ACCEPTED MANUSCRIPT: ŽMITEK et al. The effect of dietary intake of coenzyme Q10 on skin parameters and condition: results of a randomised, placebo-controlled, double-blind study. BioFactors. 43(1): 132-140, DOI: 10.1002/biof.1316

The effect of dietary intake of coenzyme Q10 on skin parameters and condition : results of a randomised, placebo-controlled, double-blind study

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Abstract

Coenzyme Q10 (CoQ10) is a natural constituent of foods, and also commonly used in both functional foods and supplements. It is also a common ingredient in cosmetics, where it is believed to reduce the signs of skin ageing. However, the existing data about the effect of dietary intake of CoQ10 on skin parameters and condition is scarce. To gain an insight into this issue, we conducted a double-blind, placebo-controlled experiment with 33 healthy subjects. Our objective was to investigate the effects of 12 weeks of daily supplementation with 50 mg and 150 mg of CoQ10 on skin parameters and condition. While the results of some previous *in vitro* studies showed possible protection in UVB-response, we did not observe significant changes of minimal erythema dose (MED). On the other hand, the intake of CoQ10 prevented seasonal deterioration of viscoelasticity and reduced some visible signs of aging. We determined significantly reduced wrinkles and microrelief lines, and improved skin smoothness. Supplementation with CoQ10 did not significantly affect skin hydration and dermis thickness.

Keywords: Coenzyme Q10, CoQ10, antioxidant, skin health, anti-aging

Coenzyme Q10 (CoQ10) is an endogenous lipophilic compound, an essential component of the mitochondrial energy metabolism (Crane, 2001) and an effective antioxidant with range of possible benefits for human health (Bentinger, Brismar, & Dallner, 2007; G.P. Littarru & Tiano, 2010; Mellors & Tappel, 1966). The presence of CoQ10 in the membranes of eukaryotic cells suggests its potential to act as an antioxidant and scavenge free radicals, preventing the activation of inflammatory signalling pathways (Schmelzer et al., 2008). The beneficial role of CoQ10 supplementation has been reported in various conditions, particularly in cardiovascular (Gao et al., 2012; Kumar, Kaur, Devi, & Mohan, 2009; Mortensen et al., 2014), neurodegenerative and mitochondrial conditions (Galpern & Cudkowicz, 2007; Shults, 2003; Shults et al., 2002), diabetes (Chew & Watts, 2004), periodontal disease (Matthews-Brzozowska, Kurhanska-Flisykowska, Wyganowska-Swiatkowska, & Stopa, 2007) and male infertility (Lafuente et al., 2013).

The human body biosynthesizes CoQ10, but its skin levels, as well as levels in other CoQ10 tissues, drop progressively with increasing age (Ely & Krone, 2000; Kalen, Appelkvist, & Dallner, 1989). CoQ10

is also supplied to the organism by exogenous sources, e.g. foods. Richest dietary sources are meat, migratory fish, nuts, and some oils, but in the diet of populations of Western countries these sources altogether contribute to just 3-5 mg CoQ10 per day (I. Pravst, Zmitek, & Zmitek, 2010). Furthermore, CoQ10 deficiency has been observed in some medical conditions (Quinzii, Hirano, & DiMauro, 2007), in persons with inadequate nutrition and in smokers (Elsayed & Bendich, 2001). It has also been shown that the endogenous synthesis of CoQ10 is inhibited by cholesterol-lowering drugs statins, which inhibit biosynthesis of mevalonate, therefore CoQ10 supplementation has been suggested in such cases (Bliznakov, 2002; Folkers, 1996; Gian Paolo Littarru & Langsjoen, 2007).

Skin is the outermost human organ that is in direct contact with the environment and therefore constantly exposed to external stress factors. In the skin CoQ10 is found both in cells and in skin surface lipids, a constituent of stratum corneum, where it acts in combination with other substances as an outermost barrier of the skin to oxidant assault (Passi, Littarru, Puddu, & De Pità, 2002; Shindo, Witt, Han, Epstein, & Packer, 1994). CoQ10 is also crucial in maintaining mitochondrial activity in cells. It has been shown that CoQ10 levels in skin and skin surface lipids decline with age (Kalen et al., 1989; Knott et al., 2015; Passi et al., 2002).

In last decade we have seen increased use of CoQ10 in health related products. Despite the fact, that in European Union there is no authorised health claims for CoQ10 as functional food ingredient, it is mostly used in products intended to support heart health. This can be explained by the fact, that the strongest evidence is available for the beneficial role of CoQ10 supplementation in cardiovascular (Gao et al., 2012; Kumar et al., 2009; Mortensen et al., 2014), but considering the fact that studies were not performed on healthy population groups such evidence can not be used in substantiation of health claims for foods (I. Pravst, 2012). On the market, CoQ10 is mostly used in food supplements (I. Pravst & Zmitek, 2011), but it can be also found in functional foods. For example, CoQ10 has been added as functional ingredient to 3.5% of yoghurts sold in Slovenian food supply in 2011 (Igor Pravst & Kušar, 2015).

