

The effect of short-term coenzyme Q₁₀ supplementation and pre-cooling strategy on cardiac damage markers in elite swimmers

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Abstract

Strenuous physical exercise and hyperthermia may paradoxically induce oxidative stress and adverse effects on myocardial function. The purpose of this study was to investigate the effect of 14-d coenzyme Q₁₀ (CoQ₁₀) supplementation and pre-cooling on serum creatine kinase-MB (CK-MB), cardiac Troponin I (cTnI), myoglobin (Mb), lactate dehydrogenase (LD), total antioxidant capacity (TAC), lipid peroxidation (LPO) and CoQ₁₀ concentration in elite swimmers. In total, thirty-six healthy males (mean age 17 (SD 1) years) were randomly selected and divided into four groups of supplementation, supplementation with pre-cooling, pre-cooling and control. During an eighteen-session protocol in the morning and evening, subjects attended speed and endurance swimming training sessions for 5 km in each session. Blood sampling was done before (two stages) and after (two stages) administration of CoQ₁₀ and pre-cooling. ANCOVA and repeated measurement tests with Bonferroni *post hoc* test were used for the statistical analysis of the data. There was no significant statistical difference among groups for the levels of CK-MB, cTnI, Mb, LD, TAC, LPO and CoQ₁₀ at the presampling (stages 1 and 2) ($P > 0.05$). However, pre-cooling and control groups show a significant increase in the levels of CK-MB, cTnI, Mb, LD and LPO compared with the supplementation and supplementation with pre-cooling groups in the post-sampling (stages 1 and 2) ($P < 0.05$), except for the TAC and CoQ₁₀. Consequently, CoQ₁₀ supplementation prevents adverse changes of myocardial damage and oxidative stress during swimming competition phase. Meanwhile, the pre-cooling strategy individually has no desired effect on the levels of CK-MB, cTnI, Mb, LD, LPO, TAC and CoQ₁₀.

Key words: Cardiac damage markers: Coenzyme Q₁₀: Elite swimmers: Heavy training: Pre-cooling

Strenuous trainings or prolonged moderate-intensity exercise in a hot and humid environment can significantly add to the challenge that physical exercise imposes on the human thermoregulatory system, as heat exchange between body and environment is substantially impaired under these conditions⁽¹⁾. This can lead to serious performance decrements of athletes and an increased risk of developing heat stress and illnesses such as heat cramps, heat exhaustion and heat stroke⁽¹⁾. Increased body core temperature becomes a stimulus of visceral vessels to produce irregular free radicals including nitric oxide (•NO) and reactive oxygen species (ROS)⁽²⁾. Minimising increased body core temperature and exercise-induced oxidative stress in hot environments can prevent athletes facing problems in the World Cup of Qatar 2022⁽³⁾.

Cooling body strategy in two stages of pre-cooling and per-cooling can inhibit fatigue, cell damages and health problems by lowering core body temperature⁽³⁾. More recent studies have shown its effects on improving time to exhaustion and

performance; however, there are also disagreements in this regard, identifying it as a non-applicable method for the field and competitive sports⁽³⁾. Since 2000, pre-cooling method(s) has been used for most sport fields in hot environmental conditions ($\geq 28^\circ\text{C}$)⁽⁴⁾. In this respect, researchers reported the use of pre-cooling (immersion in cold water) and per-cooling (drinking cold water) to be useful in reducing the pressure on the heart and blood vessels by decreasing body temperature and dehydration during training bouts on the stationary bike at 32°C with relative humidity of 80%⁽⁵⁾. Engagement in long-term sport activities leads to the production of ROS including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\text{OH}\cdot$) by the increase of the mitochondrial electron leakage, impaired haemostasis and reduced immune system function⁽⁶⁾. In fact, they initiate lipid peroxidation (LPO) (cell membranes are a common target) and protein oxidation, which play important roles in stabilising pathogens and a wide range of diseases⁽⁶⁾.

Abbreviations: CK-MB, creatine kinase-MB; CoQ₁₀, coenzyme Q₁₀; cTnI, cardiac Troponin I; LD, lactate dehydrogenase; LPO, lipid peroxidation; Mb, myoglobin; ROS, reactive oxygen species; TAC, total antioxidant capacity; THR, target heart rate.

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The role, as well as the involvement of excess ROS production in cardiac pathophysiology, has been well documented, contributing to contractile dysfunction most likely involving abnormalities in intracellular Ca^{2+} homeostasis, cardiomyopathy, arrhythmia, ischaemia–reperfusion injury and mitochondrial DNA damages⁽⁷⁾. Many studies using both animal and human subjects have demonstrated that exhaustion and ultra-endurance exercises (e.g. cycling, running, swimming and half-ironman triathlon) result in increased exercise-induced cardiac fatigue (EICF) and biomarkers of oxidative damage in both blood and cardiac muscles⁽⁸⁾. Consequently, markers of both skeletal muscles and myocardial damage increase, including the serum cardiac enzymes (lactate dehydrogenase (LD) 1 isoenzyme, α -hydroxybutyrate dehydrogenase (HBD), aspartate transaminase (AST) and CK), creatine kinase isoenzymes (CK-MB), cardiac contractile protein isoenzymes (cardiac Troponin I (cTnI) and cardiac Troponin T (cTnT)) and myoglobin (Mb), which is an early marker of myocardial damage⁽⁹⁾.

According to previous studies, a swimming session induced higher cell damage and oxidative stress in boys compared with the girls⁽¹⁰⁾. There was a positive correlation between malondialdehyde, CK and LD especially in teenage boys compared with the girls after a swimming session⁽¹⁰⁾. These sex differences could be attributed to the higher concentrations of 17- β -oestradiol in girls than in boys; because of their antioxidant properties, they could decrease the inflammatory cytokines response. In fact, these two roles could be related, because disorder inflammatory system due to strenuous exercise includes significant generation of ROS and antioxidant depletion, which can result in an increase in oxidative damage and cell membrane instability⁽¹⁰⁾. Santos Silva *et al.*⁽¹¹⁾ stated that adolescent swimmers who involved in high-level trainings and competition stages experienced proteolytic and lipolytic damages. One strategy of the researchers and athletic coaches for coping with the adverse effects of oxidative stress caused by continued and heavy training bouts is using coenzyme Q₁₀ (CoQ₁₀) supplementation⁽¹²⁾.

CoQ₁₀ has been shown in all tissues and organs in the body, with highest concentrations in the myocardium⁽¹³⁾. It plays an important role in the mitochondrial redox component and endogenously produced lipid-soluble antioxidants (scavenges oxygen radicals) of the human organisms⁽¹³⁾. Other important functions of CoQ₁₀ are protecting the stability of the cell membranes and being capable of re-cycling and regenerating other antioxidants such as tocopherol and ascorbate⁽¹³⁾. Therefore, owing to the lack of studies in this area, it was hypothesised that the use of CoQ₁₀ supplementation and

pre-cooling strategy can affect adverse changes of serum lipid peroxide, which is an index of oxidative stress, cardiac damage markers, total antioxidant capacity (TAC) and CoQ₁₀ concentration of elite adolescent swimmers during heavy training and recording bouts in the hot and humid environment.

