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Full Length Research Paper

The impact of coenzyme Q10 supplement on the indicators of muscle damage in young male skiing athletes

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This study was conducted in order to know the impact of coenzyme Q₁₀ (CoQ₁₀) supplement on the muscle damage and total oxidant (TOS) enzyme levels of young skiing athletes during exercise. 15 male athletes were used for two weeks in the study. The athletes were divided into three groups: the control group and two subject groups taking 100 mg and 200 mg CoQ₁₀. A maximal exercise program with 70-80% overload was applied to the groups for two hours every day for two weeks. Before (B.T.) and after (A.T.) the training, blood samples were taken from athletes in order to determine CoQ₁₀, TOS and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutamyl transpeptidase (GG), lactate dehydrogenase (LDH), Creatine kinase (CK) enzyme activities. HPLC kit was used to determine CoQ₁₀ levels and TOS kit was used to determine TOS levels. When the pre-exercise and post-exercise CoQ₁₀ levels were compared, it was seen that CoQ₁₀ values of subject groups increased (p<0,05; p<0,01) while the control group did not show a significant difference. Also, a decrease was seen in TOS values of subject groups (p<0.01), whereas the control group showed an increase (p<0,01). In addition, significant increases (P<0.01) were obtained in the levels of AST, ALT, LDH and CK of the control group compared to those of the subject groups. Comparing CoQ₁₀ and TOS levels by days during 2 weeks, it was found that TOS levels of control group increased; no change occurred at CoQ10 and; TOS levels of experimental groups decreased and significant increases were found in CoQ_{10} groups. In conclusion, CoQ_{10} usage may have impact on lower TOS values and liver, muscle enzyme activities of experimental group compared to control group.

Key words: Training, muscle damage, CoQ₁₀, total oxidant, enzyme activities.

INTRODUCTION

It is known that muscle damage occurs with various levels in muscle tissue during exercise. Muscle damage leads to decrease on muscle strength, speed and flexibility according to the type and quality of exercise. On the other hand, it is assumed that it leads to muscle

exhaustion, loss of function and especially the extension of adaptation process (Eston et al., 2003). Low-intensity exercise stimulates expression of antioxidant enzymes and high-intensity exercise may lead to oxidative stress and cell damage and this situation may require

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antioxidant supplement (Gomez-Cabrer et al., 2008). In addition, reactive oxygen types are generated continuously during aerobic metabolism and removed by various biologic antioxidants. Antioxidant protection may not be 100% effective all the time. If peroxidants increase or antioxidants fail, oxidative stress occurs and it leads to molecular and tissue damage (Revanet al., 2013).

Another indicator used to assess muscle damage is the increase in serum levels of muscle enzymes such as AST, ALT, ALP, GGT, LDH and CK. High enzyme levels are the indicator of the fatigue, damage and increase that occur in concentrations of big tissue structures such as liver and skeletal muscle. Different studies indicated that these enzymes increase after exercise-related muscle damage and 72 h after the exercise, enzyme levels turn back pre-exercise basal levels (Diaz et al., 2010; Schneider et al., 1995). It is known that oxidant generation increases during exercise. Being one of the most remarkable compounds in this issue, CoQ₁₀ takes place in ubiquinone family that can be synthesized in humans and all animals. Being a lipophilic antioxidant and playing a role in cell signal transduction and gene expression, CoQ₁₀ is a significant carrier that takes part in ATP synthesis and electron transfer on mitochondrial respiratory chain (Kubo et al., 2008; Crane, 2001; Turunen et al, 2004). Recently, CoQ₁₀ has gained considerable attention as a dietary supplement capable of influencing cellular bioenergetics and counteracting some of the damage caused by free radicals (Juel, 2006; Rosenfeldt et al., 2003; Zhou et al., 2005). Many studies have demonstrated that exercise training results in an increased production of free radicals and other forms of reactive oxygen species that contributes decreased physical performance, muscular fatigue, and muscle damage (Kon, 2007; Reid, 2008).

It is assumed that CoQ_{10} transfers electron to cellular molecules and helps to generate energy from ATP by means of contributing to mitochondria to generate energy due to its first degree correlation with cellular energy flow and energy generation. It is also asserted that regular training changes morphology of muscle structure and improves performance and thus decreases muscle fatigue and damage (Cooper et al., 2002). This study investigated the impact of coenzyme Q_{10} supplement on muscle damage, liver enzyme activities and total oxidant (TOS) levels during training.