In addition to such use, CoQ10 is also commonly use in cosmetics, mostly due to its perceived ability to protect skin from free radical damage and reduce signs of ageing. As shown by several *in vitro* experiments, CoQ10 is able to protect skin from reactive oxidative species (ROS), induce proliferation of skin fibroblasts, inhibit MMP-1 enzymes that degrade extracellular matrix components, accelerate the production of epidermal basement membrane components, reduce DNA damage triggered by UVA irradiation, decrease UVR-induced inflammatory response and decrease levels of superoxide generation by ArNOX proteins (Ashida, 2009; Fuller, Smith, Howerton, & Kern, 2006; Inui et al., 2008; Masaki, 2010; Morré, Morré, Rehmus, & Kern, 2008; Muta-Takada et al., 2009). There are also some studies showing beneficial effects of topical CoQ10 use on skin *in vivo*. Knott et al. showed very recently that topical application of CoQ10 raises its epidermal content both in SSL and deeper layers of the epidermis and improves antioxidant potential of the skin (Knott et al., 2015). Improvement of antioxidant potential of the skin by topical CoQ10 was also shown by Vinson et al. (Vinson & Anamandla, 2006). Hoppe *et al.* showed that three months of topical CoQ10 application decreased wrinkle depth in human skin (Hoppe et al., 1999), but statistical data for these effects were not provided. A clinical trial by Inui *et al.* involving 31 females demonstrated a reduction in wrinkle score after the use of CoQ10 cream for 5 months (Inui et al., 2008). Clinical trial by McDaniel with idebenone (synthetic CoQ10 analogue) lotion showed increase of collagen I expression and improvement in skin

Pripombe dodal [IP1]: Izpisati kaj to pomeni

roughness, wrinkles and fine lines, but there is lack of vehicle control group (McDaniel, Neudecker, DiNardo, Lewis Ii, & Maibach, 2005).

Supported by these evidence, and also by very strong marketing campaigns of the cosmetics industry, CoQ10 has also become interesting functional food ingredient in so called *beauty products*, formulated to support skin health. However, the existing data about the effect of dietary intake of CoQ10 on skin parameters and condition is scarce (Ashida, 2009). Passi et al. showed that combined oral and topical use of CoQ10 in combination with vitamin E has the ability to raise CoQ10 levels in skin and reduces wrinkle depth (Passi, De Pità, Grandinetti, Simotti, & Littarru, 2003), but to our knowledge there are no reports in the scientific literature which would access the efficiency of dietary CoQ10 alone. To gain an insight into this issue, we conducted a double-blind, placebo-controlled experiment with 33 healthy volunteers. Our objective was to investigate the effects of 12 weeks dietary supplementation with CoQ10 on erythema response to UVB, visible signs of aging - wrinkles and skin micro relief, skin hydration and elasticity, and dermis condition.

Materials and methods

Design of the study

Subjects

Thirty three healthy Caucasian female volunteers, ranging in age from 45-60 years (mean age 52.6±4.2 (SD)) with Fitzpatrick skin phototypes II and III were enrolled in the study after written consent. Inclusion criteria were signs of skin aging (mimic wrinkles/ poor skin tone/ visual dryness), photoaged skin on the face, expression of mimic wrinkles. Exclusion criteria were pregnancy or breastfeeding, known or suspected allergy to any ingredient of the tested products, high blood cholesterol and use of cholesterol-lowering medicines, diagnosed diabetes, thyroid disease, inflammatory skin diseases, regular use of dietary supplements (including products with added CoQ10) six months or less preceding study entry, invasive (botox injections, hyaluronic acid fillers, needle rollers, needle mesotherapy, etc.) and non-invasive (radiofrequency, electrotherapy, ultrasound therapy etc.) rejuvenation treatments six months or less preceding study entry, the use of cosmetic products containing coenzyme Q10 six months or less preceding study entry, gluteal hyperpigmentation, expected sunbathing (also in solariums) within the study period. Subjects were also asked not to change their routinely used skin care regime on the test sides during the entire study period. Furthermore, subjects were ~~advised~~ asked to continue their normal dietary habits. Additional dietary supplements were not allowed during the 12-week intervention trial. Sunbathing and use of tanning machines were not allowed during the study period. Consistent with the principles laid down in the Declaration of Helsinki, all subjects signed their informed consent before recruitment. The study was approved by Ethics Committee of the Higher School of Applied Sciences, and included to ClinicalTrials.gov register record NCT02604641.

Subjects were randomly assigned to either (a) a placebo group (mean age 52±4 years), (b) a low-dose group (LD group; 54±4 years) receiving 50 mg CoQ10/day, or (c) ~~and~~ a high-dose group (HD group; mean age 52±5 years) receiving 150 mg CoQ10/day; with ~~(~~11 subjects per group~~)~~.

Out of 33 subjects enrolled in the study 32 completed the entire 12 week trial (HD: 10 subjects, LD and placebo: 11 subjects each), there was one drop-out in HD group before conducting measurements after 6 weeks.

Intervention

All subjects consumed 5 mL of a syrup daily for 12 weeks. Placebo group received aqueous syrup formulation without CoQ10, LD group received syrup with 50 mg CoQ10 per 5 mL, and HD group received syrup with 150 mg CoQ10 per 5 mL. To enable production of aqueous syrup with CoQ10, a water-soluble form of CoQ10 was used in the formulations (Q10Vital® as used in Quvital® food supplements, Valens Int d.o.o., Slovenia) (Milivojevic Fir, Smidovnik, Milivojevic, Zmitek, & Prosek, 2009; I. Pravst, Prosek, Wondra, Zmitek, & Zmitek, 2009). Bioavailability of this constituent was previously investigated (Zmitek et al., 2008). All three syrups were formulated and produced by Valens Int d.o.o., using sugar syrup and same organoleptic characteristic, ensuring proper placebo control. To assure proper CoQ10 concentration all three variations of the syrup, samples were also sent for testing to an independent laboratory (Chelab S.r.l, Resana, Italy), where CoQ10 concentration was determined using standard high-performance liquid chromatography (I. Pravst & Zmitek, 2011).