Methods

Experimental design

This study was cross-sectional and experimental that followed a repeated measures design with controlled trial. After permission of the I.R.IRAN Swimming Federation and head coach, the training programme of swimmers was designed by the coach and researcher for the competition stage in the hottest season (summer) of the year. Performances of the swimmers were measured by recording freestyle swimming (24 h before and after supplementation and pre-cooling strategy) at 800, 200 and 50 m, respectively. Considering the type of statistical analysis of the study, effect size = 0.49, power = 0.81, α = 0.05 and the four groups were studied, the number of required samples was determined as thirty-six individuals using the software G*Power 3.1.9.2⁽¹⁴⁾. Total sample size with thirty-six healthy volunteers was selected by the convenient sampling method from a population with homogeneous elite swimmers. Each participant was randomly assigned to one of four groups: supplementation (received oral administration of 300 mg/d of CoQ₁₀), pre-cooling (immersion in the cold water $18 \pm 0.5^\circ\text{C}$), supplementation with pre-cooling and control with nine members. Stages of this study are represented in Fig. 1.

Participants

In total, thirty-six elite male swimmers were recruited to take part in the study; the mean age, stature, mass and body fat composition were 17 (SD 1) years, 176.13 (SD 4.37) cm, 70.54 (SD 6.55) kg and 13.55 (SD 1.75)%, respectively. Table 1 provides the descriptive characteristics of the subjects. They were the members of national swimming team of Iran in Tehran with 10 years of professional swimming records and had gained top ranks at national level. Volunteers completed a medical history and Physical Activity Readiness-Questionnaire (PAR-Q) to determine eligibility. All subjects were non-smokers, and none had any history of recurrent respiratory illness, such as asthma or chronic cough. Swimmers did not use any supplement or energetics 1 month before the research and were trained in Sport Complex of Azadi in Tehran at the same nutritional and welfare conditions.

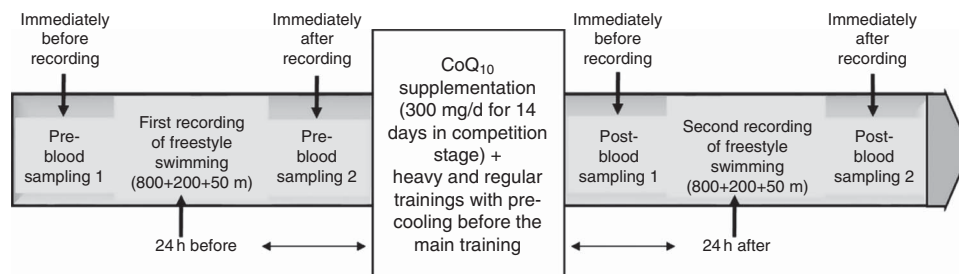


Fig. 1. General approach of the research process. CoQ₁₀, coenzyme Q₁₀.

Compliance with ethics guidelines

The study protocol for withdrawal of blood from healthy volunteers was approved by the regional ethics committee at Urmia University of Medical Sciences, Urmia, Iran. All followed procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and Declaration of Helsinki (2013 edition). Also, parents (for swimmers under the age of 18 years) and swimmers (for athletes older than 18 years) provided both verbal and written consent before participating in this investigation.

Measurement of individual characteristics

Body fat percentage was measured using a skin caliper (model number: E-RA_k3; Nakamura Mfg. Co., Ltd) and skinfold valid equation of Jackson and Pollock for men (triceps, abdomen and suprailiac) (Equation 1)⁽¹⁵⁾. The subjects' height was measured by wall-mounted stadiometer of Seca (Seca 206) with an error margin of 1 mm. Subjects' mass was measured by the digital scale of Beurer with the precision coefficient of 100 g made in Germany. BMI was calculated by dividing body mass by squared height (kg/m²). Aerobic power (VO_{2max}) was estimated by the exhaustion test of Bruce (running on a treadmill, Jog Now 700 Unity Treadmill; Technogym) through indirect way. In addition, anaerobic power was measured by the running-based anaerobic sprint test, using its reliable Equations 1, and 2⁽¹⁶⁾. Participants measured and recorded their resting heart rate accurately before leaving the bed for 3 consecutive days. Carotid artery pulse of 30 s, multiplied by 2, and its mean values were recorded. Heart rate of the training session was immediately measured after recording by the swimmers from the carotid artery and by a Polar watch (Beurer PM 90 Model; Beurer)⁽¹⁶⁾.

$$\begin{aligned} \text{Body fat \%} &= 0.39287 \times (\text{sum of three skinfolds}) \\ &- 0.00105 \times (\text{sum of three skinfolds})^2 \\ &+ 0.15772 \times (\text{age}) - 5.18845. \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Aerobic power} &= 14.76 - 1.379 \times (\text{time}) + 0.451 \times (\text{time}^2) \\ &- 0.012 \times (\text{time}^3) \end{aligned}$$

$$\text{Anaerobic power} = \text{mass} \times \text{distance}^2 \div \text{time}^3. \quad (2)$$

Supplementation protocol

Data from human and animal studies provide good evidence that exogenous CoQ₁₀ may be taken up by peripheral tissues, then slowly redistributes into plasma for at least 2 weeks depending upon the length of the dosing period. In addition, the most suitable dosage is 300 mg/d in a single dose for raising the base level in human subjects owing to its 33-h half-life, hydrophobic nature and high molecular mass⁽¹⁷⁾. Therefore, according to the previous studies and similar to the administration of Leelarungrayub *et al.*⁽¹⁸⁾, swimmers received daily 300 mg of CoQ₁₀ (2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone) with a trans-structure in the form of a gel manufactured by America (Nature's Bounty) for 14 d. During

this period, control and pre-cooling groups took daily 300 mg of placebo capsules (lactose), which were similar to CoQ₁₀ in terms of flavour, colour and size.

Dietary control

The mean food intake was obtained using a 24-h dietary recall questionnaire. All subjects were asked to mention all food and drink they had in the past 24 h. This questionnaire was completed for each subject in six non-consecutive times during 2 weeks (three times a week). Obtained values were converted into grams using the Guideline of Household Scales. Then, each food was coded according to the Guideline of Food Processor Software and was analysed in terms of quantity and energy contribution⁽¹⁹⁾. The mean values and standard deviations of daily macronutrient and micronutrient intake are presented in Table 2.

Pre-cooling administration

According to the studies on the track and field and cycling⁽²⁰⁾, swimmers were pre-cooled before the start of the main trainings in each session and the second recording bout between the warm-up inside and outside the water. Subjects entered cold water pool at a temperature of 18 ± 0.5°C from the lower limbs to shoulder girdle for 15 min⁽²¹⁾. To prevent cold shocks, this was done after taking a shower (gradual reduction of water temperature)⁽²²⁾. Also, in order to prevent dehydration and maintain body core temperature at an optimal level and improve blood flow to active muscles, during every training session the swimmers consumed 500 ml of Powerade (hypotonic sportive drink with 6–8% carbohydrate) and 1 liter of water chilled to the temperature of 5 ± 1°C⁽²³⁾.

