Effect of Short-term Coenzyme Q_{10} Supplementation and Precooling on Serum Endogenous Antioxidant Enzymes of Elite Swimmers

Ali Emami,[1] Asghar Tofighi,[1] Siamak Asri-Rezaei,[2] and Behnaz Bazargani-Gilani[3]

[1]*Department of Sport Physiology, Physical Education and Sport Sciences Faculty, University of Urmia, Urmia, Iran;* [2]*Department of Clinical Pathology, Veterinary Medicine Faculty, University of Urmia, Urmia, Iran; and* [3]*Department of Food Hygiene and Quality Control, Veterinary Science Faculty, University of Bu-Ali Sina, Hamedan, Iran*

Abstract

Emami, A, Tofighi, A, Asri-Rezaei, S, and Bazargani-Gilani, B. Effect of short-term coenzyme Q_{10} supplementation and precooling on serum endogenous antioxidant enzymes of elite swimmers. *J Strength Cond Res* XX(X): 000–000, 2017–This study aimed to investigate the effect of the use of a 2-week precooling strategy and supplementation coenzyme Q_{10} (CoQ_{10}) on superoxide dismutase (SOD), catalase (CAT), and serum glutathione peroxidase (GPx) in elite, adolescent swimmers during heavy and regular trainings and recording of freestyle swimming. Thirty-six healthy males (mean ± *SD*; age: 17.5 ± 1.1 years, body fat content: 14.55 ± 1.75%) were randomly selected and divided into 4 groups of CoQ_{10} (300 $mg \cdot d^{-1}$), precooling (immersion in the water at 18 ± 0.5° C), supplementation with precooling, and control, each with 9 participants. During an 18-session protocol in the morning and evening, participants attended speed and endurance trainings for 5 km every session. A 3-stage blood sampling was conducted before the first recording and before and after the second recording in 800, 200, and 50 m. Repeated measurement and the Bonferroni correction were used for the statistical analyses of the data ($\alpha = 0.05$). According to the results, there was no significant difference between the mean serum level of SOD, CAT, and GPx in the groups at the first stage of blood sampling ($p > 0.05$). At the third stage, a significant difference was observed among all groups ($p < 0.05$). At the second stage, precooling and control groups show a significant increase compared with the supplementation and supplementation with precooling groups ($p < 0.05$). As an antioxidant essential for adenosine triphosphate synthesis, CoQ_{10} supplementation prevented adverse changes of antioxidant enzymes during heavy trainings and swimming recording and decreased the serum level, while precooling individually increased serum level of antioxidant enzymes by itself.

Key Word Catalase, CoQ_{10}, Glutathione Peroxidase, Heavy Training, Superoxide Dismutase

Introduction

Participating in severe and long physical activities leads to the production of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) by increasing mitochondrial electron leakage, impaired hemostasis, and reducing immune activity (30). In fact, they initiate lipid peroxidation, protein oxidation, and DNA damage, all playing important roles in stabilizing pathogens and a wide range of diseases (30). Also, an increase in ROS disturbs redox balance within the muscular contraction process (12). High body temperature during trainings, especially in a hot and humid environment, stimulates visceral vessels for irregular production of nitric oxide radical ($\bullet NO$) and ROS (16). In addition, the heat stress causes a change in cardiac output, increased release of catecholamine, metabolic changes in the muscles, and finally heatstroke, followed by increasingly reduced athletic performances (38). To cope with oxidative stress caused by free radicals, cells such as mitochondria and peroxisomes are equipped with antioxidant defense system consisting of enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) (first defense line of the cells), and numerous nonenzymatic antioxidants, including vitamins A, E, and C, glutathione (GSH), ubiquinone, and flavonoids against the free radicals (12).

On the other hand, increased serum activity of antioxidant enzymes after trainings indicates the increase of $O_2^{\bullet -}$ and H_2O_2 presence in serum as a result of which oxidative stress increases (12). Nayanatara et al. (27) suggested the stress arising from the compulsive and consecutive swimming trainings per day as the cause of activating free radicals and lipid peroxidation

Address correspondence to Ali Emami, AliEmami2@yahoo.com.

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increase in most body tissues and producing products such as malondialdehyde (MDA). Santos-Silva et al. (34) stated that teenaged swimmers engaged in high-level training and competitions experience proteolytic and lipolytic injuries. Thus, minimizing high body temperature and oxidation stress during trainings and competitions in hot and humid environments has been considered by athletic centers to prevent athletic problems in World Cup 2014 of Brazil and Olympics 2016 of Brazil (5). Cooling the body in 2 states of precooling and percooling can prevent fatigue and failure by lowering body temperature (5). Most studies, including the research by Bogerd et al. (4), suggest its effect on improving failure and performance time of the athletes. Still, there are disagreements with this effect in some studies which identify it as an impractical method for the competitive and field sports (5).