Assessments

Regular checks of the subjects were carried out three times during the study: at baseline (week 0), after 6 weeks (week 6) and after 12 weeks of supplementation (week 12) and Visioface images of the face were recorded at those terms. Changes of dermis ultrasonic echogenicity and thickness as well as skin surface parameters (hydration, viscoelasticity) were measured on the face at week 0 and week 12. Minimal erythema dose was determined on gluteal area at week 0 and week 12. Wrinkle area fraction measurements were done on face using Visioface CSI system and additionally assessed according to Lemperle scale at week 0 and week 12. Results were obtained during a period of colder outside temperatures and low sun exposure from November 2014 to January 2015; average monthly temp. 8.8°C, 3.9°C and 2.8°C, respectively). All measurements were carried out on subjects lying in a room with temperature 20-25°C and relative humidity 40-60%, except Visioface imaging was done in sitting position. Measurements started after 30 min acclimatization period in the same atmospheric conditions. Subjects were advised to clean their face at least 2 h before measurement term and not to apply any cosmetic products on their face 2 h or less before the measurement term.

Skin viscoelasticity and hydration measurements, ultrasound measurements

Viscoelasticity measurements were performed on the predetermined position of the right cheek using Cortex Technology DermaLab Combo SkinLab elasticity probe (Cortex Technology, Handsund, Denmark). Measurement gives results in MPa.

Hydration measurements were performed on the predetermined area of the right cheek using Cortex Technology DermaLab Combo SkinLab hydration probe, which operates based on the conductivity principle. Eight repetitions were done and the result for each subject is the average of eight consecutive measurements. Measurement give results in μS.

Ultrasound measurements were performed using Derma-Lab® Combo SkinLab USB 20 MHz high resolution ultrasound scanner probe (Cortex Technology, Handsund, Denmark). A constant gain curve was applied for each volunteer and dermis thickness and intensity (density) were determined as published elsewhere (*Handbook of Non-Invasive Methods and the Skin*). Measurements were made on the on the predetermined position at right cheek. Skin thickness is measured in μm and intensity as a 0-100 score.

Photography, wrinkle measurements and evaluation, skin surface evaluations

High resolution lateral (left and right) and frontal images of face (10 MPx) were taken using VisioFace Quick system (Courage+Khazaka electronic GmbH, Germany), with constant distance from the camera in standardized, white and UV light after the subject placed her face to the front or to the side in a light facial booth. The diodes illuminate the face homogeneously. The camera and lights were both software-controlled and immediately ready for use. Because topography of the skin varies significantly within a few milimeteres, exact location of the face was obtained by carefully comparing details on the face with baseline image, repositioning the face position in apparatus in order to obtain an exactly matching picture of the face. Wrinkle area fraction (wrinkle area divided by assessment area) of periorbital wrinkles were measured for each subject at baseline and after 12 weeks using VisioFace CSI software.

Wrinkle assessment were performed for six different wrinkle types at different face areas from frontal and lateral Visioface images by experienced professionals at week 0 and week 12 according to Lemperle scale (0-5) -(Lemperle, Holmes, & Lemperle, 2001). In evaluation for each wrinkle type only the subjects who had expressed wrinkles of observed type at baseline were evaluated.

Evaluation of subjects' skin smoothnes and microrelief was also done at week 0 and week 12 by comparison of the Visoface images of the face (frontal, left lateral and right lateral). The 96 pairs of photographs were assessed using a 3-grade scale (-1: deterioration, 0: no change, +1: great improvement) by experienced professionals. Photographs for week 0 and week 12 were presented in blind and randomized sequence for each subject.

Minimal erythema dose (MED)

At baseline and after 12 weeks the minimal UVB erythema dose (MED,) was assessed using the automated MED Tester (Dermalight® 80 MED Tester, Dr Hoenle Medizintechnik GmbH, Germany; UVB 280–320 nm). Increasing UV doses (exact dosages depending on the individual's skin phototype following the Fitzpatrick classification) were applied on a gluteal area through means of 10 small round apertures within the MED tester.
MED readings were taken 24 h after application of UV, with the MED being defined as the lowest dose of UV resulting in visible erythema of the skin. The UV dose is given in J/cm^2. No application of skin care products on the gluteal area 12 h before and 24 h after UV application was allowed.

Statistical methods

Data were analyzed using StatPlus software (version 5.9.8) and The Real Statistics Resource Pack for ~~Excel~~Microsoft Excel. All the data measured are shown as the mean ± SE. Student's t-test or Wilcoxon

Pripombe dodal [IP2]: Proizvajalec/Založnik

Pripombe dodal [IP3]: Za prvič napišemo? Standard error (SE)

signed rank test (for non-parametric variables) were used to compare baseline values and values during the supplementation and observation in each group. Mean percentage change from baseline was determined. For comparison of changes between groups upaired t-test or Wallis-Kruskal test (for non-parametric variables) was used. Values were considered to be statistically significant when *P* was ≤ 0.05.

Pripombe dodal [IP4]: Z veliko ali z malo?

