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Research Paper

Relative bioavailability of coenzyme Q10 formulation for paediatric individualized therapy

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Abstract

Objectives Conduct a preliminary comparison of the bioavailability between two formulations: commercial grade coenzyme Q10 (CoQ10) powder (solid formulation) and a new oil-in-water liquid emulsion and their effect on other antioxidants.

Methods Six healthy individuals participated in a randomized, crossover, open, consecutive design, with a 2-week washout period. Pharmacokinetic parameters were assessed after a single and multiple intakes of 250 mg CoQ10 given daily for 1 week.

Key Findings The differences in the pharmacokinetic parameters of maximum plasma concentration, area under the curve between 0–360 and 0–4 h, elimination half-life were statistically significant with a relative bioavailability of 489% increase over solid CoQ10 formulation. A multiple dose supplementation increased plasma CoQ10 levels in both formulations, liquid emulsion performing better (2.4- vs 3.9-fold for solid and liquid formulation, respectively) without modifications on other antioxidants. Furthermore, the plasma CoQ10 at 7th day was statistically different between formulations ($P < 0.05$).

Conclusions The results obtained showed that liquid emulsion improves the bioavailability of CoQ10 respect to solid form which not only facilitates the individualized administration for the child but in turn could increase the therapeutic efficacy, which should be confirmed by further studies.

Introduction

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone) (CoQ10) (Figure 1), also known as ubiquinone, is a lipophilic molecule classified as a fat soluble quinone. CoQ10 is a component of the mitochondrial respiratory chain where it acts controlling the efficiency of oxidative phosphorylation and being essential for the production of cellular energy. Due to its hydrophobicity, CoQ10 is inserted into the mitochondrial inner membrane. Moreover, CoQ10 is also considered an antioxidant agent together with other lipophilic antioxidants and plays an intrinsic role in protecting circulating lipoproteins against oxidative damage.[1,2]

Human CoQ10 deficiency can be classified as primary or secondary resulting in different heterogeneous diseases. Primary CoQ10 deficiency seems to be relative rare and has been associated with mutations of diverse genes involved in its biosynthesis.[3,4] Growing evidence supports that secondary or acquired CoQ10 deficiency is more common. Low plasma CoQ10 has been reported in different diseases including muscular, neurodegenerative, cardiovascular, statin-induced myopathy and reproductive diseases as well as cancer,[5–8] among others.

Coenzyme Q10 has been widely used for the treatment of mitochondrial and other neurodegenerative disorders. Potential treatment indications for the use of CoQ10 include migraine,[9,10] chronic tinnitus aurium,[11] hypertension,[12] heart failure and atherosclerosis[13]; however, the role of CoQ10 in such conditions is still an open question. Despite the questions related to its therapeutic use, we cannot ignore the evidence that most patients with these

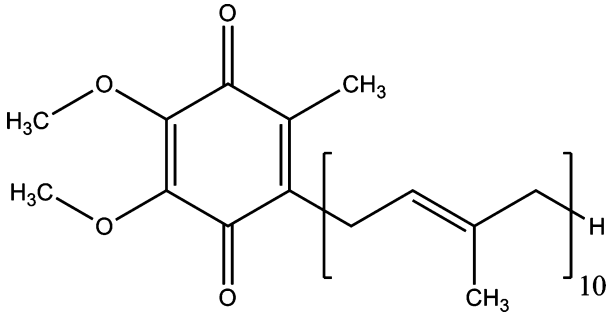

Figure 1 Structure of coenzyme Q10.

deficiencies have shown clinical improvement with oral CoQ10 supplementation and, if deficiency is present, it appears that early administration of 10 mg/kg per day is highly beneficial, especially in infants.[14]

One major issue concerning the use of CoQ10 in therapy is the potential efficacy, mainly determined by its absorption/bioavailability properties in the different formulations currently administered.[15] CoQ10 is a crystalline powder, insoluble in water, with high hydrophobicity ($\log P > 10$), and therefore poorly absorbed by the organism. As CoQ10 has a rather complex chemical structure, its formulation must be prepared with special care to obtain a product with acceptable bioavailability and efficacy.[16] Besides, the bioavailability of a pharmaceutical can be enhanced by the formulation development as well as the manufacturing process.[17]

