the tissues<sup>(10)</sup>. The main issue with CoQ<sub>10</sub> supplementation is its low and incomplete absorption, and hence, its efficacy when provided as a supplement is doubted or limited<sup>(12)</sup>. To overcome this issue, the most popular approaches focused on adding synthetic products such as liposomes, lipid-free nano-formulations, water-soluble CoQ<sub>10</sub>, CoQ<sub>10</sub>-loaded oleo gels and CoQ<sub>10</sub> micellisation. Although these strategies have improved CoQ<sub>10</sub> absorption and bioavailability, these synthetic products have no health benefits<sup>(12)</sup>.

Our group previously conducted pharmacokinetic studies on omega-3 fatty acids (OM3) esterified in monoacylglycerols (MAG) and reported that plasma OM3 concentrations were two to three times higher 0–6 h post dose intake when OM3s were esterified in MAGs compared to when esterified in ethyl esters or triacylglycerols<sup>(13,14)</sup>. The amphipathic nature of MAGs contributes to improving the emulsification of fats and producing micelles in the gastrointestinal tract<sup>(15–17)</sup>. Therefore, we hypothesise that CoQ<sub>10</sub> blood levels over a 48 h follow-up will be higher when provided with MAG-OM3 than CoQ<sub>10</sub> alone (in powder form) or added to rice oil (a neutral triacylglycerol oil). The objective of the present study was to perform, in men and women, comparative pharmacokinetics of the three CoQ<sub>10</sub> formulations and monitor concentrations of total CoQ<sub>10</sub> in the plasma over 48 h after the single-dose intake.

## Methods

### Study participants

The present study was a randomised, triple-blind, crossover trial conducted at the Research Center on Aging, Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie – Centre Hospitalier Universitaire de Sherbrooke (CIUSSSE–CHUS), in Sherbrooke (Quebec, Canada). This trial was revised and approved by the Research Ethics Committee of the CIUSSSE–CHUS (reference no. 2020-3280). The present study is registered at [clinicaltrials.gov](https://clinicaltrials.gov) under no. NCT04035525.

Recruitment took place from June to December 2020. The main inclusion criteria were to be aged between 18 and 50 years old. Exclusion criteria included people with a particular diet such as being vegetarian or vegan, and/or with allergies to fish/seafood, people taking CoQ<sub>10</sub> and/or omega-3 fatty acid supplements or who had taken these supplements daily in the previous 6 months. People with a body mass index of <18.5 kg/m<sup>2</sup> or >34.9 kg/m<sup>2</sup> were also excluded and those smoking tobacco or marijuana as well. Other exclusion criteria

assessed during the phone call were: current or past performance athletes; alcohol and/or drug abuse; malnutrition; diabetes; presence of systemic, gastrointestinal, hepatic, renal, cardiac, thyroid or hormonal problems; or a diagnosis of schizophrenia, psychotic disorder, bipolarity, major depression (<5 years), panic disorder and/or obsessive-compulsive disorder. Also, women who were pregnant or lactating or going through menopause and people who had donated blood in the past month were excluded. Females of childbearing age were required to use a contraceptive method to avoid getting pregnant during the trial. After phone screening, participants were invited to the Research Center. At that time, participants were requested to read and sign the informed consent form. All participants provided written informed consent prior to the trial beginning. The fasted blood samples were then collected to assess blood biochemistry at baseline. These analyses were performed at the Centre Hospitalier Universitaire de Sherbrooke clinical laboratory and included HDL cholesterol, LDL cholesterol, triacylglycerols, fasting blood glucose concentration and glycated haemoglobin. Individuals with values outside the clinical reference range were excluded.