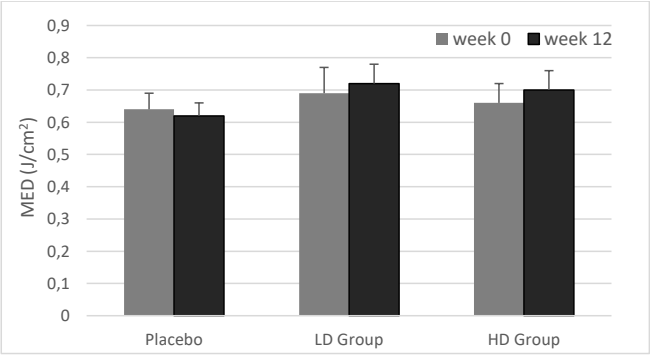
Results and discussion

Out of 33 subjects enrolled in the study 32 completed the entire 12 week trial. One subject in HD group withdrew before conducting measurements after 6 weeks for nonrelated reasons, and results for 32 subjects were analysed. None had to leave the study for adverse events or serious side effects. There was no report of side effects of any kind.

Minimal erythema dose ~~MED~~

Minimal erythema dose (MED) was slightly reduced ~~slightly~~ from the baseline at the end of the study period in placebo group (placebo: 0.64 J/cm^2 ± 0.05 at baseline vs. 0,62 J/cm^2 ± 0,04 at week 12, p = 0.64) while it was slightly improved in both CoQ10 groups (LD group: 0.69 J/cm^2 ± 0.08 at baseline vs. 0,72 J/cm^2 ± 0,06 at week 12, p = 0.36; HD group: 0.66 J/cm^2 ± 0.06 at baseline vs. 0,70 J/cm^2 ± 0,06 at week 12, p = 0.32), but those changes were not significant in neither of the groups (Figure 1, Supplementary Table 1). Also intergroup comparison between placebo, and LD or HD groups did not show any significant differences (p = 0.28 and 0.31 between placebo-LD and placebo-HD group, respectively). Consequently, based on these results we can not confirm *in vivo* anti-inflammatory effect of CoQ10, which was previously shown for UV response in some *in vitro* studies (Dong-Woo et al., 2007; Fuller et al., 2006; Zhang et al., 2012). It should be noted that while conduction of a study with higher number of subjects or with longer supplementation period might result in significant changes in MED, based on herein reported results the expected increase in MED would still be minor. Also, the increase of the dosage of CoQ10 supplementation did not have an important influence on MED; the difference ~~as there is no significant~~ difference between LD and HD group was not significant (p = 0.99).

***Figure 1**. Minimal erythema dose (MED, mean ± SE) at baseline and after 12 week of CoQ10 supplementation. No significant change was detected in neither placebo or CoQ10 groups.*

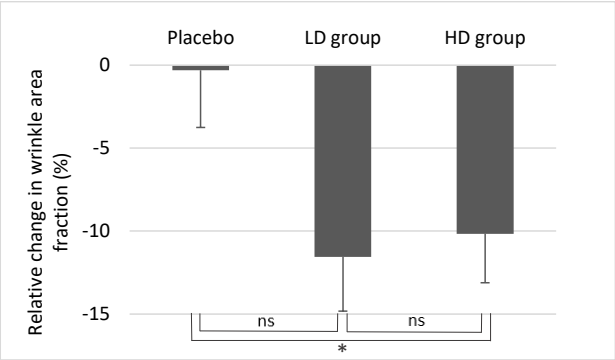


Wrinkle assessments

Assessment of the effect of CoQ10 supplementation on wrinkle expression was done in the periorbital area. Measurements of periorbital wrinkle area fraction show no significant change in placebo group (0.580±0.065 baseline vs. 0.579±0.065 at week 12, p = 0.92) while there was significant improvement in both CoQ10 groups (Figure 2, Supplementary Table 2). In LD group wrinkle area fraction was reduced from 0.575±0.077 at baseline to 0.509±0.074 at week 12 (p = 0.02) and in HD group it was reduced from 0.492±0.070 at baseline to 0.442±0.070 at week 12 (p = 0.02). Also intergroup comparison of LD and HD groups shows significant reduction in wrinkle area in comparison to placebo (p = 0.04 for LD and 0.04 for HD group vs. placebo). However, there is no significant difference in relative change of wrinkle area fraction over 12 week supplementation time between HD and LD group (p = 0.77).

Pripombe dodal [IP5]: Poenotiti velik ali mali P (ponekod imamo »p«, drugid »P«)

Figure 2*. Relative changes in periorbital wrinkle area for placebo, LD and HD group after 12 weeks of CoQ10 supplementation. Data shown as relative change in wrinkle area fraction (± SE) in comparison to baseline values. #p< 0.05 significant difference for comparison of week 12 to week 0; *p < 0.05, ** p < 0.01 significant difference; ns no significant difference between groups.*