Thus, to improve CoQ10 solubility and bioavailability, a variety of formulations have been developed. Most common formulations are based on powder, tablets, two-piece capsules or soft gel capsules containing an oil suspension. Currently, crystalline CoQ10 powder, oil emulsions, solubilizates of CoQ10 and nanoparticulate formulations are available.[18–20]

Different formulations result in bioavailability variation and dosage consistency, and there is a serious possibility that patients may have been treated suboptimally.[21]

Taking into account that CoQ10 oral supplementation is especially successful if started during childhood, we have previously developed a stable and safe oil-in-water (O/W) liquid emulsion of CoQ10 when narrow dose adjustment is required[22] to increase the bioavailability of CoQ10, facilitate its administration in paediatrics and therefore increase the therapeutic efficacy. The administration of drugs in paediatrics is a unique challenge and requires individualized therapy. Liquid formulations rather than solid dosage forms are preferred for oral administration to children, especially for those with swallowing difficulty which it is very common in patients with mitochondrial and neurodegenerative disorders.

The objective of this work was to establish the relative bioavailability of this new developed liquid formulation in comparison with the commercial grade CoQ10 powder (solid formulation). An additional objective was to evaluate the effect of 1-week CoQ10 supplementation at the levels of other antioxidants such as vitamins A, E and C.

Materials and Methods

Coenzyme Q10 preparations

Two different CoQ10 formulations were examined: (1) 250 mg powder-filled hard-shell gelatine capsule commercial grade (Shenzhou Biology & Technology Co, Hohhot Inner Mongolia, China) (solid formulation) and (2) O/W liquid emulsion (20 mg/ml) previously developed[22] (liquid formulation). Briefly, the O/W liquid emulsion was prepared by solubilizing CoQ10 powder (Prest S.A batch: AHK-1115) in soya bean oil (Gersoja, batch: LENV141210) at40 °C. The vehicle of the O/W emulsion was prepared with 0.25% w/v xanthan gum (Magel S.A. batch: 585/2007), 45% soya bean oil, 30% syrup, 23.85% distilled water, 0.08% methylparaben (Magel S.A. batch: IA2011), 0.02% propylparaben (Chutrau, batch: LI1814), 0.3% sodium saccharin (Van Rossum, batch: 80118) and 0.5% orange essence (Prest S.A., batch: 9206). The first step was to solubilize the CoQ10 powder in soya bean oil and subsequently the orange essence. Then, xanthan gum and syrup were added to the oil solution to obtain a primary emulsion by mixing with an automatic mixer for five minutes. Methylparaben and propylparaben were solubilized in distilled water at 90 °C. When the solution reached room temperature, sodium saccharine was dissolved. This resultant solution was added to the primary emulsion and mixed with an automatic mixer for 15 min. The final CoQ10 concentration of the O/W emulsion was 20 mg/ml. The formulation was stored in amber glass vials and kept at controlled room temperature (25 °C).

The composition of the two CoQ10 preparations was confirmed by HPLC–UV direct analysis of each formulation.[23] This analysis demonstrated CoQ10 concentrations of 249 ± 6 mg for solid Q10 capsules and 20.6 ± 0.4 mg/ml for liquid CoQ10 formulation (mean ± SD).

Subjects

Six healthy volunteers (three men and three women aged 18–40 years) were recruited. Subjects were self-reportedly healthy and free from acute or chronic illness requiring prescription medication and attended a screening visit with a medical doctor. All of them were non-smokers and did not take vitamin supplements within 2 weeks or CoQ10 supplements within 4 weeks before the study. Subjects were

within ideal body mass according to weight and height and were excluded if they displayed any of the following: cardiovascular, pulmonary, hepatic, renal, thyroid or adrenal disease. Serum tests, including haematological tests (haematocrit, haemoglobin, red blood cells, platelet and total and differential leucocyte counts), liver enzymes, cholesterol (total, HDL and LDL), serum creatinine and urinary protein and creatinine, were performed 15 days before the commencement of the study. Systolic and diastolic blood pressure and heart rate were also measured. Subjects were requested not to undergo any lifestyle changes or initiate any new medications or supplements during the study.

Study design

Two designs, one single dose and 1-week repeated dose, were carried out.