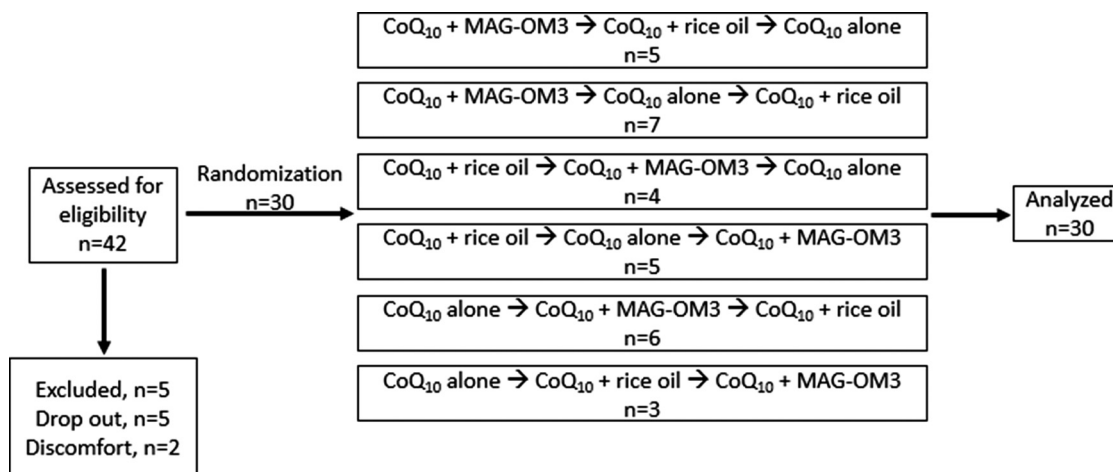
### Randomisation and blinding

Randomisation of the treatment groups was performed using '<https://www.randomizer.org/>'. Treatment randomisation is described in a flow chart in Fig. 1. Participants, clinical staff and laboratory staff were blinded to treatment allocation. The CoQ<sub>10</sub> + MAG-OM3 and CoQ<sub>10</sub> + rice oil capsules were identical in size, shape and taste, but differed from the CoQ<sub>10</sub> alone capsule. To maintain blinding, participants wore an eye mask when taking the supplements and a research staff not involved in the trial provided the capsules to participants to keep blinding. Also, each plasma sample was assigned a random number between 1 and 990 to avoid knowing the participant's number, treatment and the time at which the sample was analysed. These were unblinded only after performing all the CoQ<sub>10</sub> analyses described below.

### Study treatments

The study treatments consisted of (1) CoQ<sub>10</sub> + MAG-OM3, (2) CoQ<sub>10</sub> + rice oil (a neutral triacylglycerol oil) and (3) CoQ<sub>10</sub> alone, in powder. Each capsule provided 100 mg of oxidised CoQ<sub>10</sub>, the most stable form of CoQ<sub>10</sub>, and 500 mg of oil, except for the CoQ<sub>10</sub> alone which contained only 100 mg of CoQ<sub>10</sub> in powder. The supplement content in CoQ<sub>10</sub> was analysed by Labs Mart and it ranges from 108 to 117 mg/capsules (*n* 2 capsules/supplement type). The fatty acid profile of the capsules was determined using the modified AOCS Ce 1b-89 method and is detailed in Table 1.

Participants consumed two capsules providing 200 mg of CoQ<sub>10</sub> and 1 g of oil. This dose was selected because a study compared the pharmacokinetics of a single oral dose of 100 and 200 mg of CoQ<sub>10</sub> and they reported an increase of 150 % of CoQ<sub>10</sub> in the plasma with the 200 mg dose compared to an increase of 80 % with the 100 mg dose<sup>(18)</sup>. Another study also obtained similar results<sup>(19)</sup>. Therefore,



**Fig. 1.** Clinical trial flow chart and randomisation of the treatments order to the CoQ<sub>10</sub> + MAG-OM3, CoQ<sub>10</sub> + rice oil or CoQ<sub>10</sub> alone supplement. The *n* represents the number of participants allocated to the treatment sequence. CoQ<sub>10</sub>, coenzyme Q<sub>10</sub>; MAG-OM3, monoglycerides omega-3.

the dose of 200 mg/d of CoQ<sub>10</sub> was selected for the present study. Also, a dose of 1 g of oil was selected because it was similar to a previous study done by our group<sup>(13,14)</sup>. A minimum of 1 week washout period was mandatory between treatments since CoQ<sub>10</sub>'s half-life is 33 h<sup>(20)</sup>. It is generally accepted for drugs that four to five times the half-life eliminate the active molecule at 94–97 %, which is considered to be below clinical significance<sup>(21)</sup>. Therefore, in this trial, 6.9 d were required to eliminate CoQ<sub>10</sub> at 97 %.

**Table 1:** Fatty acids content in the supplements

Fatty acids	CoQ <sub>10</sub> + MAG-OM3 supplement (mg/capsule)		CoQ <sub>10</sub> + rice oil supplement (mg/capsule)	
	Mean	SD	Mean	SD
8:0	0.0	0.0	0.4	0.5
10:0	0.9	0.4	3.4	3.7
14:0	1.5	0.0	1.5	0.1
16:0	6.1	0.7	89.6	2.8
16:1 n-7	0.5	0.0	0.9	0.0
17:1	0.4	0.0	0.1	0.3
18:0	6.6	0.0	9.5	0.3
18:1 n-9	11.3	0.8	190.4	5.8
18:1 n-7	3.3	0.0	4.0	0.1
18:2 n-6	3.6	0.7	144.1	4.4
18:3 n-6	0.7	0.0	1.6	0.1
18:3 n-3	1.2	0.0	4.3	0.1
20:0	5.2	0.1	3.6	0.1
20:1	14.3	0.2	2.4	0.1
20:2	2.4	0.1	0.0	0.0
20:3 n-6	2.9	0.0	0.0	0.0
20:4 n-6	12.9	0.2	0.0	0.0
20:3 n-3	1.3	0.1	0.0	0.0
20:5 n-3	224.3	3.5	0.0	0.0
22:0	0.0	0.0	1.3	0.1
22:1	1.7	0.1	0.0	0.0
22:5 n-6	3.4	0.1	0.0	0.0
22:5 n-3	14.3	0.1	0.0	0.0
22:6 n-3	95.3	1.6	0.0	0.0
24:0	0.0	0.0	2.5	0.1
<b>TOTAL Concentration</b>	<b>414.1</b>	<b>8.9</b>	<b>459.8</b>	<b>18.7</b>

n = 3 capsules/supplement form.