Protocol of training and recording

Training protocol with a 2-week heavy and regular swimming training contract (9 sessions/week) of training programme was designed by coaches and researchers for the competition stage (from four stages of swimming trainings). Training variables (volume, intensity and frequency) were kept constant every week and the training distance for every session was considered to be 5 km. Performances of the swimmers were measured by recording freestyle swimming at 800, 200 and 50 m distances, respectively, with the maximum effort and heart rate control following studies and similar to the swim matches with a 10- to 15-min active rest between the records⁽¹⁸⁾. Training sessions were conducted in two shifts, one in the morning and one in the evening, without also working with bodybuilding. Indoor swimming pool of 25 × 50 m with the depth of 4 m and water temperature of 27 ± 1°C (or 80 ± 2°F) was selected according to the rules of the Federation International Natation Amateur (FINA). Ambient pool temperature was 33 ± 1°C with a the relative humidity of 60–70%. Swimming speed was controlled through counting stroke rate (SR) and stroke length (SL) by a digital chronometer and a Polar watch. Before the main training, swimmers performed a warm-up out of the water (general and dynamic) and warm-up inside the water. In addition, after the main training a cool-down inside water was performed for



15–20 min (a total of 45–60 min). Intensity of the trainings was set according to the Karvonen formula or target heart rate (THR) based on Equation 3. Main trainings were practised for 2 h using different drills of four styles swimming in the intervals and repetitive sets, focused on the speed (Sp1–3) and endurance (En1–3) swim⁽²⁴⁾.

Sp1 (80–90% THR, 100–200 m), Sp2 (90–100% THR, 50–100 m) and Sp3 (100% THR, 12.5–50 m).

En1 (50–60% THR, 800–1500 m), En2 (60–70% THR, 400–800 m) and En3 (70–80% THR, 200–400 m).

$$\text{HRmax} = 220 - \text{age}$$

$$\text{THR} = \% \text{ of target intensity} \times \text{HRR} + \text{HRrest}$$

$$\text{Heart rate reserve (HRR)} = \text{HRmax} - \text{HRrest}$$

$$V = \text{SL} \times \text{SR}. \quad (3)$$

Preparation of blood sampling

Blood sampling was carried out 24 h before (stages 1 and 2) and after (stages 1 and 2) supplementation of CoQ₁₀ and pre-cooling strategy (the highest level of effectiveness of CoQ₁₀ in blood) in four stages⁽¹⁷⁾. At both time points, approximately 5 ml of blood was sampled from an antecubital vein of right arms of the subjects by nursing expert before noon (from 08.00 to 11.00 hours). Then, 2 ml of it was poured and thoroughly stirred in test tubes with anticoagulant substances (K₂EDTA) for blood cell count. The remaining blood (3 ml) was immediately transferred into gel tubes specific for serum without addition of an anticoagulant material for serum preparation and was centrifuged at 1500 g for 10 min at 4°C in a Beckman (J2-21) centrifuge (Beckman Coulter). Plasma samples were immediately frozen and stored at –80°C until analysis. Participants avoided any physical activity 24 h before blood sampling and recordings. Biochemical analysis of blood samples was conducted in equipped biochemistry laboratory of veterinary faculty (Department of Clinical Pathology, University of Urmia, Urmia, Iran). Also, laboratory equipment was evaluated 3 months before starting study.

Measurement of blood indicators

Serum isoenzymes of CK-MB and cTnI were measured by chemiluminescence: bioMérieux launches VIDAS[®] Troponin I Ultra kit is made in France and the MB kit Audit Diagnostics facility in Cork, Ireland. Serum Mb concentration was determined by using a commercial kit (Eiken Chemical Co.). Serum LPO concentration was determined by using a commercial kit (Kyowa Medex Co.). Serum enzyme of LD (with sensitivity 5 IU/l) and TAC were measured according to the biochemical kit of Biorex Company of England (Biorex, Co.). The concentration of serum CoQ₁₀ was estimated by summing the contents of CoQ₁₀ in reduced form (ubiquinol) and oxidised form (ubiquinone), each measured through HPLC. The method used for such determination was explained in the protocol of Graves *et al.*⁽²⁵⁾ with trivial modifications. Blood cell count was carried out using American Cell Counter of Mindray (BC-3000 plus).

Statistical analysis

Statistical analysis was performed using SPSS statistics version 22.0 and excel 2013 for Windows software (SPSS, Inc.). The distribution of the data was evaluated by the Kolmogorov–Smirnov test. Owing to normal distribution of study variables, univariable ANCOVA and also repeated measurement ANOVA tests in the general linear model were used to find significant differences among and within groups, respectively. Comparison of significant interactions and main effects for all statistical tests was performed using Bonferroni's *post hoc* test. The data were expressed as mean values and standard deviations. Differences were considered statistically significant at the $P < 0.05$ level.

Results

ANOVA indices levels of CK-MB, cTnI, Mb, LD, TAC, LPO and CoQ₁₀ in the pre-blood sampling (stages 1 and 2) showed no significant statistical difference among the groups ($P > 0.05$). In the post-blood sampling (stages 1 and 2), a significant statistical difference was observed among the groups for levels of CK-MB, cTnI, Mb, LD, TAC, LPO and CoQ₁₀ indicators ($P < 0.05$). So that, levels of CK-MB, cTnI, Mb, LD and LPO in supplementation and also supplementation with pre-cooling groups a significant decrease were observed compared with both the pre-cooling and control groups respectively; although, a significant increase were seen for TAC and CoQ₁₀ levels. Also, a significant difference was observed within the groups for levels of CK-MB, cTnI, Mb, LD, TAC, LPO and CoQ₁₀ indicators ($P < 0.05$). So that, levels of CK-MB, cTnI, Mb, LD and LPO in pre-cooling and also control groups, an increase were observed compared with both the supplementation and supplementation with pre-cooling groups; although, a decrease were seen for TAC and CoQ₁₀ levels. Details of significant differences of the indices are shown in Figs 2–8. No statistically significant differences for any measured variable of the dietary and the physical characteristic of the subjects were seen among the groups ($P > 0.05$). Details of their results are shown in Tables 1 and 2, respectively. It is noteworthy that health symptoms or side effects such as vomiting, headaches and skin rash and reduction in blood pressure and heart rate during the study were inquired upon daily by telephone. Participants tolerated the oral administration of CoQ₁₀ well, and there were no negative effects reported. In addition, the initial results of complete blood count testing and urinalysis showed normal levels.