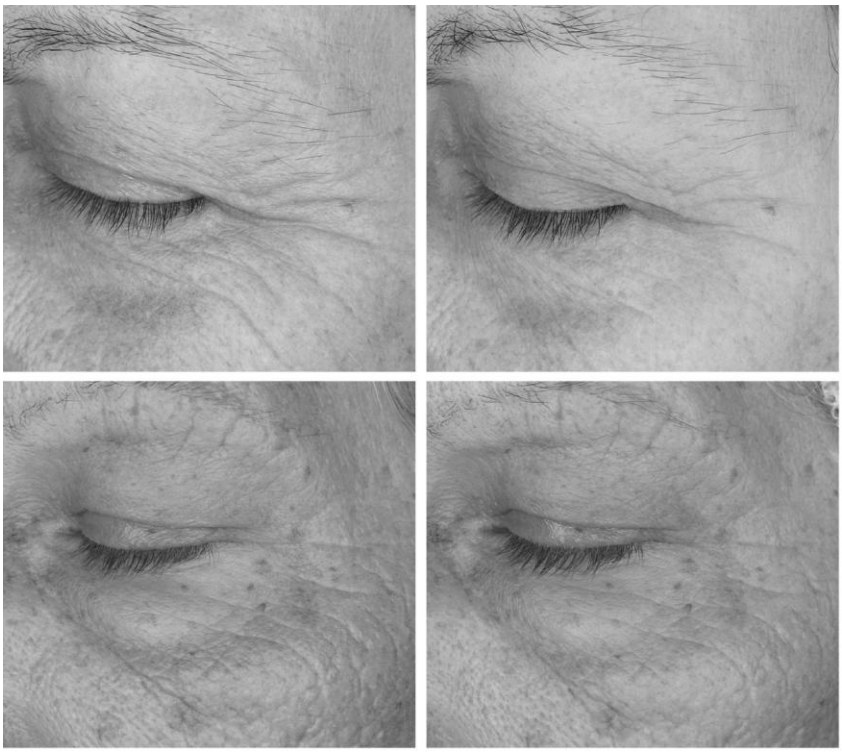
To provide further insight into anti-aging effects of CoQ10, we performed expert assessment of wrinkles of different type in different face areas according to Lemperle scale. Table 1 provides comparisons for subjects with expressed wrinkles at selected area at baseline. In placebo group no significant changes in wrinkle expression were observed for neither of the six evaluated wrinkle types. Evaluation of periorbital (PO) lines showed significant improvements in both LD and HD groups in comparison to week 0 (p < 0.05), confirming results of the measurement of wrinkle area fraction. In HD group, additional improvements were also noted in nasolabial folds (NL), corner of the mouth lines (CM) and upper radial lip lines (UL) (p < 0.05, <0.05 and <0.01, respectively).

Table 1*. Wrinkle assessment according to Lamperle scale (0-5) of HF, horizontal forehead lines; GF, glabellar frown lines; PO, periorbital lines; NL, nasolabial folds; CM, corner of the mouth lines; UL, upper radial lip lines.*

	HF		**GF**		**PO**		**NL**		**CM**		**UL**	
week	0	12	0	12	0	12	0	12	0	12	0	12

Placebo	1.3±0.2	1.4±0.2	2.1±0.3	2.1±0.3	2.5±0.2	2.5±0.2	2.0±0.4	2.0±0.4	2.0±0.4	2.0±0.4	1.9±0.4	1.9±0.4
LD Group	2.1±0.4	2.0±0.3	2.9±0.4	3.0±0.4	2.8±0.3	2.4±0.3*	2.9±0.4	2.8±0.4	2.5±0.4	2.4±0.4	1.8±0.3	1.7±0.3
HD Group	1.3±0.2	1.0±0.2	2.6±0.5	2.6±0.5	2.2±0.4	1.7±0.3*	2.8±0.4	2.1±0.5*	2.6±0.4	2.1±0.5*	1.4±0.3	0.6±0.3**

*p < 0.05; **p < 0.01: Significantly different from week 0



***Images 1-4**. Images show periorbital area of two subjects from HD group before the CoQ10 supplementation (week 0, left images) and after 12 week of supplementation (right images). After 12 weeks of supplemetation wrinkles are visibly reduced and also improvement of microrelief lines and smoothness can be observed.*

Pripombe dodal [IP6]: A ni to kar Figure? Slike je potem treba nekako označiti od 1 do 4? Razen če so po navodilih revije možni »Images«.

Dermis thickness and intensity

In the placebo group the average dermis thickness remained without significant change (mean 1461±42 μm at baseline vs. 1453 ± 43 μm at week 12. p = 0.42) as determined with ultrasound imaging of the dermis (Supplementary Table 3). However, there was also no significant change of dermis thickness in neither of CoQ10 groups (LD group: 1494±51 μm at baseline vs. 1510 ± 47 μm at week 12. p = 0.308, HD group: 1432±57 μm at baseline vs. 1448 ± 53 μm at week 12. p = 0.16). Dermis intensity score was also not significantly changed for neither of the groups (placebo: mean 27±2 at baseline vs. 30 ± 3 at week 12. p = 0.11; LD group: 28±2 at baseline vs. 26 ± 2 at week 12. p = 0.19; HD group: mean 26±2 at baseline vs. 28 ± 3 at week 12. p = 0.20). As dermis intensity is related to amount of properly structured dermal proteins eg. collagen and elastin (density), we cannot conclude that CoQ10

promoted the synthesis or reduced degradation of structural proteins as shown in some in vitro studies.(Ashida, 2009; Fuller et al., 2006; Inui et al., 2008) However, we should note that due to large inter-personal variations in baseline dermis intensity the study was under-powered to show the effect; a study with over 100 subjects per arm would be needed for clear conclusions.

Elasticity and hydration

Measurement of skin viscoelasticity revealed significant 24.5% decrease in the placebo group after 12 week study period (p= 0.03), but on the other hand viscoelasticity was stable in both CoQ10 groups as there was no significant change in viscoelasticity in neither of them (p=0.69 and 0.22 for LD and HD group, respectively) as shown in Table 4. Inter-group differences of viscoelasticity changes were significant between placebo and HD group (p=0.01), and near to significant between placebo and LD group (p=0.06). It is worth noting that study was conducted over late autumn and winter season simultaneously for all three groups. As several studies have confirmed dramatic changes in viscoelasticity and other skin surface parameters (Nam, Baek, Koh, & Hwang, 2015; Song et al., 2015) during the colder winter months, obtained results support positive effects of oral CoQ10 supplementation in the prevention of negative viscoelasticity seasonal changes during winter.