### Study design

Participants were required to be fasted at arrival. A blood sample was then collected, and a standardised breakfast with the two capsules was provided. The breakfast provided 410 kcal (75 % carbohydrates, 22 % proteins, 3 % lipids) and was composed of two toasts with raspberry jam, a banana, a protein chocolate milk and a coffee or a tea. In the present study, meals were provided throughout the day to control the dietary fat intake. A low-fat menu was selected to limit bias in the CoQ<sub>10</sub> metabolism. After consuming the capsules, the timer indicated the subsequent blood samples to be collected at 1, 3, 5, 6, 7, 8, 10, 11, 24 and 48 h post-single oral dose intake. Blood was collected in tubes containing citrate. Lunch and dinner were provided at 4 and 9 h post-single-dose intake, and they provided, respectively, 614 kcal (78 % carbohydrates, 17 % proteins, 5 % lipids) and 696 kcal (72 % carbohydrates, 23 % proteins, 5 % lipids). Lunch was composed of one serving of chow mein, baby carrots, an apple, a yogurt, a juice and a fig bar, and dinner was composed of one serving of General Tao, one slice of bread, one mini cucumber, apple sauce, protein chocolate milk, vegetable juice and a granola bar. Also, at 11 h post-dose intake, a snack (one granola bar and one package of gummy fruits) provided 210 kcal (87 % carbohydrates, 6 % proteins, 7 % lipids). The menu and food items consumed by a participant in the first pharmacokinetic study day were kept the same for the other two pharmacokinetic days to limit potential bias. Participants returned for a blood sample 24 and 48 h post-single-dose intake. During the study day, blood samples were kept on ice and centrifuged at 1700 g for 10 min at 4°C, and the plasma was aliquoted and stored at –80°C until further analysis.

### CoQ<sub>10</sub> extraction and analysis

Total CoQ<sub>10</sub> was extracted from plasma samples, using the Hansen *et al.* method<sup>(22)</sup>. Because reduced CoQ<sub>10</sub> is unstable, plasma CoQ<sub>10</sub> was oxidised with 1,4-benzoquinone to generate a stable CoQ<sub>10</sub> analyte. To allow quantification, coenzyme Q<sub>9</sub> (CoQ<sub>9</sub>) was added as an internal standard.



Briefly, samples were thawed at room temperature for 45 min. In total, 50  $\mu$ l of plasma were transferred to a 1.5 ml tube along with 50  $\mu$ l of 1,4-benzoquinone dissolved in methanol (0.4 mg/ml) and 50  $\mu$ l of CoQ<sub>9</sub> dissolved in 1-propanol (800  $\mu$ g/l). After mixing for 15 min at room temperature, 1 ml of 1-propanol was added to the tube. Tubes were then mixed again for 1 min and centrifuged at 10 000 g for 3.5 min at 4°C. After centrifugation, 1 ml of the supernatant was transferred to an HPLC vial. In total, 300  $\mu$ l of methanol was added to have the same solvent as the mobile phase of the liquid chromatography system. All vials were stabilised in the autosampler at 4°C for 30 min before being injected. One quality control sample was done per batch.

Analyses were performed on Waters Acquity UPLC H-Class system coupled to Waters Xevo TQ-S Micro tandem mass spectrometer. The column used was Waters Acquity UPLC BEH C18 2.1  $\times$  50 mm, 1.7  $\mu$ m and the pre-column was Waters Acquity UPLC BEH C18 Vanguard 2.1  $\times$  5 mm, 1.7  $\mu$ m and it was maintained at 25°C. The mobile phase was composed of methanol with 5 mM of ammonium formate, in isocratic mode. The injection volume was 1  $\mu$ l, the flow was 0.4 ml/min and the total run time was 14 min per injection. The mass spectrometer was operated in the electrospray positive mode. The optimised detector parameters were set as follows: nitrogen desolvation was at 600°C with a flow rate of 1000 l/h, the nitrogen cone gas was set at 80 l/h, the argon collision gas was at 5 psi, the source temperature was at 150°C and the capillary voltage was set at 0.9 kV. The cone voltage was at 88 V for CoQ<sub>10</sub> and 82 V for CoQ<sub>9</sub>. The collision energy was at 32 V for CoQ<sub>10</sub> and at 30 V for CoQ<sub>9</sub>. Quantification was performed in multiple reaction monitoring mode with the following mass transitions: CoQ<sub>10</sub> at m/z 863.6  $\rightarrow$  197.1 and CoQ<sub>9</sub> at 795.6  $\rightarrow$  197.1. The dwell time for each transition was 0.037 s.

Calibration curves were freshly prepared daily using CoQ<sub>10</sub> stock solution at the following concentrations: 0, 250, 500, 1500, 2000 and 2500  $\mu$ g/l. An internal standard (CoQ<sub>9</sub>) at a concentration of 800  $\mu$ g/l was also added in each of the calibration curve samples. Calibration curves were generated using MassLynx software by plotting the peak area ratio of CoQ<sub>10</sub> to internal standard *v.* the CoQ<sub>10</sub> concentration. Sample CoQ<sub>10</sub>

concentrations were calculated with a linear regression using the 1/*x* weighting factor.