Discussion

The aim of this study was to determine whether short-term oral CoQ₁₀ supplementation and pre-cooling strategy would affect the oxidative stress, cardiac damage markers, TAC and CoQ₁₀ concentration following swimming competition phase in a hot and humid environment. According to the previous studies, hyperthermia and transient hypoxia during prolonged and strenuous exercises lead to the increased oxidative stress (driving CVD progression), activation of anaerobic glycolysis enzymes, imbalance between pro-oxidant and antioxidant



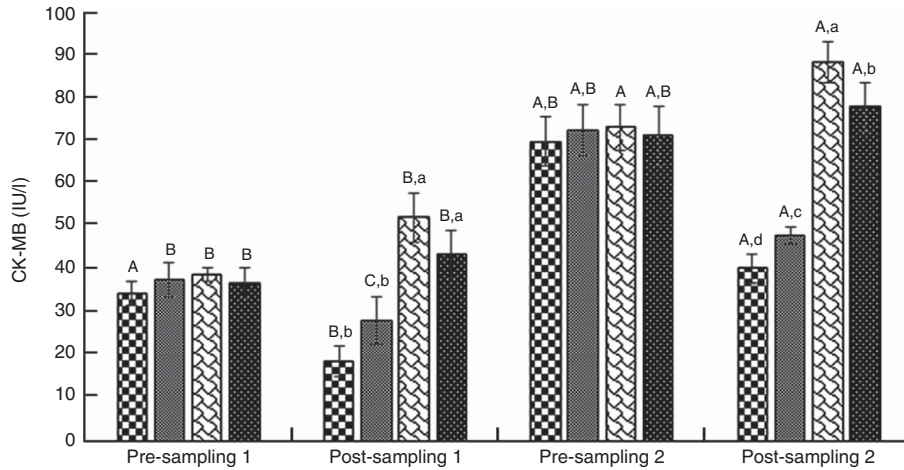


Fig. 2. Creatine kinase-MB (CK-MB). Values are means (n 9) and standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c,d} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. ▨, CoQ₁₀; ▩, CoQ₁₀+pre-cooling; ▧, pre-cooling; ▦, control.

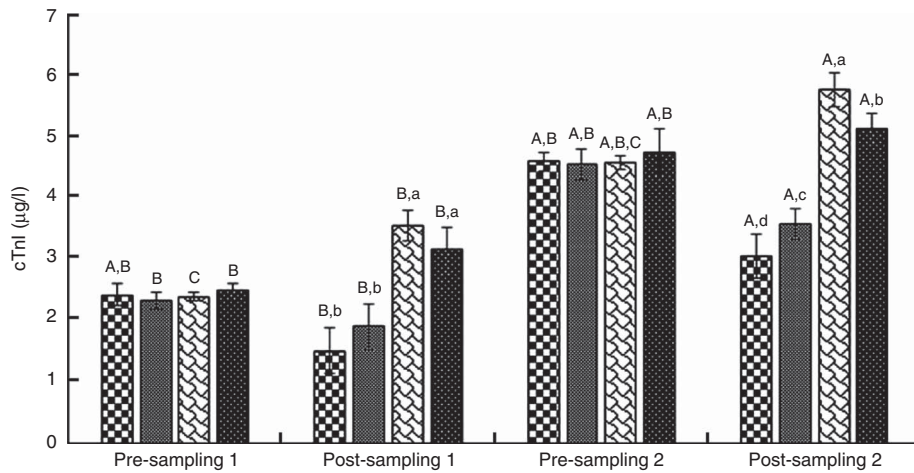


Fig. 3. Troponin I (cTnI). Values are means (n 9) and standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c,d} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. ▨, CoQ₁₀; ▩, CoQ₁₀+pre-cooling; ▧, pre-cooling; ▦, control.

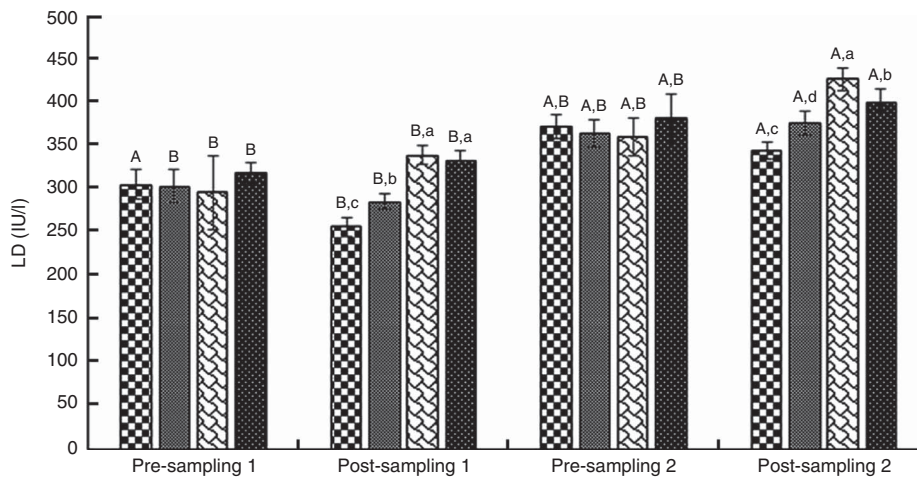


Fig. 4. Lactate dehydrogenase (LD). Values are means (n 9) and standard deviations represented by vertical bars. ^{A,B} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c,d} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. ▨, CoQ₁₀; ▩, CoQ₁₀+pre-cooling; ▧, pre-cooling; ▦, control.

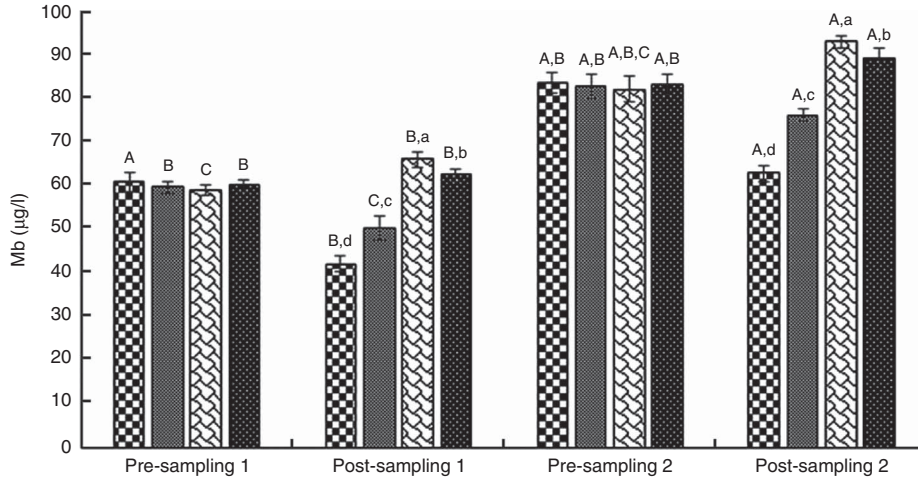


Fig. 5. Myoglobin (Mb). Values are means (*n* 9) and standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c,d} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. CoQ₁₀; CoQ₁₀+pre-cooling; pre-cooling; control.

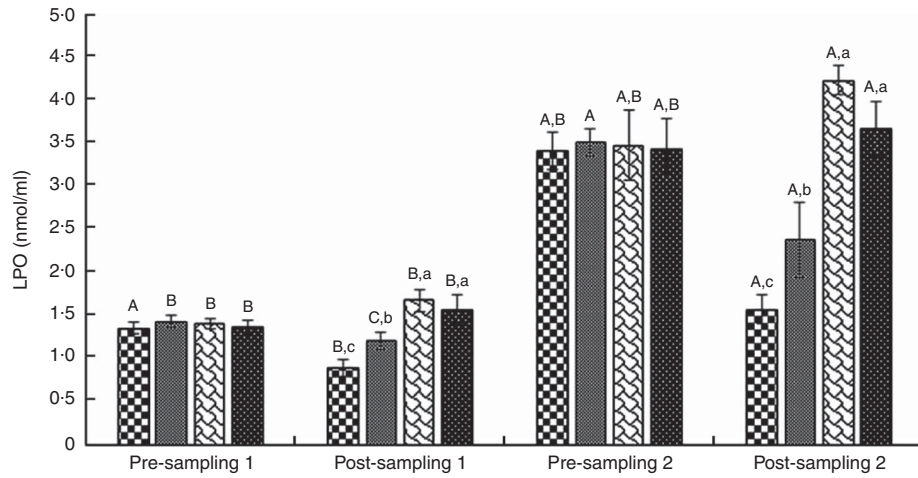


Fig. 6. Lipid peroxidation (LPO). Values are means (*n* 9) and standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. CoQ₁₀; CoQ₁₀+pre-cooling; pre-cooling; control.