Since 2000, precooling has been used for most sport fields in a hot environment at 28° C (20). In this respect, Hasegawa et al. (17) reported the use of precooling and percooling to be helpful for training on the stationary bike at 32° C and relative humidity of 80% and reducing heart pressure and blood vessels pressure through decreasing body temperature and preventing dehydration. In accordance with the findings of Demirci and Beytut (9), one strategy of the researchers and athletic trainers for coping with the adverse effects of oxidative stress caused by continued and heaving trainings is using CoQ_{10} supplementation. CoQ_{10} plays an important role in the mitochondrial redox component and endogenously produced lipid-soluble antioxidants (scavenges oxygen radicals) of the human organism (7). Other important functions of CoQ_{10} are protecting the stability of the cell membranes, protecting DNA from oxidative damage induced by free radicals, and being capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate (7). CoQ_{10} is a necessary cofactor for the lack of pairing proteins in the regulation of body core temperature and accelerating the biological and biochemical interactions, affecting the temperature of muscle contraction (11). CoQ_{10} supplementation prevents induced cell damage by the expression of free radicals from various sources as a result of improvement in enzymatic and nonenzymatic antioxidant system activity (36).

Because of the contradictory results and scarce studies on the physiological effect of short-term precooling and CoQ_{10} supplementation strategy on antioxidant enzymes, this research aimed to conduct a field and experimental approach to test whether the use of precooling and CoQ_{10} supplementation has any effect on improving the body's endogenous antioxidant enzyme system performance in elite swimmers during heavy trainings (competition stage) in hot and humid environments and swimming recording.

Methods

Experimental Approach to the Problem

This study was experimental, conducted after gaining the approval of Swimming Federation and the team's coach. The aim of the current study was to analyze the effect of 14 days CoQ10 supplementation and pre-cooling strategy on serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in elite swimmers during heavy and regular trainings bouts and recording of freestyle swimming in competition stage. The study followed a repeated measures design, with each participant randomly assigned to specific 4 groups of CoQ10 (300 $mg \cdot d^{-1}$), precooling (immersion in the water at 18 ± 0.5° C), supplementation with precooling, and control. Training sessions were conducted by coaches and presence of researcher in two shifts, one in the morning and one in the evening. Performances of the swimmers were measured by recording freestyle swimming (24 hours before and after supplementation) at 800, 200 and 50 m respectively.

Subjects

Study population included healthy, male (mean ± *SD*: 17.60 ± 1.14) swimmers aged 16–19 who were the members of the national swimming team of Iran in Tehran with 10 years of professional swimming records and had gained top ranks at the national level. All subjects were nonsmokers, 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. A sample with 36 participants was selected by the convenient sampling method from a population with 36 individuals, divided into 4 groups with 9 members by simple randomization. Participants were divided into 4 groups: 1–CoQ_{10} supplementation, 2–supplementation with precooling, 3–precooling, and 4–control. Stages of this study are represented in Figure 1.

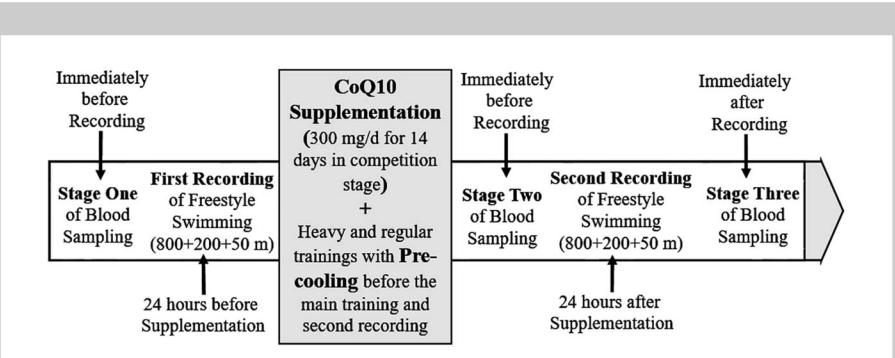

Figure 1. General approach of research process.

Also, for the swimmers under the age of 18 years, consent of their parents and for athletes older than 18 years, the consent of swimmers for participation in the study were taken.

Compliance With Ethics Guidelines

Study protocol for withdrawal of blood from healthy volunteers was approved by the institutional review board at Urmia University, Urmia, Iran. All followed procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki (1975), revised in 2000 and 2008. Informed consent was obtained from all patients for participating in the study.