### Statistical analysis

Sample size was calculated with a website sample size calculator<sup>(23)</sup>. The sample size was calculated based on the available data on the pharmacokinetics of CoQ<sub>10</sub> that was the closest to our study design. López-Lluch *et al.* performed a pharmacokinetic study on seven different CoQ<sub>10</sub> formulations and monitored CoQ<sub>10</sub> plasma concentration over a 48 h period in fourteen healthy adults after a single oral dose intake of approximately 100 mg of CoQ<sub>10</sub><sup>(24)</sup>. Using the 0–48 h AUC of the olive oil/coconut oil + CoQ<sub>10</sub> treatment (6.28  $\pm$  3.07 mg/l\*48 h) compared to the finely ground CoQ<sub>10</sub> powder (8.94  $\pm$  3.33 mg/l\*48 h), we calculated, with a power of 80 % with an  $\alpha$  = 0.05, a sample size of twenty-five participants was required. We, therefore, tested thirty participants to account for a potential 20 % attrition.

Area under the curves (AUC) were calculated using GraphPad Prism 7.03 software. The incremental AUC was calculated with the 48 h-curves, by ignoring the areas below the baseline.  $\Delta C_{\max}$  was defined as the CoQ<sub>10</sub> maximum concentration minus the baseline CoQ<sub>10</sub> plasma concentration.  $T_{\max}$  was defined as the time required to reach  $C_{\max}$ .

The primary outcome of the present study was the 0–48 h AUC of CoQ<sub>10</sub>. Secondary outcomes included 0–6 h AUC,  $\Delta C_{\max}$ ,  $T_{\max}$ ,  $C$  0 h and  $C$  48 h. Statistical analyses were performed using IBM SPSS Statistics 25 and GraphPad Prism 7.03 software. For the pharmacokinetic parameters, the Shapiro–Wilk normality test ( $\alpha$  = 0.05) was performed, and data were not normally distributed. Therefore, Friedman's ANOVA statistical rank test for paired samples was used. Dunn's multiple comparison test was used for the comparison between treatments. For the comparison between sexes, a two-way ANOVA test was performed, with Bonferroni's multiple comparison test. However, for the  $\Delta C$  48 h, these data were analysed by fitting a mixed model, rather than by repeated-measures ANOVA, because there were missing values. Indeed, three participants refused to come back on one of their 48 h follow-up visits. The *P* interaction represents the

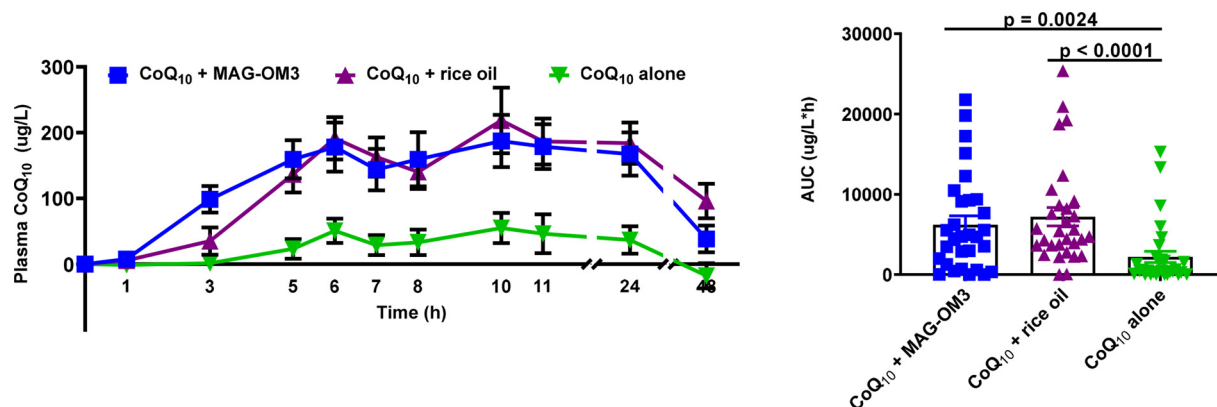
**Table 2:** Baseline characteristics of the participants

Baseline characteristics	Total cohort (n = 30)		Men (n = 15)		Women (n = 15)		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	26.1	4.8	26.7	4.3	25.5	5.2	0.4771
BMI (kg/m <sup>2</sup> )	25.3	3.6	25.6	3.5	25.1	3.8	0.7043
Plasma TG* (mmol/L)	0.9	0.6	0.9	0.4	1.0	0.7	0.9584
Plasma HDL-C (mmol/L)	1.4	0.3	1.3	0.3	1.4	0.2	0.1738
Plasma LDL-C (mmol/L)	2.5	0.8	2.5	0.8	2.4	0.8	0.7934
Plasma glucose (mmol/L)	4.5	0.4	4.4	0.4	4.5	0.4	0.5183
HbA1c (%)	5.0	0.3	5.1	0.3	5.0	0.3	0.6834

BMI = body mass index, TG = triglycerides, HDL-C = high-density lipoproteins cholesterol, LDL-C = low-density lipoproteins cholesterol, HbA1c = glycated hemoglobin. P values were evaluated by T test for unpaired measurements.