On the other hand no significant changes in skin hydration (Table 4) were detected in neither of the groups. As dermis is mostly responsible for skin elasticity, the hydration level of the skin relates to the hydration level of the epidermis layer and therefore are not correlated.

Table 2. Skin viscoelasticity and hydration for placebo, LD and HD group at baseline (week 0) and after 12 week of CoQ10 supplementation

				% change	
		Week 0	Week 12	Wk 12- Wk	p-value*
Viscoelasticity	Placebo	2.15±0.28	1.63±0.23	-24.5	0.03
	LD Group	1.87±0.28	1.96±0.14	4.8	0.69
	HD Group	1.80±0.11	1.97±0.17	9.4	0.22
Hydration	Placebo	220.92±16.55	184.83±15.72	-16.3	0.06
	LD Group	193.40±14.67	178.21±16.10	-7.9	0.47
	HD Group	232.75±18.62	200.98±21.77	-13.7	0.16

**Comparison week 12 to week 0*

Improvement of skin smoothness, microrelief and skin firmness in HD and LD group

There was an improvement in skin smoothness as determined by expert evaluation in both groups receiving CoQ10, namely in 70% of subjects in HD and 82% in LD group, while no change was observed in the placebo group (0%). Similar trends were also observed for microrelief lines, as they became notably less expressed in 60%, 64% and 9% of subjects, respectively. Figure 3 shows average score for changes in microrelief lines, which became less expressed and skin smoothness between week 0 and 12 as determined by expert evaluation. Skin firmness was assessed by self-evaluations and it was improved in 70%, 36% and 18% of subjects in HD, LD and placebo group, respectively.

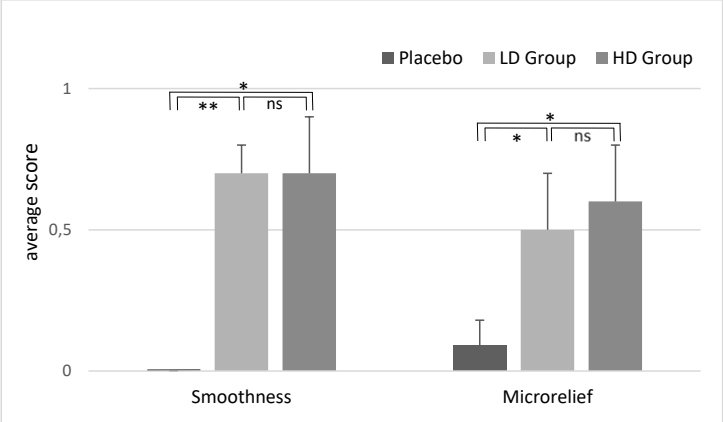


Figure 3. Average score for changes in microrelief lines and smoothness after 12 week CoQ10 supplementation as determined by expert evaluation (-1: deterioration, 0: no change, +1: great improvement). *$p < 0.05$, ** $p < 0.01$ significant difference; ^{ns} no significant difference between groups.

Conclusion

In the present study, administration of dietary supplement containing CoQ10 over 12 week period showed several anti-aging effects, as it reduced wrinkles, improved skin smoothness and microrelief as well as skin firmness. It also helped the skin to combat seasonal changes, as it prevented negative viscoelasticity seasonal changes during winter. Influence of CoQ10 dose on response was observed in viscoelasticity measurements, where result are significantly different from placebo only for higher dose, and in expert assessment of wrinkles and smoothness, as results were better for higher dose. We were unable to show the effect of supplementation on the skin hydration, dermis thickness and density. The results also showed that CoQ10 actually offers little to no photoprotection, as it was unable to reduce UVB-induced inflammation.

Study limitation

It should be noted that some baseline skin parameters are quite variable therefore it would be beneficial to perform study on higher number of subjects for cleaner conclusions regarding some parameters, study was especially under-powered for dermis parameters (intensity, thickness). Also supplementation over longer period and several seasons would be worth testing, as this study was conducted during winter time and also 12 weeks are quite short time to detect nutritional effects on skin, as the average skin cycle in healthy skin is 30-40 days.

Acknowledgments

The work was financially supported by the Slovenian Research Agency (Research program P3-0395: Nutrition and Public Health). We acknowledge support of Valens Int. d.o.o. which supplied syrups used in this study. The funding organizations had no role in the design, analysis or writing of this paper. The authors would like to thank K. Potočnik (UL Biotechnical Faculty) for help with the statistical analysis and M. Bales for providing assistance with the language.

Ashida, Y. (2009). Inhibitory Effects of Coenzyme Q10 on Skin Aging *Nutritional Cosmetics* (pp. 199-215).

Bentinger, M., Brismar, K., & Dallner, G. (2007). The antioxidant role of coenzyme Q. *Mitochondrion, 7*(Supplement 1), S41-S50.

Bliznakov, E. G. (2002). Lipid-lowering drugs (statins), cholesterol, and coenzyme Q(10). The Baycol case - a modern Pandora's box. *Biomedicine and Pharmacotherapy, 56*(1), 56-59.

Chew, G. T., & Watts, G. F. (2004). Coenzyme Q(10) and diabetic endotheliopathy: oxidative stress and the 'recoupling hypothesis'. *QJM - An International Journal of Medicine, 97*(8), 537-548.