Single dose design

The study was a randomized (by lot), comparative, crossover design (open, consecutive, controlled, single oral dose), in which all subjects received a single oral dose of 250 mg of solid CoQ10 (1 capsule) or liquid CoQ10 (12.5 ml), with a 2-week washout between treatments. Baseline blood samples were obtained after a 12-h overnight fast for the measurement of CoQ10 and lipids. Blood samples were taken at 2, 4, 6, 8, 10, 24, 168 and 360 h after administration of the first dose. The supplement was taken with a standardized breakfast. Further standardized meals (lunch, afternoon tea and dinner) were provided at 5, 9 and 13 h, after administration of the supplement.

After a 2-week washout, subjects returned for a second pharmacokinetic visit identical to the first, except that they received the alternative CoQ10 formulation to the one received on the first pharmacokinetic day.

Repeated dose design

To evaluate the percentage increase in CoQ10 levels from baseline and the effect on other antioxidants such as vitamins A, E and C after 1-week supplementation of both formulations, all subjects received a single oral daily dose of 250 mg (solid or liquid formulation) during a 1-week period. Baseline and 7th day blood sample (168 h) were obtained after a 12-h overnight fast for the measurement of CoQ10 and vitamins. After a 2-week washout, subjects received the alternative CoQ10 formulation to the one received on the first day.

All participants were instructed to avoid consuming alcohol and caffeine-containing products 72 and 24 h before the first dose of each CoQ10 preparation.

The study protocol (No. 0739436/2011) was performed according to the principles of the Declaration of Helsinki and was approved by the Ethic Committee of our Institution and all subjects signed an informed written consent document before the beginning of the study.

Sample collection

Plasma sample

Heparinized blood samples were obtained by serial puncturing, and aliquots were centrifuged at 2000*g* for 10 min. Plasma was stored at −80 °C until analysis.

Analytical procedure

Equipment

The quantification of CoQ10 and vitamins was performed in an HPLC Spectra System SCM1000 (Thermo Scientific, Waltham, MA, USA) with a quaternary pump, a P4000 degasser, AS3000 autosampler and a UV2000 Dual λ Absorbance detector. Chromatograms were processed using ChromQuest Chromatography Data System software.

Plasma coenzyme Q10

Coenzyme Q10 in plasma was performed by an optimized micro-HPLC-UV method as previously detailed (linear range: 0.08–15.0 μM, LOQ 0.01 μM).[24] In brief, heparinized plasma (100 μl) was supplemented with 50 μl of *p*-benzoquinone solution in 1-propanol (4 mg/ml). Then, 150 μl of cold 1-propanol was added and centrifuged, and the organic layer was evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 50 μl of ethanol.

The chromatographic conditions were the following: 30 °C column temperature, the isocratic mobile phase consisted of methanol 100% and the flow rate was set at 0.4 ml/min. UV detection was performed at 275 nm with an injection volume of 10 μl. Separation was achieved using an analytical XTerra C18 microcolumn (Waters Corp., Milford, MA, USA) (50 × 2.1 mm, 3.5 μm) with an C18 guard column (Waters Corp.).

Plasma vitamins A and E

Determination of both hydrophobic vitamins was performed by a previously detailed HPLC-UV method.[25] Briefly, 50 μl of heparinized plasma was supplemented with 100 μl of cold 1-propanol, centrifuged and the organic layer was evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 100 μl of methanol. The chromatographic conditions were the following: 25 °C

column temperature, the isocratic mobile phase consisted of methanol: water (94 : 6) and the flow rate was set at 0.4 ml/min. UV detection was performed at 296 and 325 nm with an injection volume of 10 µl. Separation was achieved using a Thermo Scientific BDS Hypersil C18 analytical microcolumn (100 × 2.1 mm i.d., 2.4 µm particle size) with an C18 guard column (Waters Corp.).

Plasma vitamin C

Determination of vitamin C was performed as previously detailed.[26] Briefly, 50 µl of heparinized plasma was supplemented with 100 µl of cold 10% metaphosphoric acid, centrifuged and injected into the equipment. The chromatographic conditions were the following: 25 °C column temperature, the isocratic mobile phase consisted of 0.1% phosphoric acid and the flow rate was set at 0.7 ml/min. UV detection was performed at 265 nm with an injection volume of 10 µl. Separation was achieved using a Merck LiChrospher C18, HPLC cartridge (250 × 4 mm i.d., 5 µm particle size) with a guard column.