\*For TG, the data was not normally distributed, and a nonparametric Mann-Whitney test was performed.





**Fig. 2.** Plasma CoQ<sub>10</sub> concentrations over a 48 h period when combined with MAG-OM3, rice oil or alone. The results are expressed as the mean  $\pm$  SEM. *P* values were assessed by the Friedman nonparametric statistical analysis. CoQ<sub>10</sub>, coenzyme Q<sub>10</sub>; MAG-OM3, monoglycerides omega-3; AUC, area under the curve.

combined effect of sex and supplement type, while *P* sex and *P* supplement represent the individual effects of sex or supplement.

## Results

### Participants baseline characteristics

Table 2 presents the baseline data of the participants. There was no significant difference between men and women. The participants' mean age was  $26.1 \pm 4.8$  years. Treatment randomisation is described in the flow chart (Fig. 1).

### Primary outcome

Fig. 2 and Table 3 present the 0–48 h pharmacokinetic curves and pharmacokinetic parameters of CoQ<sub>10</sub>. The 0–48 h AUC of CoQ<sub>10</sub> combined with MAG-OM3 or rice oil was significantly higher than CoQ<sub>10</sub> provided alone (Table 3).

### Secondary outcomes

The 0–6 h AUC of CoQ<sub>10</sub> was significantly higher when CoQ<sub>10</sub> was combined with MAG-OM3 or rice oil compared to CoQ<sub>10</sub> alone ( $P < 0.0001$ ). Plasma CoQ<sub>10</sub> peaked 6 h and again 10 h post-single-dose intake (Fig. 2). *T*<sub>max</sub> and CoQ<sub>10</sub> plasma concentrations at baseline were not different between treatments. CoQ<sub>10</sub> concentrations still in the plasma 48 h

after taking the supplements were 1.5–4.5 times higher for the CoQ<sub>10</sub> + rice oil supplement compared to MAG-OM3 or CoQ<sub>10</sub> alone ( $P = 0.0004$ ) (Table 3). The  $\Delta C_{max}$  of plasma CoQ<sub>10</sub> was, respectively, 1 and 1.5 times higher when CoQ<sub>10</sub> was provided with MAG-OM3 or rice oil compared to the powder supplement form ( $P = 0.0015$ ) (Table 3).

CoQ<sub>10</sub> pharmacokinetic parameters were also assessed in relation to sexes (Fig. 3). There was no sex difference in the AUC 0–48 h, *T*<sub>max</sub> and  $\Delta C_{max}$ . Forty-eight hours after the single-dose intake of CoQ<sub>10</sub>, women had, on average, eleven times higher CoQ<sub>10</sub> plasma concentration than men ( $P = 0.0143$ ). There was an interaction between sex and supplement form for the baseline concentration (*C* 0 h) and the AUC 0–6 h in the pharmacokinetics of CoQ<sub>10</sub> between men and women (Fig. 3; Table 4). A significant sex by supplement interaction means that the effect of the supplements differs between men and women, even though the effect of sex alone is not significant. For the AUC 0–6 h, men had higher postprandial CoQ<sub>10</sub> levels, whereas the highest concentrations in CoQ<sub>10</sub> in women were reached 10 h after the single-dose intake.