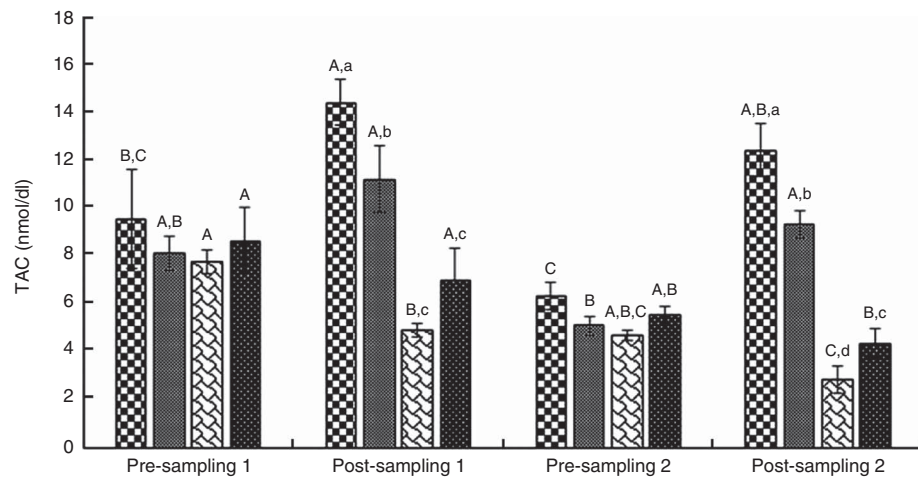


Fig. 7. Total antioxidant capacity (TAC). Values are means (*n* 9) and standard deviations represented by vertical bars. ^{A,B,C} Mean values with unlike letters were significantly different within the group measurement ($P < 0.05$). ^{a,b,c,d} Mean values with unlike letters were significantly different among the groups ($P < 0.05$). CoQ₁₀, coenzyme Q₁₀. CoQ₁₀; CoQ₁₀+pre-cooling; pre-cooling; control.

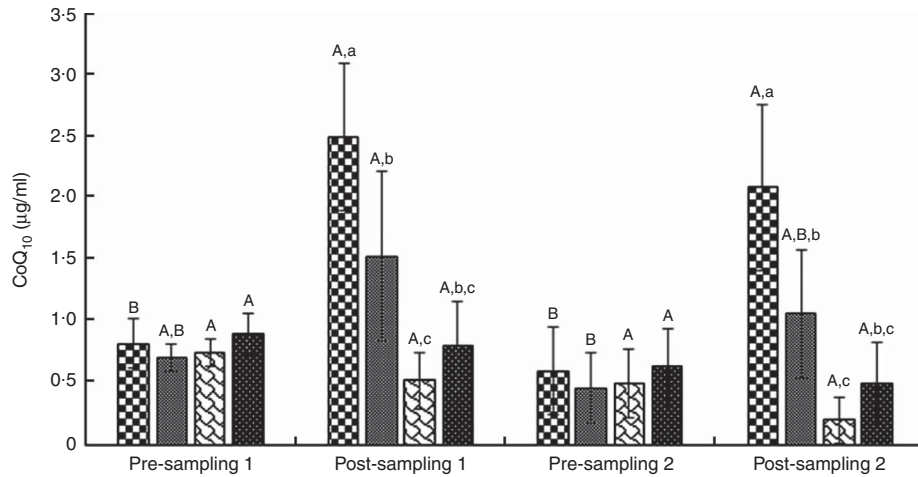


Fig. 8. Coenzyme Q₁₀ concentration (CoQ₁₀). Values are means (*n* 9) and standard deviations represented by vertical bars. ^{A,B} Mean values with unlike letters were significantly different within the group measurement (*P* < 0.05). ^{a,b,c} Mean values with unlike letters were significantly different among the groups (*P* < 0.05). CoQ₁₀, coenzyme Q₁₀; , CoQ₁₀; , CoQ₁₀ + pre-cooling; , pre-cooling; , control.

Table 1. Participants' food and energy amount received (Mean values and standard deviations; *n* 9)

Group variables	CoQ ₁₀		CoQ ₁₀ + pre-cooling		Pre-cooling		Control		<i>P</i>
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Carbohydrate (g/d)	436.80	8.95	435.50	21.20	437.40	22.51	428.00	7.56	0.75
Protein (g/d)	92.20	2.16	93.00	1.26	93.60	2.07	91.33	4.32	0.56
Fat (g/d)	82.40	1.14	83.83	3.37	82.00	2.12	83.16	2.48	0.62
Fibre (g/d)	34.20	2.58	37.66	3.32	34.40	2.96	36.83	3.12	0.18
Cholesterol (mg/d)	173.60	7.02	176.00	5.86	170.00	4.24	176.83	6.55	0.28
Ca (mg/d)	1018.40	32.79	1046.66	58.43	1025.00	24.49	1036.66	16.63	0.61
Vitamin C (mg/d)	104.00	2.54	105.50	2.25	104.80	1.48	106.66	3.77	0.43
Vitamin E (mg/d)	15.40	2.07	16.83	1.94	16.00	1.00	15.16	2.31	0.47
Vitamin B ₆ (mg/d)	2.40	1.14	3.50	0.54	2.20	0.83	2.83	1.16	0.14
Vitamin B ₁₂ (µg/d)	3.40	0.89	3.83	0.75	3.40	0.54	3.83	1.16	0.72
Se (µg/d)	72.60	1.67	74.16	2.13	73.40	1.51	74.50	1.51	0.31
Zn (mg/d)	12.20	0.83	12.50	1.04	13.00	1.00	12.33	0.81	0.55
Fe (mg/d)	11.20	1.48	11.83	1.47	10.60	0.89	12.33	1.75	0.25
Carbohydrate (kJ/d)	7310.28	149.87	7288.53	354.80	7320.33	376.73	7163.01	126.57	0.75
Carbohydrate (kcal/d)	1747.20	35.82	1742.00	84.80	1749.60	90.04	1712.00	30.25	0.75
Protein (kJ/d)	1543.06	36.26	1556.45	21.13	1566.49	34.69	1528.54	72.30	0.56
Protein (kcal/d)	368.80	8.67	372.00	5.05	374.40	8.29	365.33	17.28	0.56
Fat (kJ/d)	3102.85	42.93	3156.83	126.94	3087.79	79.87	3131.72	93.47	0.62
Fat (kcal/d)	741.60	10.26	754.50	30.34	738.00	19.09	748.50	22.34	0.62
Total energy intake (kJ/d)	11 956.20	178.82	12 001.80	364.38	11 974.61	347.73	11 823.28	177.74	0.70
Total energy intake (kcal/d)	2857.60	42.74	2868.50	87.09	2862.00	83.11	2825.83	42.48	0.70
Total energy consumption (kJ/d)	12 912.66	180.54	12 754.21	275.73	12 993.83	361.12	12 821.87	169.74	0.45
Total energy consumption (kcal/d)	3086.20	43.15	3048.33	65.90	3105.60	86.31	3064.50	40.57	0.45

CoQ₁₀, coenzyme Q₁₀.

response and the release of catecholamine and metabolic stress in the liver and intestine⁽²⁾. Finally, reactive oxygen and nitrogen species are triggered under the conditions of heavy skeletal muscle contraction, hypoxia or ischaemia⁽⁷⁾. These states show themselves with increasing levels of cardiac enzymes of CK-MB, LD and troponin⁽⁹⁾.