Pharmacokinetic parameters

To assess the relative bioavailability of the two CoQ10 formulations, the area under the CoQ10 concentration–time curve from 0 to 360 h ($AUC_{0–360\ h}$), the maximum plasma CoQ10 concentration (C_{max}) following ingestion of a single 250 mg dose of CoQ10 and the elimination half-life were calculated. Half-life of elimination was estimated from the terminal part of the concentration–time curve. The area under the concentration–time curve was determined using the linear trapezoidal method. Also additional parameters were included: incremental $AUC_{0–360\ h}$ ($IAUC_{0–360\ h}$), incremental $AUC_{0–4\ h}$ ($IAUC_{0–4\ h}$), ΔC_{max} and time to maximum plasma concentration (T_{max}). The $IAUC_{0–360\ h}$ represents the increase in area following CoQ10 ingestion above baseline CoQ10 concentrations.

Statistical analysis

Pharmacokinetic parameters were estimated by a non-compartmental analysis of CoQ10 plasma concentrations profiles using the TOPFIT program (version 2.0; Dr Karl Thomae Gmbh, Schering AG, Germany), and they were log transformed for statistical analysis. Statistical analysis was performed using GraphPad Prism version 5.00 for Windows; GraphPad Software, San Diego, CA, USA, www.graphpad.com. Shapiro–Wilk *W*-test of normality was performed. Differences between groups were analysed by Student's paired *t*-test. Levels of significance were established at $P < 0.05$.

Results

Single dose design

No adverse effects were reported or noted during the course of the study.

Baseline plasma CoQ10 levels showed no statistical differences between each formulation (0.56 ± 0.08 and 0.62 ± 0.07 µM, solid and liquid, respectively, Figure 2).

Table 1 summarizes pharmacokinetic parameters of both formulations.

The $AUC_{0–360\ h}$ and C_{max} were higher in liquid CoQ10 formulation compared with solid CoQ10 formulation ($P < 0.01$). In addition, the incremental area under the concentration curve at 360 h ($IAUC_{0–360\ h}$), which takes baseline variance into account, was different between the two formulations representing 489% increase over solid

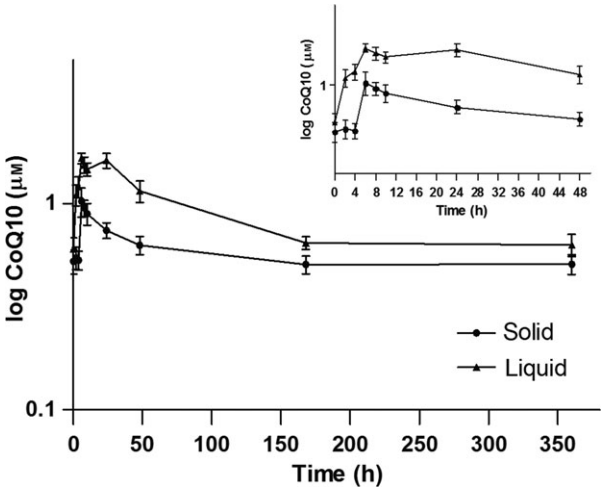

Figure 2 Changes in mean plasma total coenzyme Q10 (CoQ10) concentration over 360 h following supplementation with solid CoQ10 formulation (–●–) and liquid CoQ10 formulation (–▲–). Error bars show SEM.

Table 1 Pharmacokinetic parameters for two Coenzyme Q10 (CoQ10) formulations following a single oral dose of 250 mg CoQ10

	Solid CoQ10 $n = 6$	Liquid formulation $n = 6$
$AUC_{0–360\ h}$ (µM h)	201 ± 18	276 ± 29**
$IAUC_{0–360\ h}$ (µM h)	24.6 ± 7.6	87.9 ± 13.8**
$IAUC_{0–4\ h}$ (µM h)	0.15 ± 0.10	1.60 ± 0.24*
C_{max} (µM)	1.13 ± 0.11	1.76 ± 0.10**
ΔC_{max}	0.57 ± 0.15	1.14 ± 0.12*
T_{max} (h)	7.33 ± 0.84	7.00 ± 0.45
Half-life (h)	37.9 ± 0.7	68.1 ± 0.6**

Values are mean ± SEM. *$P < 0.05$ and **$P < 0.01$ using paired *t*-test for comparison between groups differences.