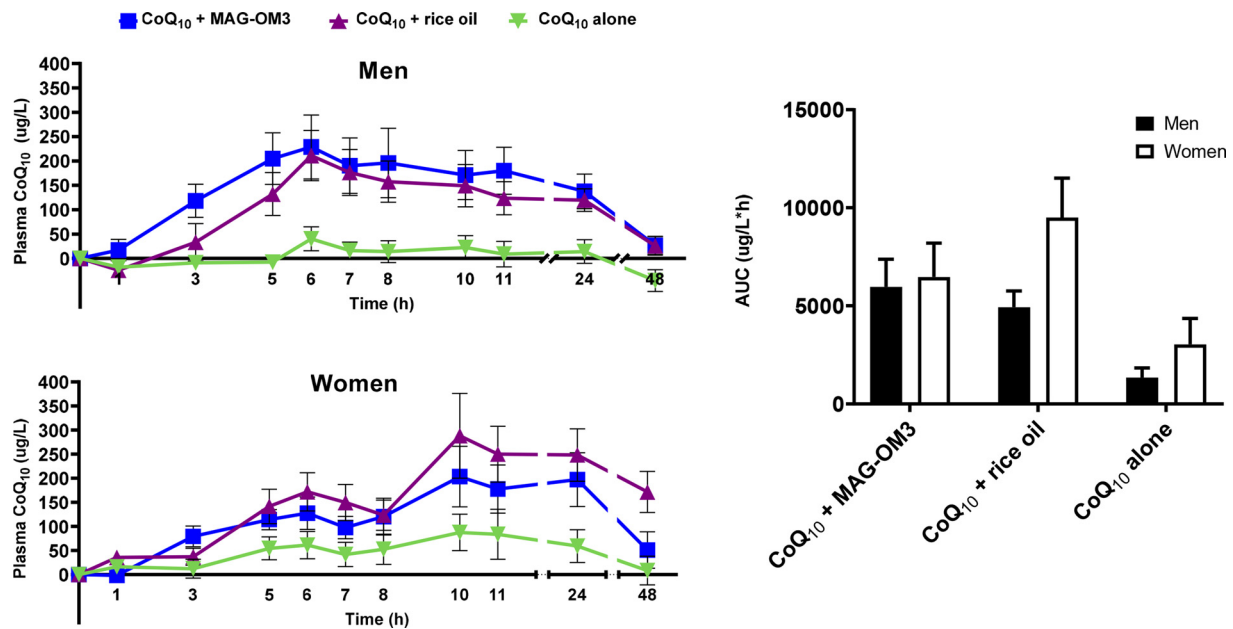
## Discussion

In the present study, we hypothesise that CoQ<sub>10</sub> blood levels over a 48 h follow-up is higher when provided with MAG-OM3 than CoQ<sub>10</sub> alone (in powder form) or added

**Table 3:** Pharmacokinetic parameters of CoQ<sub>10</sub> in combination with MAG-OM3, rice oil or alone.

Pharmacokinetic parameters	CoQ <sub>10</sub> + MAG-OM3		CoQ <sub>10</sub> + Rice oil		CoQ <sub>10</sub> alone		P-value
	Mean	SEM	Mean	SEM	Mean	SEM	
<i>T</i> <sub>max</sub> (h)	9.5	1.3	9.6	1.3	6.9	1.1	0.6128
<i>C</i> <sub>0h</sub> (μg/L)	611.6	42.0	617.9	43.4	581.8	40.2	0.0877
$\Delta C_{max}$ (μg/L)	281.1 <sup>A</sup>	44.5	325.1 <sup>A</sup>	50.1	127.8 <sup>B</sup>	26.5	0.0015
$\Delta C_{48h}$ (μg/L)	38.3 <sup>A</sup>	20.5	96.1 <sup>B</sup>	26.3	−17.7 <sup>A</sup>	19.2	0.0004
AUC 0–6h (μg/L*h)	576.7 <sup>A</sup>	100.0	456.1 <sup>A</sup>	73.1	161.7 <sup>B</sup>	41.6	<0.0001
AUC 0–48h (μg/L*h)	6211.0 <sup>A</sup>	1097.0	7216.0 <sup>A</sup>	1148.0	2199.0 <sup>B</sup>	702.6	<0.0001

Results are expressed as mean  $\pm$  SEM. *T*<sub>max</sub> = time at which the maximum concentration is reached, *C*<sub>0h</sub> = plasma concentration at baseline,  $\Delta C_{max}$  = maximum concentration, *C*<sub>48h</sub> = plasma concentration 48h post single dose intake, AUC 0–6 h = area under the curve over 6h, AUC 0–48h = area under the curve over 48h. *P* values were assessed by Friedman nonparametric statistical analysis, with Dunn's multiple comparison test. Data with different letters A and B are statistically different.



**Fig. 3.** Plasma CoQ<sub>10</sub> concentration in men and women over a 48 h period when combined with MAG-OM3, rice oil or alone and AUC over a 48 h period. The results are expressed as the mean  $\pm$  SE. CoQ<sub>10</sub>, coenzyme Q<sub>10</sub>; MAG-OM3, monoglycerides omega-3; AUC, area under the curve.

to rice oil (a neutral triacylglycerol oil). We report here that both CoQ<sub>10</sub> + MAG-OM3 and CoQ<sub>10</sub> + rice oil had approximately two times higher AUC 0–48 h than CoQ<sub>10</sub> alone, and hence, our hypothesis is rejected. The rationale for using MAG-OM3 in the present study stem from a previous study done by our group supporting that when provided esterified in MAG, OM3 plasma concentrations were twice than when provided in a triacylglycerol or ethyl ester form and that concentration in the plasma 24 h after the single dose was also twice that of the other supplements<sup>(14)</sup>. Moreover, our previous study also showed that there is an early effect of MAG-OM3 on fat absorption, and this would improve the AUC 0–6 h of CoQ<sub>10</sub> compared to other formulations. However, our data showed that AUC 0–6 h was higher when CoQ<sub>10</sub> was provided with MAG-OM3 and rice oil compared to when provided alone. Therefore, it seems that providing CoQ<sub>10</sub> with MAG-OM3 did not reproduce the same postprandial benefit observed in Chevalier *et al.*<sup>(14)</sup> when investigating the metabolism of OM3. This suggests that liposoluble molecules like CoQ<sub>10</sub> might only require to be solubilised in an oil to improve its absorption. However, its metabolism seems to be biphasic.