MATERIALS AND METHODS

Experimental design

Of the 30 volunteer male skiing athletes who do exercise regularly and do not take any vitamin supplement tablet, 15 were randomly selected for the study. There were not to change their nutritional habits during the study. The subjects were asked to stop taking any medicine minimum one week before the test day, stop consuming alcohol and foods containing caffeine 24 h before the exercise.

Thesis Protocol was accepted by Kafkas University Faculty of Medicine Ethics Committee with approval dated 09.06.2009 and numbered B.30.2.KAÜ.0.20.71.00. The athletes were asked to sign the informed consent form. Conducted in line with the relevant directive specified in Helsinki Declaration, the study obtained approval from the Local Ethics Committee and ensured voluntary participation by providing study subjects with information on the objective of the study before the measurements. The athletes were categorized into three groups as control group, experimental groups using 100 mg and 200 mg CoQ_{10} .

Training program and the appliance of CoQ₁₀

The study includes 14-day supplement of CoQ_{10} following one-week control period. The athletes took 100 and 200 mg CoQ_{10} 30 min after breakfast between Day 1 and Day 14. The athletes were asked to maintain their usual nutritional habits and daily activities. During the study, 70-80% maximal loading training program was applied on each of the three groups. This program was applied for 2 h once in a day during 2 weeks. The training program was prepared systematically and specifically to each group so as to obtain the most reliable effect physiologically. A program including the basic, general and specific endurance exercises was applied to the athletes involved in the exercise program for 14 days.

Training program

Day 1: 30 min running (Basic Endurance (TD) + Power Training 3x30 min.

Day 2: 12km walking (General Endurance (GD) / Specific Endurance (ÖD) + Medicine ball warm-up

Day 3: 10 min warm-up+ Traditional technique with cross-skating + 20 min. training without baton

Day 4: Baton walking + Running (TD/GD)

Day 5: Traditional technique + 30 min. running without baton

Day 6: Skating techniques (TD) +30 min. running without baton

Day 7: 20 min. running + Static power training

Day 8: Traditional technique (TD) + 6x2 km interval (ÖD)

Day 9: 20 min. running (TD) + 40 min. Medicine ball warm-up + 3x20 min. power training

Day 10: Traditional technique (TD) + 20 min. training without baton + 10 min. running

Day 11: Traditional technique (TD) + 6x2 km interval (ÖD)

Day 12: Traditional technique (TD) + 20 min. warm-up+ 1 hour GD

Day 13: Traditional technique with cross-skating (TD) + 10 min.

warm-up+ 10km training without baton (GD) +15 min. running Day 14: Traditional technique + 20 min warm-up + 1 hour GD

Taking blood samples

Blood samples were taken from athletes before training once and after training during two weeks to anticoagulant lithium heparin tubes. The blood samples were centrifuged at 4000 rpm in cooled centrifuge for 15 min and the supernatant was taken into covered polypropylene tubes and stored in -80 $^{\circ}$ C deep freeze to determine CoQ₁₀ and TOS levels; in -20 $^{\circ}$ C deep freeze to determine AST, ALT, ALP, GGT, LDH and CK analyses.

Biochemical analysis

CoQ₁₀ assay

The protocol for determining CoQ₁₀ in plasma was modified from

Table 1. Mean ± SD characteristics of subjects in different groups.

Specification group	Age (Yr)	Weight (kg)	Weight (cm)
Control group	21.60 ± 0.51	$64.20 \pm 3,14$	1.76 ± 0.02
100 mg CoQ ₁₀ group	21.80 ± 0.73	66.80 ± 2.97	1.76 ± 0.01
200 mg CoQ ₁₀ group	21.60 ± 0.51	63.80 ± 2.31	1.75 ± 0.01

the Mosca et al. protocol by using the reverse-phase-HPLC C-18 (Spherisorb ODS-2, 5 μ m, 4.6x250 mm column; Water company). A mixture of 200 μ l of plasma and 20 μ l of internal standard CoQ9 (0.5 μ g/ml) was denatured by adding 3.0 ml of fresh ethanol/n-hexane (2:5, v:v). After vortexing for 2 min and centrifuging at 300 g for 10 min at 4°C, 1.30 ml of organic n-hexane was separated for evaporation under nitrogen gas and reconstituted with 100 μ l of ethanol/n-hexane (6:4, v:v). Only 20 μ l was injected into a 20 μ l sample loop. Interestingly, CoQ₁₀ and internal CoQ₉ control peaks were eluted, and showed a mobile phase (methanol hexane (6:4,v:v) with a running flow at 1.0 ml/min by using a Conta Meric LDL analyzer with UV detector (\aleph = 275 nm). The CoQ₁₀ concentration in plasma was calculated by comparing with the high peak of standard CoQ₁₀ (0-20 μ g/ml).