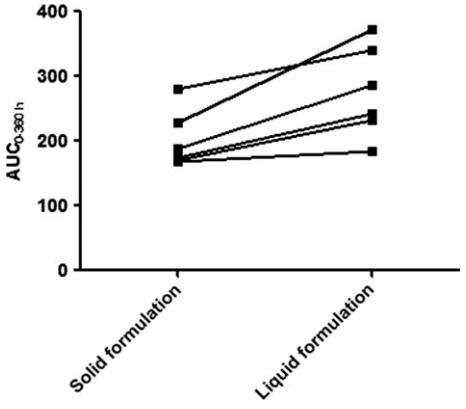

Figure 3 The area under the coenzyme Q10 (CoQ10) concentration–time curve from 0 to 360 h ($AUC_{0–360\ h}$) for each participant, after a single oral 250 mg dose of each CoQ10 formulation.

CoQ10 formulation. The increase in C_{max} from baseline (ΔC_{max}) was two times higher in the liquid formulation with respect to the solid form ($P < 0.05$).

The elimination half-life also showed a significant increment in liquid formulation ($P < 0.01$) while T_{max} remained similar between the treatments (Table 1).

The $AUC_{0–360\ h}$ for each participant after a single dose of each CoQ10 formulation is shown in Figure 3, highlighting the interindividual variation in CoQ10 absorption.

Repeated dose design

In accordance with the results obtained in the single dose design, CoQ10 administration during 1 week showed an increment of plasma CoQ10 concentration of 2.4- and 3.9-fold for solid and liquid formulation, respectively. Moreover, the final CoQ10 plasma concentration after 1-week administration of the liquid formulation was significantly higher than those found after a similar administration of solid formulation (1.45 ± 0.16 vs 0.71 ± 0.15 μM, $P < 0.05$).

No modification was observed in plasma levels of vitamins A, E and C subsequent to 1-week administration of CoQ10 (Table 2).

Discussion

The development of pharmaceutical formulations for paediatric patients poses a unique challenge. Active pharmaceutical ingredients and excipients must be compatible with one another to produce effective, stable, well-tolerated formulations, easy to administer and with good palatability. Moreover, the use of liquid formulations facilitates paediatric use that requires a range of dosage forms suitable for different ages and weight and a range of strengths or concentrations allowing administration of the correct age-related dose.[27] Furthermore, children with mitochondrial disorders have swallowing difficulties, so in these cases, the use of a liquid form is even more beneficial. Thus, liquid formulations rather than solid dosage form are preferred for oral administration to children.[28,29]

In the case of the active pharmaceutical ingredients such as CoQ10 with poor aqueous solubility, high hydrophobicity and consequently poor absorption, various formulation strategies are used. Absorption of crystalline CoQ10 is enhanced by dispersion and solubilization techniques.[30–33] A common strategy to improve bioavailability is a well-absorbed preparation. In this way, the use of soya bean oil, polysorbate oil 80, propylene glycol, lecithin or other emulsifying agents and surfactants has been reported.[30–33] Thus, an oil solution formulation is a simple and efficient alternative to improve bioavailability.[18] Moreover, O/W liquid emulsions are utilized as carriers of highly lipophilic drugs.[34]

In this study, the bioavailability of a liquid formulation of CoQ10 (O/W liquid emulsion) compared with a solid formulation (crystalline CoQ10) was assessed.

Our results demonstrated that in healthy volunteers, the CoQ10 in the liquid formulation was more bioavailable than CoQ10 in the solid dosage form.

The pharmacokinetic profiles obtained in our study were comparable to those described in previous pharmacokinetic trials of orally ingested CoQ10. Half-lifetime T_{max} and a second plasma CoQ10 peak observed at about 24 h following oral ingestion were consistent with previously reported trials.[30–33,35–37] T_{max} levels showed no differences between the two formulations. However, liquid formulation showed

Table 2 Effect of 1-week treatment with two Coenzyme Q10 (CoQ10) formulations (250 mg/day) on plasma vitamins A (μM), E (μM) and C (μM) (values are means ± SEM)

	Vitamin A		Vitamin E		Vitamin C	
	Solid CoQ10	Liquid formulation CoQ10	Solid CoQ10	Liquid formulation CoQ10	Solid CoQ10	Liquid formulation CoQ10
Baseline	1.32 ± 0.15	1.25 ± 0.18	17.50 ± 2.31	16.69 ± 0.68	26.08 ± 6.5	31.54 ± 6.55
1 week	1.26 ± 0.15	1.24 ± 0.16	18.5 ± 2.11	18.37 ± 0.74	30.4 ± 12.1	32.71 ± 7.47

higher $IAUC_{0–4\ h}$ over crystalline CoQ10 ($P < 0.05$) which reflects the superiority in early uptake. This may be explained by the structure of the solubilizate, small enough to be directly incorporated into the intestinal border.[35]

Differences in the pharmacokinetic parameters $AUC_{0–360\ h}$, $IAUC_{0–360\ h}$, C_{max}, ΔC_{max} and elimination half-life were statistically significant between the two formulations showing a clear superiority of the liquid formulation over the crystalline CoQ10.