Indeed, we observed a biphasic shape of the CoQ<sub>10</sub> pharmacokinetic curve with a CoQ<sub>10</sub> peak at 6 h and again 10 h post-single-dose intake. This two-peak pattern has been reported in previous pharmacokinetic studies of CoQ<sub>10</sub><sup>(9,25–27)</sup>. For instance, Evans *et al.*<sup>(25)</sup> reported a biphasic distribution of CoQ<sub>10</sub>, with a first peak 6 h and a second peak between 12 and 24 h post-dose intake<sup>(25)</sup>. One main explanation for this biphasic distribution is related to CoQ<sub>10</sub> redistribution from tissues and its enterohepatic recycling after food intake 4 and 9 h post-single-dose intake<sup>(27,28)</sup>. Lamber and Parks<sup>(28)</sup> hypothesised that ‘lipids secreted at the very onset of a meal are those that were consumed in an earlier meal, suggesting the presence of an enterocyte storage pool for triglycerides’<sup>(28)</sup>.

Thus, we hypothesise that CoQ<sub>10</sub> might have been stocked in the liver or other tissues enriched in lipids and thereafter redistributed to the blood circulation after food intake<sup>(9,29)</sup>. However, our results offer another explanation for this biphasic shape of CoQ<sub>10</sub> metabolism. Indeed, our data support a difference in the CoQ<sub>10</sub> metabolism between men and women since we found that CoQ<sub>10</sub> concentrations peaked at 10 h post-dose in women but at 6 h post-dose in men (Fig. 3). Other evidence is related to a sex by supplement interaction for AUC 0–6 h and a trend towards this interaction for  $\Delta C$  48 h ( $P = 0.0696$ ). These results support that the CoQ<sub>10</sub> metabolism in response to a CoQ<sub>10</sub> supplement differs between men and women. Hence, men had a higher AUC 0–6 h than women and their concentration remained higher up to 24 h after the single-dose intake but returned close to baseline 48 h after the single-dose intake. On the other hand, 24 h after the single CoQ<sub>10</sub> dose with MAG-OM3 or rice oil, women had ~30–60 % higher CoQ<sub>10</sub> plasma concentrations than men and 48 h after the single-dose intake, women still had at least twice the CoQ<sub>10</sub> plasma concentration level of men. Unlike the data of Wahlqvist *et al.*<sup>(30)</sup> suggesting that men have a better absorption and/or lower clearance of CoQ<sub>10</sub> than women, here our data suggest that women actually have lower clearance since their plasma CoQ<sub>10</sub> concentrations are higher than men 24 and 48 h after the single-dose intake. This could be partially explained by a slower gastric motility<sup>(31)</sup> in women supporting why CoQ<sub>10</sub> absorption peaked later in women, although  $T_{max}$  was not statistically different between men and women. However, from our data, it seems that there are transiently higher concentrations of CoQ<sub>10</sub> in women than men and there was a trend towards a sex by supplement interaction ( $P = 0.0696$ ) in the  $\Delta C$  48 h concentration. Delta plasma CoQ<sub>10</sub> concentrations in women receiving the CoQ<sub>10</sub> + rice oil were about eleven times higher than the other supplements. Looking back at the data to see if this



**Table 4:** Pharmacokinetic parameters of CoQ<sub>10</sub> in combination with MAG-OM3, rice oil or alone in men and women

Pharmacokinetic parameters	CoQ <sub>10</sub> + MAG-OM3				CoQ <sub>10</sub> + Rice oil				CoQ <sub>10</sub> Alone				P-value		
	Men		Women		Men		Women		Men		Women		P interaction	P sex	P supplement
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Tmax (h)	7.2	1.5	11.7	2.1	8.7	1.7	10.6	1.9	7.7	1.9	6.1	1.0	0.1772	0.3127	0.1795
C0h (µg/L)	589.6	54.6	633.6	65.3	658.7	58.4	577.1	64.4	614.8	64.5	548.9	48.9	0.0198	0.6691	0.2706
ΔCmax (µg/L)	279.8	67.6	282.4	60.2	373.1	52.1	373.2	85.8	87.0	18.4	168.7	48.3	0.5550	0.3588	0.0003
ΔC48h* (µg/L)	26.5	18.8	51.0	37.9	25.6	18.9	171.5	42.6	-45.5	22.2	8.3	30.0	0.0696	0.0143	0.0002
AUC 0-6h (µg/L*h)	731.3	177.2	422.0	81.3	452.2	113.5	460.1	96.3	99.9	35.3	223.4	73.4	0.0250	0.6176	<0.0001
AUC 0-48h (µg/L*h)	5957.2	1414.1	6464.9	1725.0	4927.9	824.8	9503.1	2007.1	1348.6	489.7	3049.5	1304.5	0.1707	0.1453	<0.0001

Tmax = time at which the maximum concentration is reached, C0h = plasma concentration at baseline, ΔCmax = maximum concentration, ΔC48h = plasma concentration 48h post single dose intake, AUC 0-6 h = area under the curve over 6h, AUC 0-48h = area under the curve over 48h. P values were assessed by a 2-way ANOVA statistical analysis, with Bonferroni's multiple comparison test. The P interaction represents the combined effect of sex and type of supplement while P sex and P supplement represent the individual effect of sex or supplement.