Crane, F. L. (2001). Biochemical functions of coenzyme Q(10). *Journal of the American College of Nutrition, 20*(6), 591-598.

Dong-Woo, K., In Koo, H., Dae Won, K., Ki-Yeon, Y., Chung-Kil, W., Won-Kook, M., & Moo-Ho, W. (2007). Coenzyme Q_{10} effects on manganese superoxide dismutase and glutathione peroxidase in the hairless mouse skin induced by ultraviolet B irradiation. [Article]. *Biofactors, 30*(3), 139-147.

Elsayed, N. M., & Bendich, A. (2001). Dietary antioxidants: potential effects on oxidative products in cigarette smoke. *Nutrition Research, 21*(3), 551-567.

Ely, J. T. A., & Krone, C. A. (2000). A Brief Update on Ubiquinone (Coenzyme Q10). *Journal of Orthomolecular Medicine, 15*(2), 63-68.

Folkers, K. (1996). Relevance of the Biosynthesis of Coenzyme Q10and of the Four Bases of DNA as a Rationale for the Molecular Causes of Cancer and a Therapy. *Biochemical and Biophysical Research Communications, 224*(2), 358-361.

Fuller, B., Smith, D., Howerton, A., & Kern, D. (2006). Anti-inflammatory effects of CoQ10 and colorless carotenoids. *J Cosmet Dermatol, 5*(1), 30-38. doi: 10.1111/j.1473-2165.2006.00220.x

Galpern, W. R., & Cudkowicz, M. E. (2007). Coenzyme Q treatment of neurodegenerative diseases of aging. *Mitochondrion, 7*(Supplement 1), S146-S153.

Gao, L., Mao, Q., Cao, J., Wang, Y., Zhou, X., & Fan, L. (2012). Effects of coenzyme Q10 on vascular endothelial function in humans: A meta-analysis of randomized controlled trials. *Atherosclerosis, 221*(2), 311-316. doi: 10.1016/j.atherosclerosis.2011.10.027

Handbook of Non-Invasive Methods and the Skin. (Second Edition ed.). Boca Raton: CRC Press.

Hoppe, U., Bergemann, J., Diembeck, W., Ennen, J., Gohla, S., Harris, I., . . . Steckel, F. (1999). Coenzyme Q10, a cutaneous antioxidant and energizer. *BioFactors, 9*(2-4), 371-378.

Inui, M., Ooe, M., Fujii, K., Matsunaka, H., Yoshida, M., & Ichihashi, M. (2008). Mechanisms of inhibitory effects of CoQ10 on UVB-induced wrinkle formation in vitro and in vivo. *Biofactors, 32*(1-4), 237-243.

Kalen, A., Appelkvist, E. L., & Dallner, G. (1989). Age-related chenges in the lipid compositions of rat and human tissues. *Lipids, 24*(7), 579-584. doi: 10.1007/bf02535072

Knott, A., Achterberg, V., Smuda, C., Mielke, H., Sperling, G., Dunckelmann, K., . . . Blatt, T. (2015). Topical treatment with coenzyme Q10-containing formulas improves skin's Q10 level and provides antioxidative effects. *BioFactors*. doi: 10.1002/biof.1239

Kumar, A., Kaur, H., Devi, P., & Mohan, V. (2009). Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacology & Therapeutics, 124*(3), 259-268. doi: 10.1016/j.pharmthera.2009.07.003

Lafuente, R., Gonzalez-Comadran, M., Sola, I., Lopez, G., Brassesco, M., Carreras, R., & Checa, M. A. (2013). Coenzyme Q10 and male infertility: a meta-analysis. *Journal of Assisted Reproduction and Genetics, 30*(9), 1147-1156. doi: 10.1007/s10815-013-0047-5

Lemperle, G., Holmes, R. E., & Lemperle, S. S. M. (2001). A Classification of Facial Wri. *Plastic and Reconstructive Surgery, 108*(6), 1735-1750.

Littarru, G. P., & Langsjoen, P. (2007). Coenzyme Q10 and statins: Biochemical and clinical implications. *Mitochondrion, 7*(Supplement 1), S168-S174.