Recent literature indicates that ROS production facilitates normal cardiac function through the activation of ryanodine receptor 1 (RyR1), receptors inducing Ca²⁺ release⁽²⁶⁾. However, ROS production at high levels can lead to RyR1 malfunction and aberrant calcium release, inducing contractile dysfunction, arrhythmia and myopathy with further upstream molecular targets

being protein kinase A and calcium calmodulin kinase⁽²⁶⁾. In agreement with the present results, König *et al.*⁽²⁷⁾ reported CK, CK-MB and Mb as myocardial stress markers that significantly increased after the fourth stage of a 5-d stage of cycling race, but the increase was far lower than the marathons and half-marathons. Clarkson *et al.*⁽²⁸⁾ reported a significant increase in the markers of muscle damage (LD, CK and Mb) after eccentric exercise and sport competition in healthy individuals. In addition, Shave *et al.*⁽⁸⁾ concluded that the physiologic stress imposed during and after half-ironman triathlon in a reduced left ventricular contractility altered diastolic filling, coupled with minimal cardiac damage (CK and CK-MB) in a number of highly trained

Table 2. Participants' physical characteristics (Mean values and standard deviations)

Group variables	CoQ ₁₀ (n 9)		CoQ ₁₀ +pre-cooling (n 9)		Pre-cooling (n 9)		Control (n 9)		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (years)	17.60	1.14	17.40	1.14	17.20	1.30	17.71	1.11	0.88
Height (cm)	177.20	1.92	173.20	4.65	179.20	5.89	175.28	3.19	0.15
Mass (kg)	72.11	3.08	69.44	8.54	71.76	7.23	69.12	5.76	0.80
Body fat (%)	13.54	2.30	13.46	1.67	13.68	2.35	13.82	1.14	0.93
BMI (kg/m ²)	22.97	1.16	23.15	2.15	22.34	1.41	22.50	2.07	0.68
VO _{2max} (ml/kg per min)	55.43	2.29	53.51	3.78	54.77	2.90	54.13	3.37	0.75
Max power (W)	455.3	22.3	464.1	16.9	471.1	25.5	473.4	32.1	0.53

CoQ₁₀, coenzyme Q₁₀.

male triathletes. However, they did not report a significant change of cTnT. Inconsistency of present results is probably owing to the fact that the changed concentration of cardiac damage markers is proportional to the numerous compounding variables (e.g. age, training status, absolute exercise intensity and environmental conditions)⁽²⁸⁾.

Oral administration of CoQ₁₀ plays a pro-oxidant role during the increase of O₂^{•-}, turning it into H₂O₂ at the presence of superoxide dismutase enzyme in complex I and III of the respiratory chain⁽¹³⁾. It also plays an important role in the oxidative phosphorylation by transferring electron into complex III. In the presence of antioxidant enzymes (catalase and glutathione peroxidase), H₂O₂ is transformed into H₂O as a result of which ATP is produced; otherwise, it turns into OH[•]⁽¹³⁾. Also, CoQ₁₀ appears to increase ATP levels by preventing the loss of adenine nucleotide pool from cardiac cells⁽²⁹⁾. Additionally, CoQ₁₀ has evidenced activity in preventing lipid peroxidation as an antioxidant, and as an indirect stabiliser of calcium channels to decrease overload or imbalance intracellular calcium⁽³⁰⁾.

Results of this study showed that CoQ₁₀ supplementation alone and also combined with pre-cooling leads to a significant decrease in serum levels of CK-MB, cTnI, Mb, LD and LPO markers, and increased levels of TAC and CoQ₁₀ compared with the other groups in the post-sampling (stages 1 and 2) ($P < 0.05$). In this regard, Kon *et al.*⁽³¹⁾ demonstrated that after supplementation of 300 mg/d CoQ₁₀ for 20 d in elite Kendo athletes during training camp, serum LPO concentration did not change in either group, although LPO in the CoQ₁₀ group was lower than in the placebo group. Therefore, the effect of CoQ₁₀ supplementation on exercise-induced oxidative stress in athletes was unclear in their study. Also, it showed that CoQ₁₀ supplementation increased serum CoQ₁₀ concentration and reduced exercise-induced muscular serum CK activity and Mb concentration in the athletes⁽³¹⁾. Possible causes of these contradictory results may be old age of the subjects and the type of sport contract.

In a recent study⁽¹²⁾, it was reported that CoQ₁₀ supplementation is efficient in reducing the degree of oxidative stress for amateur male athletes during high-intensity exercise bouts (50 km running), leading to the maintenance of cell integrity. In addition, they found a significant increase in the total antioxidant status in both CoQ₁₀ groups compared with the placebo group⁽¹²⁾. Agreeing with the results of this study, Leelarungrayub *et al.*⁽¹⁸⁾ suggested that the 12 d of CoQ₁₀ supplementation reduces oxidative stress and improves TAC (including proteins and enzymatic

and non-enzymatic antioxidant compounds) level and CoQ₁₀ concentration, within a sample of young swimmers⁽¹⁸⁾. Altogether, the results of the study by Sohal *et al.*⁽³²⁾ indicated that, contrary to the present results, supplemental intake of CoQ₁₀ has no discernable effect on the main antioxidant defenses or pro-oxidant generation in most tissues of mice⁽³²⁾. Bloomer *et al.*⁽³³⁾ indicated that in physically active men and women, 30 d of CoQ₁₀ supplementation (300 mg/d) increased the reduced serum CoQ₁₀, but that increase did not affect exercise performance and resting or exercise-induced measures of oxidative stress. In addition, Östman *et al.*⁽³⁴⁾ demonstrated that supplementation of 90 mg/d CoQ₁₀ for 8 weeks in moderately trained men did not affect oxidative stress indices.

In contrast, Gul *et al.*⁽³⁵⁾ showed that supplementation of 100 mg/d CoQ₁₀ for 8 weeks decreased lipid peroxidation immediately after the repeated bouts of supramaximal exercises in sedentary men. Different results of these studies are most likely for different types, dosages and time frames of antioxidant(s) treatment, sampled tissues, exercise protocol used to induce oxidative stress, measurement time, used assays and recruited (trained *v.* untrained, old *v.* young and healthy *v.* diseased) test subjects among other variables⁽³³⁾. These results may support the results of Taiwanese group (2011), suggesting that CoQ₁₀ acts as an inflammatory mediator in the activation of PKC, NADP⁺, oxidised LDL and dependent on the inhibitory and effective function of AMP-activated protein kinase (AMPK). Activation of AMPK function by activating a signalling pathway of Akt/eNOS/NO and stabilising energy protects the cells against oxidative damage to maintain endothelial function in hypoxia and heat stress (or ATP depletion) conditions. Although there is no study on the relationship between CoQ₁₀ and AMPK, they showed a correlation between CoQ₁₀ and AMPK⁽³⁶⁾.