TOS assay

Preparing working standard solution

SSSS is diluted 40,000 times with deionised water. A liquid of 50 microliter SSSS is added to 10 ml deionised water and vortexed (The first step dilution). A liquid of 50 microliter of the prepared solution is added to 10 ml deionised water and vortexed (The second step dilution). The final concentration of the working standard is 20 micromolar H2O2.

Preparing working solution daily

Place 500 microliter Reagent 1 in cell and add 75 microliter to the prepared standard (or sample). Read the initial absorbance at 530 nm for the first absorbance point. Add 25 microliter Reagent 2 to the cell and incubate 10 min at room temperature or 5 min at 37°C. Read the absorbance a second time at 530 nm.

AST, ALT, ALP, GGT, LDH and CK blood samples were analyzed in Medicine Faculty Laboratory of Kafkas University.

Statistical analysis

SPSS 17.0 program was used to conduct statistical analysis of the data. The data were defined as the mean ± standard deviation. Matched t-test was used to determine any difference between data before and after substance. Variance was homogenous for use of standard ANOVA methodology. After statistical significance was established by ANOVA, individual comparisons were made using Tukey's multiple comparison test. Lower P value than 0.05 was regarded to be significant.

RESULTS

The mean values of control group's age (21.60 \pm 0.51), weight (64.20 \pm 3,14) and height (1.76 \pm 0.02); group 1's age (Year) (21.80 \pm 0.73), weight (kg) (66.80 \pm 2.97) and

height (cm) (1.76 \pm 0.01); group 2's age (Year) (21.60 \pm 0.51), weight (kg) (63.80 \pm 2.31) and height (cm) (1.75 \pm 0.01) of participants were presented respectively (Table 1).

When the pre-exercise and post-exercise CoQ₁₀ levels were compared, it was seen that CoQ₁₀ values of subject groups increased (p<0.05; p<0,01) while the control group did not show a significant difference. Also, a decrease was seen in TOS values of subject groups (p<0.01) whereas the control group showed an increase (p<0.01). In addition, significant increases (P<0.01) were obtained in the levels of AST, ALT, LDH and CK of the control group compared to those of the subject groups. However p<0.01 decrease was found in LDH and CK levels of experimental groups and p<0.05 decrease was found in AST, ALT values with CoQ₁₀ usage. On the other hand, no significant difference of ALP and GGT levels was found in control and 100 mg CoQ₁₀ groups and p<0.05 decrease was found in 200 mg CoQ₁₀ group (Table 2).

During the two-week training, groups were compared. Accordingly, no increase was found in CoQ_{10} levels of control group and an increase was found in experimental groups. Comparing CoQ_{10} levels of experimental groups with the first day of control group, no difference was found in levels of 100 mg CoQ_{10} group until 3rd day and significant increases were observed from 6th to 14th day. In addition, CoQ_{10} levels of 200 mg CoQ_{10} group started to increase clearly on 1st day (p<0.01) (Figure 1)

Comparing TOS levels of experimental and control groups during two-week training, no significant difference was found between groups from 1st to 3rd day. However, significant increases were found in TOS levels of control groups from 6th to 14th day (p<0.01). On the other hand, p<0.05 decrease was found in 100 and 200 mg CoQ_{10} groups from 6th day (Figure 2).

DISCUSSION AND CONCLUSION

This study was conducted in order to research the impact of coenzyme Q_{10} (CoQ_{10}) supplement on the muscle damage and total oxidant TOS enzyme levels of young skiing athletes during exercise. CoQ_10 supplementation leads to augment in plasma coenzyme Q concentrations, the extent of which depends upon the type of formulation, dosage, and also duration (Tauler et al., 2008). The CoQ_{10} supplement in capsule form was provided in this

Table 2. The mean and standard deviation (±SD) values of liver enzyme activity levels of groups before and after training.