Measurement of Individual Characteristics

Body fat was measured using a skin caliper (made in Japan) and skinfold equation of Jackson and Pollock for men (triceps, abdomen, and suprailiac). The subjects' height was measured by wall-mounted stadiometer of Seca (Seca 206, made in Germany) with an error margin of 1 mm. Subjects' weight was measured by the digital scale of Beurer with the precision coefficient of 100 g made in Germany. Body mass index was calculated by dividing body weight by squared height ($kg \cdot m^{-2}$). Aerobic power ($\dot{V}o_2max$) was estimated by the exhaustion test of Bruce (running on a Technogym treadmill made in Italy) through indirect way, using its mathematical formulas. Also, anaerobic power was measured by the RAST (Running-based Anaerobic Sprint Test) test. Participants measured and recorded their resting heart rate accurately before leaving the bed for 3 consecutive days. Carotid artery pulse of 10 seconds, multiplied by 6 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 (18).

$$\text{Body fat } \% = 0.39287 \times (\text{sum of 3 skinfolds}) - 0.00105 \times (\text{sum of 3 skinfolds})^2 + 0.15772 \times (\text{age}) - 5.18845$$

$$\dot{V}o_2max = 14.76 - 1.379 \times (\text{time}) + 0.451 \times (\text{time})^2 - 0.012 \times (\text{time})^3$$

$$\text{Power} = \text{weight} \times (\text{distance})^2 \div (\text{time})^3.$$

Coenzyme Q_{10} and Placebo Dose

Miles (26) suggested that the minimum duration of CoQ_{10} supplementation is 2 weeks, and the most suitable dosage of 300 $mg \cdot d^{-1}$ in a single dose for raising the base level in human subjects because of its 33-hour half-life, hydrophobic nature, and high molecular weight. According to the methods of Leelarungrayub et al. (23), the athletes received daily 300 mg of CoQ_{10} ($C_{59}H_{90}O_4$) with a transstructure in the form of a gel manufactured by America (Nature's Bounty, USA) for 14 days. During this period, control and precooling groups took daily 300 mg of placebo capsules (lactose) which were similar to CoQ_{10} in terms of flavor, color, and size.

Table 1. Mean ± *SD* of participant's received food and energy levels.

Variables	Groups				*p*
	CoQ_{10} ($n = 9$)	CoQ_{10} + precooling ($n = 9$)	Precooling ($n = 9$)	Control ($n = 9$)	
Carbohydrate ($g \cdot d^{-1}$)	436.80 ± 8.95	435.50 ± 21.20	437.40 ± 22.51	428.00 ± 7.56	0.75
Protein ($g \cdot d^{-1}$)	92.20 ± 2.16	93.00 ± 1.26	93.60 ± 2.07	91.33 ± 4.32	0.56
Fat ($g \cdot d^{-1}$)	82.40 ± 1.14	83.83 ± 3.37	82.00 ± 2.12	83.16 ± 2.48	0.62
Fiber ($g \cdot d^{-1}$)	34.20 ± 2.58	37.66 ± 3.32	34.40 ± 2.96	36.83 ± 3.12	0.18
Cholesterol ($mg \cdot d^{-1}$)	173.60 ± 7.02	176.00 ± 5.86	170.00 ± 4.24	176.83 ± 6.55	0.28
Calcium ($mg \cdot d^{-1}$)	1,018.40 ± 32.79	1,046.66 ± 58.43	1,025.00 ± 24.49	1,036.66 ± 16.63	0.61
Vitamin C ($mg \cdot d^{-1}$)	104.00 ± 2.54	105.50 ± 2.25	104.80 ± 1.48	106.66 ± 3.77	0.43
Vitamin E ($mg \cdot d^{-1}$)	15.40 ± 2.07	16.83 ± 1.94	16.00 ± 1.00	15.16 ± 2.31	0.47
Vitamin B_6 ($mg \cdot d^{-1}$)	2.40 ± 1.14	3.50 ± 0.54	2.20 ± 0.83	2.83 ± 1.16	0.14
Vitamin B_{12} ($\mu g \cdot d^{-1}$)	3.40 ± 0.89	3.83 ± 0.75	3.40 ± 0.54	3.83 ± 1.16	0.72
Selenium ($\mu g \cdot d^{-1}$)	72.60 ± 1.67	74.16 ± 2.13	73.40 ± 1.51	74.50 ± 1.51	0.31
Zink ($mg \cdot d^{-1}$)	12.20 ± 0.83	12.50 ± 1.04	13.00 ± 1.00	12.33 ± 0.81	0.55
Iron ($mg \cdot d^{-1}$)	11.20 ± 1.48	11.83 ± 1.47	10.60 ± 0.89	12.33 ± 1.75	0.25
Carbohydrate ($kcal \cdot d^{-1}$)	1,747.20 ± 35.82	1,742.00 ± 84.80	1,749.60 ± 90.04	1,712.00 ± 30.25	0.75
Protein ($kcal \cdot d^{-1}$)	368.80 ± 8.67	372.00 ± 5.05	374.40 ± 8.29	365.33 ± 17.28	0.56
Fat ($kcal \cdot d^{-1}$)	741.60 ± 10.26	754.50 ± 30.34	738.00 ± 19.09	748.50 ± 22.34	0.62
Total energy intake ($kcal \cdot d^{-1}$)	2,857.60 ± 42.74	2,868.50 ± 87.09	2,862.00 ± 83.11	2,825.83 ± 42.48	0.70
Total energy consumption ($kcal \cdot d^{-1}$)	3,086.20 ± 43.15	3,048.33 ± 65.90	3,105.60 ± 86.31	3,064.50 ± 40.57	0.45

Table 2. Mean ± *SD* of participant's physical characteristics.