The present results support the findings of Zheng and Moritani⁽³⁷⁾ because, as they reported, when CoQ₁₀ increases 2, 3-diphosphoglycerate levels in erythrocyte cells, H₂O₂'s decomposition curve diverts to the right and increases oxygen transfer in a certain pressure (PO₂). As a result, not only the increase of oxygen in the skeletal muscles but also in the heart and respiratory muscles may increase ATP synthesis and reduce lactate production, possibly leading to the increase of VO_{2max}⁽³⁷⁾. According to some studies, CoQ₁₀ has been shown to reduce neuronal cell death by activating the PI3K pathway in neurons, because the PI3K pathway mediates the movement of glucose into the cells, which is also activated by insulin. In

addition, it is a key step in oxidative phosphorylation in the inner mitochondrial membrane. It is possible that the increased response to occlusion in the subjects after CoQ₁₀ administration is linked to activation of the PI3K signalling pathway, which directly increases tissue blood flow⁽³⁰⁾. This mechanism is possibly owing to a significant decrease in serum levels of CK-MB, cTnI, Mb, LD and LPO indices in the supplementation and also supplementation with pre-cooling groups compared with the pre-cooling and control groups. It is noteworthy that Mb performance is dependent on the blood flow, because it is now known not only as an oxygen storage protein to mediate facilitated diffusion *in vivo* and an intracellular catalyst but it has also been described to be a regulating mitochondrial function through its •NO scavenger⁽³⁸⁾.

CoQ₁₀ is a necessary cofactor for the lack of coupling proteins in regulating body temperature, accelerating the biological and biochemical interactions' trends and affecting the temperature of muscle contraction⁽³⁹⁾. Interest in pre-cooling has increased for more than three decades among the athletes using various ways, including exposure to cold weather, use of cooling jackets, immersion in cold water, ice or cool drinks⁽²³⁾. In this respect, Hue *et al.*⁽⁴⁰⁾ proposed that during cooling, heart rate, body core temperature and skin temperature significantly decrease by having cold drinks while practicing long swimming especially in the evening sessions. Marsh and Sleivert⁽⁴¹⁾ reported that pre-cooling increases blood flow to active muscles through skin vasoconstriction in high-intensity cycling performances. As a result of an increase in the blood flow, the muscle can improve its aerobic metabolism and energy production. Displacement of lactate acid for oxidation prevents from its accumulation and pH drop and delays fatigue⁽⁴¹⁾. Muscle pH from the rest amount (7.1) was reduced during high-intensity trainings and speed swimming, especially at 200 m speed. Then, the performance of some pH-sensitive enzymes such as phosphofructokinase was inhibited, reducing ATP production and increasing the incidence of EICF⁽²⁴⁾.

Results of present study showed that serum levels of CK-MB, cTnI, Mb, LD and LPO increased, and TAC and CoQ₁₀ decreased significantly in the pre-cooling group compared with the other groups in the post-sampling (stages 1 and 2) ($P < 0.05$). This inconsistency with the results of other studies may relate to the differences in the type of sport contract and pre-cooling (immersion in the water at $18 \pm 0.5^\circ\text{C}$) method or low temperature of pool water ($27 \pm 1^\circ\text{C}$) compared with the high environmental temperature during their study⁽²⁰⁾. Moreover, the consequences such as exposure to cold caused likely contraction vibrations by sympathetic neural system, increased consumption of muscle glycogen, increased blood pressure and heart rate (tachycardia), rapid and unbalanced breathing (reduction of cardiac output and available oxygen to the tissues and acidosis conditions), increased metabolic stress hormone and TAC decline (due to resistance against free radicals and maintaining cell homeostasis). In addition, coronary blood flow was also reduced and later on resulted in an altered basophil activity in the myocardium⁽⁴²⁾. Overall, indicators of the supplementation group were significantly lower, except for TAC and CoQ₁₀ levels. On the other hand, increased plasma CoQ₁₀ level was likely to lead to the increased oxidative phosphorylation, acceleration of the electron transfer from the flavoproteins into cytochromes, less

dependence on anaerobic glycolysis pathway, increase of NEFA burns and eventually less lactate acid accumulation with a potential to reduce swimming-induced muscular and myocardial cell damage^(13,24). In accordance with the present results, Booth *et al.*⁽²²⁾ reported no effect of pre-cooling on plasma lactate acid, VO_{2max} and other physiological factors in the production of muscle energy during endurance cycling at 35°C and the relative humidity of 50%. Accordingly, if it had some effects on the physical performance, it would probably play a role only by reducing body core temperature and pressure on the cardiovascular system⁽²²⁾.

Conclusions

Our results showed that CoQ₁₀ supplementation reduced serum levels of myocardial damage markers and increased TAC and CoQ₁₀ concentration during heavy trainings and recording bouts, preventing from the adverse consequences of pre-cooling in elite adolescent swimmers. As an antioxidant and cell membrane stabiliser, body core temperature regulator and essential cofactor in mitochondrial oxidative phosphorylation, CoQ₁₀ can inhibit the expression of free radicals and reduce swimming-induced oxidative stress. Pre-cooling strategy was not effective in eliminating or reducing myocardial damage markers and oxidative stress and increasing TAC and CoQ₁₀ concentration. Presumably, pre-cooling decreased the body core temperature, increased time to exhaustion and reduced the pro-inflammatory cytokines. However, further research is needed in this area.

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References

1. Wendt D, van Loon LJ & Lichtenbelt WD (2007) Thermo-regulation during exercise in the heat: strategies for maintaining health and performance. *Sports Med* **37**, 669–682.
2. Hall DM, Buettner GR, Oberley LW, *et al.* (2001) Mechanisms of circulatory and intestinal barrier dysfunction during whole