	Control group		100 mg CoQ₁₀ group		200 mg CoQ ₁₀ group	
Groups	B.T.	A.T.	B.T.	A.T.	B.T.	A.T.
CoQ ₁₀ (µmol/L)	1,42±0,17	1,61 ± 0,32	$1,43 \pm 0,18$	$3,2 \pm 0,36*$	1,44 ± 0,19	4,8 ±0,23**
TOS (nmol)	0,16±0,75	0,63±0,20**	0,16±0,75	0,18±0,08**	0,16±0,75	0,11±0,04**
LDH (IU/L)	157,0±10,3	442,6±32,4**	$154,0 \pm 17,8$	271,4±11,2**	155,0± 9,33	240,4±9,23**
CK (IU/L)	12,94±5,78	32,4±14,50**	$13,75 \pm 6,15$	11,28±5,04**	$12,96 \pm 5,79$	9,23 ± 4,13**
AST (U/L)	$33,4 \pm 3,20$	36,2 ± 3,83**	$33,4 \pm 3,20$	$32,0 \pm 2,73^*$	$33,4 \pm 3,20$	31,80 ± 3,11*
ALT (U/L)	25,8±6,09	28,0±5,87**	24,8±4,43	23,8± 3,16*	24,2±3,89	23,4±3,64*
ALP (U/L)	28,6±12,81	28,8± 12,90	28,08±12,55	28,91±12,93	27,79±12,42	18,47±8,26*
GGT (mg/DI)	13,35±5,97	$12,75 \pm 5,70$	$12,62 \pm 5,64$	$12,47 \pm 5,58$	$12,50 \pm 5,59$	4,96 ± 2,22*

^{**} p<0.01, * p<0.05, Befor training (B.T), After Taining (A.T.), Coenzyme Q_{10} , (Co Q_{10}), Total Oxidant (TOS), Aspartat Eaminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Glutamyl Transpeptidase (GGT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK).

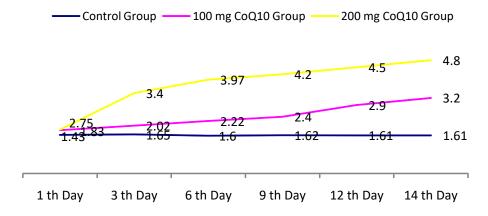


Figure 1. The change in CoQ_{10} levels of control group, and 100 and 200 mg CoQ_{10} groups during fourteen-day training.

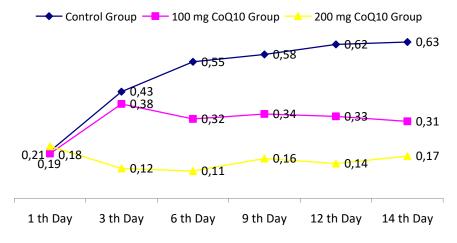


Figure 2. The change in TOS levels of control group, and 100 and 200 mg CoQ_{10} groups during fourteen-day training.

study at a dosage of 5 mg/kg/day for 14 days. Previous reports have shown that the bioavailability of CoQ₁₀ can reach maximal concentration at 26.5 or 25.8 h following supplementation (Turunen et al., 2004). Thus, daily CoQ₁₀ supplementation could provide maximal concentration in human plasma during experiments. Therefore, CoQ₁₀ supplementation at 5 mg/kg/day for 14 days was noted to increase plasma CoQ₁₀ levels approximately 2fold compared to before supplementation. Previous reports suggest that CoQ₁₀ in the ubiquinone form is essential for generating energy within mitochondria and providing antioxidant defense similar to the other fatsoluble antioxidants, such as vitamin E. This appears to be due to the scavenging of free radicals and prevention of oxidation of lipids and other molecules (Kagan et al., 1990).