Littarru, G. P., & Tiano, L. (2010). Clinical aspects of coenzyme Q10: an update. *Nutrition, 26*, 250-254.
Masaki, H. (2010). Role of antioxidants in the skin: anti-aging effects. *Journal of Dermatological Science, 58*(2), 85-90.
Matthews-Brzozowska, T., Kurhanska-Flisykowska, A., Wyganowska-Swiatkowska, M., & Stopa, J. (2007). Healing of periodontal tissue assisted by Coenzyme Q(10) with Vitamin E - clinical and laboratory evaluation. *Pharmacological Reports, 59*, 257-260.
McDaniel, D. H., Neudecker, B. A., DiNardo, J. C., Lewis Ii, J. A., & Maibach, H. I. (2005). Clinical efficacy assessment in photodamaged skin of 0.5% and 1.0% idebenone. [Article]. *Journal of Cosmetic Dermatology, 4*(3), 167-173. doi: 10.1111/j.1473-2165.2005.00305.x
Mellors, A., & Tappel, A. L. (1966). The Inhibition of Mitochondrial Peroxidation by Ubiquinone and Ubiquinol. *Journal of Biological Chemistry, 241*(19), 4353-4356.
Milivojevic Fir, M., Smidovnik, A., Milivojevic, L., Zmitek, J., & Prosek, M. (2009). Studies of CoQ10 and cyclodextrin complexes: solubility, thermo- and photo-stability. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, doi: 10.1007/s10847-009-9555-4 (In Press)*.
Morré, D. M., Morré, D. J., Rehmus, W., & Kern, D. (2008). Supplementation with CoQ10 lowers age-related (ar) NOX levels in healthy subjects. *Biofactors, 32*(1-4), 221-230.
Mortensen, S. A., Rosenfeldt, F., Kumar, A., Dolliner, P., Filipiak, K. J., Pella, D., . . . Littarru, G. P. (2014). The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. *JACC Heart Fail, 2*(6), 641-649. doi: 10.1016/j.jchf.2014.06.008
Muta-Takada, K., Terada, T., Yamanishi, H., Ashida, Y., Inomata, S., Nishiyama, T., & Amano, S. (2009). Coenzyme Q10 protects against oxidative stress-induced cell death and enhances the synthesis of basement membrane components in dermal and epidermal cells. *Biofactors, 35*(5), 435-441. doi: 10.1002/biof.56
Nam, G. W., Baek, J. H., Koh, J. S., & Hwang, J. K. (2015). The seasonal variation in skin hydration, sebum, scaliness, brightness and elasticity in Korean females. *Skin Research and Technology, 21*(1), 1-8. doi: 10.1111/srt.12145
Passi, S., De Pità, O., Grandinetti, M., Simotti, C., & Littarru, G. P. (2003). The combined use of oral and topical lipophilic antioxidants increases their levels both in sebum and stratum corneum. [Article]. *Biofactors, 18*(1-4), 289-297.
Passi, S., Littarru, G. P., Puddu, P., & De Pità, O. (2002). Lipophilic Antioxidants in Human Sebum and Aging. [Article]. *Free Radical Research, 36*(4), 471-477.
Pravst, I. (2012). Functional foods in Europe: A focus on health claims. In B. Valdez (Ed.), *Scientific, Health and Social Aspects of the Food Industry*. Rijeka: InTech. (Reprinted from: Not in File).
Pravst, I., & Kušar, A. (2015). Consumers' exposure to nutrition and health claims on pre-packed foods: Use of sales weighting for assessing the food supply in Slovenia. *Nutrients, 7*(77), 9353-9368. doi: 10.3390/nu7115474
Pravst, I., Prosek, M., Wondra, A. G., Zmitek, K., & Zmitek, J. (2009). The stability of coenzyme Q10 in fortified foods. *Acta Chimica Slovenica, 56*(4), 953-958.
Pravst, I., & Zmitek, K. (2011). The coenzyme Q10 content of food supplements. *Journal fur Verbraucherschutz und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 6*(4), 457-463.
Pravst, I., Zmitek, K., & Zmitek, J. (2010). Coenzyme Q10 contents in foods and fortification strategies. *Crit Rev Food Sci Nutr, 50*(4), 269-280. doi: 10.1080/10408390902773037
Quinzii, C. M., Hirano, M., & DiMauro, S. (2007). CoQ10 deficiency diseases in adults. *Mitochondrion, 7*(Supplement 1), S122-S126.
Schmelzer, C., Lindner, I., Rimbach, G., Niklowitz, P., Menke, T., & Döring, F. (2008). Functions of coenzyme Q_{10} in inflammation and gene expression. [Article]. *Biofactors, 32*(1-4), 179-183.

Shindo, Y., Witt, E., Han, D., Epstein, W., & Packer, L. (1994). ENZYMATIC AND NONENZYMATIC ANTIOXIDANTS IN EPIDERMIS AND DERMIS OF HUMAN SKIN. [Note]. *Journal of Investigative Dermatology, 102*(1), 122-124. doi: 10.1111/1523-1747.ep12371744

Shults, C. W. (2003). Coenzyme Q(10) in neurodegenerative diseases. *Current Medicinal Chemistry, 10*(19), 1917-1921.

Shults, C. W., Oakes, D., Kieburtz, K., Beal, M. F., Haas, R., Plumb, S., . . . Lew, M. (2002). Effects of coenzyme Q(10) in early Parkinson disease - Evidence of slowing of the functional decline. *Archives of Neurology, 59*(10), 1541-1550.

Song, E. J., Lee, J. A., Park, J. J., Kim, H. J., Kim, N. S., Byun, K. S., . . . Moon, T. K. (2015). A study on seasonal variation of skin parameters in Korean males. *International Journal of Cosmetic Science, 37*(1), 92-97. doi: 10.1111/ics.12174

Vinson, J., & Anamandla, S. (2006). Comparative topical absorption and antioxidant effectiveness of two forms of coenzyme q10 after a single dose and after long-term supplementation in the skin of young and middle-aged subjects. [Article]. *International Journal of Cosmetic Science, 28*(2), 148-148. doi: 10.1111/j.1467-2494.2006.00307_3.x

Zhang, M., Dang, L., Guo, F., Wang, X., Zhao, W., & Zhao, R. (2012). Coenzyme Q10 enhances dermal elastin expression, inhibits IL-1a production and melanin synthesis in vitro. *International Journal of Cosmetic Science, 34*(3), 273-279. doi: 10.1111/j.1468-2494.2012.00713.x

Zmitek, J., Smidovnik, A., Fir, M., Prosek, M., Zmitek, K., Walczak, J., & Pravst, I. (2008). Relative bioavailability of two forms of a novel water soluble Coenzyme Q10. *Annals of Nutrition and Metabolism, 52*, 281-287.