\*For the ΔC48h, these data were analyzed by fitting a mixed model, rather than by repeated measures ANOVA because there were missing values, i.e. 3 participants refused to come back on one of their 48h follow-up visit (n = 3).

was the result of one or two outliers, we can confirm that it was not the case and that nine participants out of fifteen had a plasma concentration higher than 80 µg/l of CoQ<sub>10</sub>.

In the present study, we also found a sex by supplement interaction on the AUC 0–6 h. In the literature, there are discrepancies between studies in the AUC 0–6 h, and this result should therefore be interpreted with caution. For instance, one study reported a higher AUC<sup>(32)</sup>, while another reported a higher concentration<sup>(30)</sup> in men than women after a single-dose intake of CoQ<sub>10</sub>. Conversely, one study reported higher CoQ<sub>10</sub> bioavailability in women than in men, although this result was not significant<sup>(26)</sup>. Therefore, it is highly recommended that future pharmacokinetics of CoQ<sub>10</sub> should be evaluated in men and women since their absorption peak is different by sex together with their levels up to 48 h after the single-dose intake. This recommendation also needs to consider the baseline CoQ<sub>10</sub> concentrations since previous studies reported higher levels in men than women<sup>(33–36)</sup>. In the present study, there was an interaction between sex and supplement for C 0 h but our *post hoc* analysis was not able to determine where the differences were. We did not detect a significant P sex effect of C 0 h in the present study (Table 4).

The present study had strengths and limitations. In terms of strengths, the crossover design was robust enough to detect significant differences between treatments. Also, every participant was their own control, which aimed at limiting inter-individual variability. Indeed, CoQ<sub>10</sub> supplementation is known to generate plasma response with high inter-individual variability<sup>(15,35,37,38)</sup> and although we had a strong research design, inter-individual variability remained important in the present study. The high inter-individual variability found in other studies could be explained by age, gender, redox state and health disorders<sup>(9,37)</sup>. Another strength of the present study is the standardised procedures. For example, all the participant's dietary intake during the study days was controlled, limiting bias among the study days especially with respect to dietary fat intake. Treatment randomisation and the triple-blind design were also strengths since neither the participants nor the clinical staff were aware of which supplement order the participants were subjected to and all tubes were coded to avoid knowing the sampling time, participant number and supplement type the tube corresponded to. The present study also has limitations. Since this is a pharmacokinetics study of a single oral dose, it does not provide information regarding the efficacy nor the long-term effects of the CoQ<sub>10</sub> supplements. Also, it does not represent the pharmacodynamics of the supplements. The study population was young men and women which perhaps does not represent the population that could benefit the most from this type of supplementation. It also would have been interesting to test the pharmacokinetics parameters of these supplements in older adults, people under statin therapy or people with fat malabsorption since these groups of people could benefit more from the CoQ<sub>10</sub> supplementation<sup>(2,9)</sup> and their pharmacokinetics are likely to differ from the ones we obtained in the present study. Knowing that CoQ<sub>10</sub> absorption is enhanced in the presence of lipids<sup>(15)</sup>, the low-fat diet throughout the study day could have influenced the overall CoQ<sub>10</sub>'s absorption downwards. However,



the purpose of our control supplement (CoQ<sub>10</sub> in powder form) was to evaluate CoQ<sub>10</sub> absorption when it was administered alone, with limited oil consumption. It would not have been possible to evaluate this effect if provided with a high-fat diet. This is a limitation in the transfer of these results to the general population since most people eat a diet that has higher fat levels than the ones we provided here.

To conclude, the purpose of this pharmacokinetic study was to evaluate whether the 48 h pharmacokinetics of CoQ<sub>10</sub> was enhanced when provided with MAG-OM3. Our results do not support this hypothesis since CoQ<sub>10</sub> AUC 0–48 h was similar to when CoQ<sub>10</sub> was provided with rice oil. We conclude that the administration of CoQ<sub>10</sub> in combination with lipid carriers helps enhance its absorption and overall metabolism. However, one important finding of the present study is the different pharmacokinetics of CoQ<sub>10</sub> between men and women, with higher level of CoQ<sub>10</sub> in women than men when provided with MAG-OM3 or rice oil. The plus value of the MAG-OM3 supplements resides in its OM3 content and pharmacokinetics providing higher OM3 levels in the blood in postprandial. Specifically, these results are of potential interest for those with fat malabsorption issues but require to be tested in this population before a definite conclusion can be drawn.

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The authors' responsibilities were as follows: M.P. designed the present study with Biodroga Nutraceuticals; S.B. handled the samples in the laboratory, wrote the first draft of the manuscript, and analysed and interpreted the data with the help of A.V.; A.V. supervised the quality control of the CoQ<sub>10</sub> analysis; A.V. and M.P. revised the manuscript and all authors read and approved the final manuscript.

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