- body hyperthermia. *Am J Physiol Heart Circ Physiol* **280**, H509–H521.
3. Bongers CC, Thijssen DH, Veltmeijer MT, *et al.* (2014) Pre-cooling and percooling (cooling during exercise) both improve performance in the heat: a meta-analytical review. *Br J Sports Med* **49**, 377–384.
 4. Jones PR, Barton C, Morrissey D, *et al.* (2012) Pre-cooling for endurance exercise performance in the heat: a systematic review. *BMC Med* **10**, 166.
 5. Hasegawa H, Takatori T, Komura T, *et al.* (2006) Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. *J Sports Sci* **24**, 3–9.
 6. Rahal A, Kumar A, Singh V, *et al.* (2014) Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed Res Int* **2014**, 761264.
 7. Burgoyne JR, Mongue-Din H, Eaton P, *et al.* (2012) Redox signaling in cardiac physiology and pathology. *Circ Res* **111**, 1091–1106.
 8. Shave R, Dawson E, Whyte G, *et al.* (2004) Altered cardiac function and minimal cardiac damage during prolonged exercise. *Med Sci Sports Exerc* **36**, 1098–1103.
 9. Kemp M, Donovan J, Higham H, *et al.* (2004) Biochemical markers of myocardial injury. *Br J Anaesth* **93**, 63–73.
 10. Tauler P, Ferrer MD, Romaguera D, *et al.* (2008) Antioxidant response and oxidative damage induced by a swimming session: influence of gender. *J Sports Sci* **26**, 1303–1311.
 11. Santos-Silva A, Rebelo MI, Castro EM, *et al.* (2001) Leukocyte activation, erythrocyte damage, lipid profile and oxidative stress imposed by high competition physical exercise in adolescents. *Clin Chim Acta* **306**, 119–126.
 12. Díaz-Castro J, Guisado R, Kajarabille N, *et al.* (2012) Coenzyme Q₁₀ supplementation ameliorates inflammatory signaling and oxidative stress associated with strenuous exercise. *Eur J Nutr* **51**, 791–799.
 13. Borekova M, Hojerova J, Koprda V, *et al.* (2008) Nourishing and health benefits of coenzyme Q₁₀ – a review. *Czech J Food Sci* **26**, 229–241.
 14. Faul F, Erdfelder E, Buchner A, *et al.* (2009) Statistical power analyses using G* Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* **41**, 1149–1160.
 15. Swisher AK, Yeater R, Moffett K, *et al.* (2003) A comparison of methods to determine body fat in individuals with cystic fibrosis: a pilot study. *J Exerc Physiol Online* **6**, 105–113.
 16. Heyward VH & Gibson A (2014) *Advanced Fitness Assessment and Exercise Prescription*, 7th ed. Champaign, IL: Human Kinetics.
 17. Miles MV (2007) The uptake and distribution of coenzyme Q(10). *Mitochondrion* **7**, S72–S77.
 18. Leelarungrayub D, Sawattikanon N, Klaphajone J, *et al.* (2010) Coenzyme Q₁₀ supplementation decreases oxidative stress and improves physical performance in young swimmers: a pilot study. *Open Sports Med J* **4**, 1–8.
 19. Mahan LK, Escott-Stump S & Raymond JL (2012) *Krause's Food & The Nutrition Care Process*. Philadelphia, PA: Elsevier Health Sciences.
 20. Tyler CJ, Sunderland C & Cheung SS (2015) The effect of cooling prior to and during exercise on exercise performance and capacity in the heat: a meta-analysis. *Br J Sports Med* **49**, 7–13.
 21. Arngrimsson SA, Pettitt DS, Stueck MG, *et al.* (2004) Cooling vest worn during active warm-up improves 5-km run performance in the heat. *J Appl Physiol* (1985) **96**, 1867–1874.
 22. Booth J, Wilsmore B, Macdonald A, *et al.* (2001) Whole-body pre-cooling does not alter human muscle metabolism during sub-maximal exercise in the heat. *Eur J Appl Physiol* **84**, 587–590.
 23. Ross M, Abbiss C, Laursen P, *et al.* (2013) Precooling methods and their effects on athletic performance. *Sports Med* **43**, 207–225.
 24. Riewald S & Rodeo S (2015) *Science of Swimming Faster*. Champaign, IL: Human Kinetics.
 25. Graves S, Sikorska M, Borowy-Borowski H, *et al.* (1998) Analysis of coenzyme Q₁₀ content in human plasma and other biological samples. In *Free Radical and Antioxidant Protocols*, pp. 353–365 [D Armstrong, editor]. Totowa, NJ: Humana Press Inc.
 26. Sag CM, Köhler AC, Anderson ME, *et al.* (2011) CaMKII-dependent SR Ca leak contributes to doxorubicin-induced impaired Ca handling in isolated cardiac myocytes. *J Mol Cell Cardiol* **51**, 749–759.
 27. König D, Schumacher YO, Heinrich L, *et al.* (2003) Myocardial stress after competitive exercise in professional road cyclists. *Med Sci Sports Exerc* **35**, 1679–1683.
 28. Clarkson PM, Kearns AK, Rouzier P, *et al.* (2006) Serum creatine kinase levels and renal function measures in exertional muscle damage. *Med Sci Sports Exerc* **38**, 623–627.
 29. Bonakdar RA & Guarneri E (2005) Coenzyme Q₁₀. *Am Fam Physician* **72**, 1065–1070.
 30. Petrofsky J, Laymon M, Lee H, *et al.* (2012) The effect of coenzyme Q₁₀ on endothelial function in a young population. *Phys Ther Rehabil Sci* **1**, 6–12.
 31. Kon M, Tanabe K, Akimoto T, *et al.* (2008) Reducing exercise-induced muscular injury in kendo athletes with supplementation of coenzyme Q₁₀. *Br J Nutr* **100**, 903–909.
 32. Sohal RS, Kamzalov S, Sumien N, *et al.* (2006) Effect of coenzyme Q₁₀ intake on endogenous coenzyme Q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice. *Free Radic Biol Med* **40**, 480–487.
 33. Bloomer RJ, Canale RE, McCarthy CG, *et al.* (2012) Impact of oral ubiquinol on blood oxidative stress and exercise performance. *Oxid Med Cell Longev* **2012**, 465020.
 34. Östman B, Sjödin A, Michaëlsson K, *et al.* (2012) Coenzyme Q₁₀ supplementation and exercise-induced oxidative stress in humans. *Nutrition* **28**, 403–417.
 35. Gul I, Gokbel H, Belviranlı M, *et al.* (2011) Oxidative stress and antioxidant defense in plasma after repeated bouts of supramaximal exercise: the effect of coenzyme Q₁₀. *J Sports Med Phys Fitness* **51**, 305–312.
 36. Tsai KL, Chen LH, Chiou SH, *et al.* (2011) Coenzyme Q₁₀ suppresses oxLDL-induced endothelial oxidative injuries by the modulation of LOX-1-mediated ROS generation via the AMPK/PKC/NADPH oxidase signaling pathway. *Mol Nutr Food Res* **55**, Suppl. 2, S227–S240.
 37. Zheng A & Moritani T (2008) Influence of CoQ₁₀ on autonomic nervous activity and energy metabolism during exercise in healthy subjects. *J Nutr Sci Vitaminol (Tokyo)* **54**, 286–290.
 38. Kamga C, Krishnamurthy S & Shiva S (2012) Myoglobin and mitochondria: a relationship bound by oxygen and nitric oxide. *Nitric Oxide* **26**, 251–258.
 39. Echtay KS, Winkler E, Frischmuth K, *et al.* (2001) Uncoupling proteins 2 and 3 are highly active H⁺ transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). *Proc Natl Acad Sci* **98**, 1416–1421.
 40. Hue O, Monjo R, Lazzaro M, *et al.* (2013) The effect of time of day on cold water ingestion by high-level swimmers in a tropical climate. *Int J Sports Physiol Perform* **8**, 442–451.
 41. Marsh D & Sleivert G (1999) Effect of precooling on high intensity cycling performance. *Br J Sports Med* **33**, 393–397.
 42. Alva N, Palomeque J & Carbonell T (2013) Oxidative stress and antioxidant activity in hypothermia and rewarming: can RONS modulate the beneficial effects of therapeutic hypothermia? *Oxid Med Cell Longev* **2013**, 957054.