Non-compartmental pharmacokinetic analysis of CoQ10 plasma levels reveals a longer apparent terminal half-life of elimination after emulsion administration in comparison with capsule. As the rate of decline of drug plasma concentrations after oral administration may not only be influenced by elimination, prolongation of terminal half-life after O/W emulsion could be explained by a more sustained intestinal absorption of CoQ10 after administration of the liquid formulation when compared with capsule. Nevertheless, further studies are needed to explain these findings. Moreover, by analysing the pharmacokinetic data, we can infer that the liquid formulation has a higher absorption efficiency with respect to the solid (defined as per cent of initial dose present in plasma at T_{max} assuming 2.5 l as total plasma volume) showing values of $1.52 \pm 0.08\%$ and $0.97 \pm 0.10\%$ for liquid formulation and solid formulation, respectively ($P < 0.001$), which are consistent with previous reports with the similar dose.[33]

The effect of multiple dose supplementation with CoQ10 over a time period of 7 days was investigated. Increases in plasma CoQ10 concentration from baseline to the end of 1-week supplementation were more pronounced for the liquid formulation than the solid formulation (3.9- vs 2.4-fold). In addition, supplementation with the liquid formulation achieves higher plasma levels of CoQ10 at 7th day respect to supplementation with the solid formulation, consistent with the results obtained in the single dose design. Nevertheless, several points need to be discussed for adequate interpretation of our results. In first place, as half-life of CoQ10 after liquid formulation is approximately 68 h, steady-state conditions have not been achieved after 1-week administration. Taking into account the terminal half-life of CoQ10 for both formulations, in our protocol, 75% and 94% of steady-state concentrations of CoQ10 have been achieved for the liquid formulation and capsule, respectively. In this context, the liquid formulation may perform better than capsules if the treatment achieves steady-state conditions.

Our results suggest low accumulation of CoQ10 during multiple dosing for both formulations, as plasma concentrations of CoQ10 at 24 h were roughly similar to the single dose administration.

Taking into account half-life of CoQ10 for both formulations, it is expected that CoQ10 will be 4.6- and 2.8-fold higher at steady state when compared with a single dose of liquid formulation and capsule, respectively. Other authors have also found low CoQ10 accumulation after short supplementation treatment with oral formulations of CoQ10.[15,38] For instance, Chopra *et al.* have found a sharp increase in mean plasma CoQ10 concentrations just after 2-week supplementation with both CoQ10 suspensions in oil and soft gel capsules. As it is known, CoQ10 is an endogenous molecule and its cellular content is homeostatically regulated; however, it is not clear how its tissue distribution is controlled.[2,35] The lower plasma concentrations of CoQ10 after multiple dosing of both liquid formulation and capsule can be explained by the fact that a rapid distribution of CoQ10 into cells and tissues is produced, as reported by Lu *et al.*[38] Further studies with longer treatment with our oral formulations are needed to confirm this hypothesis.

In spite of the suggestion of adapting different formulations over time proposed by some authors,[35,38,39] our results are in agreement with Chopra *et al.*[15] who found a 3.2-fold increase in bioavailability with solubilized CoQ10 compared with an oil suspension and tablets after a 3-week supplementation with 120 mg/day.

The increase in plasma CoQ10 levels observed at the end of each treatment did not modify plasma vitamins A, E and C levels. The lack of effect might be related to the short treatment period and/or to the fact that young healthy adult participants already had high vitamin plasma levels not improved by CoQ10 therapy.[33]

Conclusion

In summary, the results obtained showed that O/W emulsion improves the bioavailability of CoQ10 respect to solid dosage form which not only facilitates the individualized administration for the child but in turn could increase the therapeutic efficacy which should be confirmed by further studies.

Declaration

Acknowledgements

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