The present study shows that comparing the levels of CoQ₁₀ and TOS before and after the training, it was found that CoQ₁₀ levels of experimental group and TOS levels of control group increased. In addition, decreases were found in TOS values of experimental groups with CoQ₁₀ usage. These findings support prior work, as discussed above, as well as work involving healthy subjects in which CoQ₁₀ supplementation at 300 mg daily, but not 100 mg daily, to reduce fatigue and enhance physical performance (Mizuno et al., 2008). Clearly, dosing is an important concern when considering CoQ₁₀ supplementation. Although, CoQ₁₀ is located within the inner mitochondrial membrane, is the cofactor of three mitochondrial enzymes (complex I, II and III), and plays an essential role in production of adenosine triphosphate (ATP) during exercise (Crane et al.,1993). The present study noted significant changes during two-week training; groups were compared. Accordingly, no increase was found in CoQ₁₀ levels of control group and an increase was found in experimental groups. Comparing CoQ₁₀ levels of experimental groups with the first day of control group, no difference was found in levels of 100 mg CoQ₁₀ group until 3rd day and significant increases started to be observed from 6th to 14th day. However, CoQ₁₀ levels of 200 mg CoQ₁₀ group started to increase clearly on 1st day. Comparing TOS levels of experimental and control groups, no significant difference was found between groups from 1st to 3rd day. However, significant increases were found in TOS levels of control groups from 6the to 14th day. On the other hand, decrease started to be found in 100 and 200 mg CoQ₁₀ groups from 6th day. CoQ₁₀ plays various critical roles in metabolism, serves as a redox electron transporter in the mitochondria related to the synthesis of ATP, acting as an essential antioxidant, influencing the stability of membranes. Reactive oxygen types are generated continuously during aerobic metabolism and removed by various biologic antioxidants. Antioxidant protection may not be 100% effective all the time. If prooxidants increase or antioxidants fail, oxidative stress occurs and it leads to

molecular and tissue damage (Kon et al., 2007; Revan et al., 2013). However, CoQ_{10} interacts with oxygen-related radicals and singlet oxygen and prevents lipid peroxidation and any damage on biomolecules (Crane, 2001).

On the other hand, high enzyme levels are the indicator of the fatigue, damage and increase occur that in concentrations of big tissue structures such as liver and skeletal muscle. Changes in concentrations CK, AST, LDH, ALP, GGT and ALT were examined so as to assess the muscle damage (Hazar, 2004). However, CoQ₁₀ taking place in mitochondria of cells and leading to energy and ATP generation is used as a fundamental catalyzer in order to enable the organism to adopt abovementioned changes. Studies indicated that CoQ₁₀ taken before exercise reduces fatigue level and increases plasma CoQ₁₀ level (Bonetti et al., 2000; Ylikoski et al., 1997). These findings support the findings of the present research (Table 2). In another study (Niklowitz et al., 2007), it was found that 3 mg/kg oral CoQ₁₀ supplement decreases 8-OH-dG levels being an indicator of DNA damage. Also, in another study the use of CoQ₁₀ before exercise leads to a clear decrease in CPK, LDH, AST, ALP levels during the exercise (Mizuno et al., 2008; Shimomura et al., 1991; Revan et al., 2013). However, in another study CoQ₁₀ in serum or plasma may have been distributed to several tissues during intensive exercise. Shimomura et al. have suggested that CoQ₁₀ supplementation reduced increased creatine kinase (CK) and lactate dehydrogenase (LDH) in rat's subsequent downhill running (Kon et al., 2007; Shimomura et al., 1991). In this study, significant increases were found in AST, ALT, LDH and CK levels of control groups compared to those of experimental groups. However, decreases were found in LDH, CK, AST and ALT levels of experimental groups with CoQ₁₀ usage. On the other hand, no significant difference of ALP and GGT levels was found in control and 100 mg CoQ₁₀ groups and decrease was found in 200 mg CoQ₁₀ group.

Therefore, it is quite likely that CoQ₁₀ supplementation increases CoQ concentration in muscle cell membranes and reduces strenuous exercise-induced muscular injury by enhancing cell membrane stabilization. The use of oxygen and mitochondrial electron based on increasing metabolic rate increases electron leak from transport chain and thus many reactive oxygen types mainly hydroxyl radicals emerge (Eston et al., 2003; Cooper et al., 2002). It was found that to conduct training continuously and increasingly in steps not only causes a physiological dilatation and hypertrophy but also regular circulation and the use of CoQ₁₀ could turn enzyme activities which increase just after the training into normal levels. In addition, it can be said that physical exercise increases oxygen consumption and thus generation of reactive oxygen types leads to muscle fatigue and thus to oxidative damage. On the other hand, the use of CoQ₁₀

contributes to energy generation process in mitochondria, decreases muscle damage and TOS levels.

As a result, long-term and intense skiing trainings lead to the increase in CK, LDH, AST and ALT enzyme activities due to excessive muscle activity and oxidative damage occurs as a result; however, CoQ₁₀ which takes place in mitochondria of cells and leads to energy and ATP generation plays a fundamental catalyzer role in order to ensure adaptation to high physiologic activities in muscle tissues and thus decreases enzyme activities, being TOS and muscle-damage indicators.

Conflict of Interests

The author has not declared any conflict of interests.

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