Variables	Groups				
	CoQ_{10} ($n = 9$)	CoQ_{10} + precooling ($n = 9$)	Precooling ($n = 9$)	Control ($n = 9$)	p
Age (y)	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
Weight (kg)	71.20 ± 2.16	67.60 ± 9.01	69.80 ± 8.61	68.00 ± 6.02	0.81
Body fat (%)	14.75 ± 1.39	14.31 ± 2.42	14.51 ± 2.10	14.60 ± 1.57	0.98
Body mass index ($kg \cdot m^{-2}$)	22.67 ± 0.66	22.45 ± 2.01	21.66 ± 1.45	22.11 ± 1.65	0.74
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.12 ± 3.23	52.78 ± 2.46	53.96 ± 2.46	53.96 ± 2.46	0.83
Max power (W)	442.1 ± 18.3	450.2 ± 12.7	463.1 ± 27.9	463.1 ± 27.9	0.48

Dietary Control

The average food intake was obtained using a 24-hour dietary recall questionnaire. All subjects were asked to mention all food and drink they had in the last 24 hours. This questionnaire was completed for each subject in 6 nonconsecutive times during 2 weeks (3 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 analyzed in terms of quantity and energy contribution (24). Results (represented in Table 1) showed no significant difference in any of the macronutrients, minerals, and vitamins that the subjects had consumed ($p > 0.05$).

Precooling Administration

According to the studies on the running race and biking (37), and in agreement with Arngrïmsson et al. (2), swimmers were precooled in every training session before starting main trainings and recording between warm-up outside and inside the water. To prevent cold shock and after taking a shower (gradual reduction of water temperature) (6), participants entered cold water pool at a temperature of 18 ± 0.5° C from the lower limbs to shoulder girdle for 15 minutes (2). Also, 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 L of water chilled to the temperature of 5 ± 1° C (20).

Training and Recording Protocol

Training protocol with a 2-week training contract (9 sessions per week) of timed and designed trainings was considered by the researcher and trainer for the competition stage (from 4 stages of swimming trainings) in the hottest month of the

Table 3. Mean ± *SD* of participant's serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

Variables	Groups			
	CoQ_{10} ($n = 9$)	CoQ_{10} + precooling ($n = 9$)	Precooling ($n = 9$)	Control ($n = 9$)
SOD ($IU \cdot gHb^{-1}$)				
Stage 1	$821.77 \pm 72.13^{Aa*}$	851.31 ± 103.73^{Ba}	784.80 ± 48.75^{Ca}	791.92 ± 67.47^{Ba}
Stage 2	608.92 ± 175.51^{Ab}	646.98 ± 79.55^{Cb}	$1{,}040.81 \pm 57.98^{Ba}$	869.68 ± 158.80^{Ba}
Stage 3	858.42 ± 72.59^{Ad}	$1{,}255.47 \pm 125.83^{Ac}$	$1{,}744.34 \pm 197.94^{Aa}$	$1{,}493.69 \pm 111.51^{Ab}$
CAT ($IU \cdot gHb^{-1}$)				
Stage 1	321.10 ± 29.99^{Aa}	341.65 ± 34.79^{Ba}	365.64 ± 48.25^{Ba}	344.51 ± 43.09^{Ba}
Stage 2	179.22 ± 14.31^{Bb}	208.74 ± 17.81^{Cb}	468.29 ± 46.43^{Ba}	394.90 ± 63.92^{Ba}
Stage 3	328.15 ± 50.10^{Ac}	457.48 ± 40.56^{Ab}	689.47 ± 35.64^{Aa}	592.37 ± 78.86^{Aa}
GPx ($IU \cdot gHb^{-1}$)				
Stage 1	$1{,}328.90 \pm 106.68^{Aa}$	$1{,}440.83 \pm 104.68^{Ba}$	$1{,}344.47 \pm 83.41^{Ba}$	$1{,}394.57 \pm 132.31^{Ba}$
Stage 2	744.37 ± 42.72^{Bb}	792.32 ± 51.28^{Cb}	$2{,}074.83 \pm 467.46^{ABa}$	$1{,}544.41 \pm 401.09^{ABa}$
Stage 3	$1{,}351.93 \pm 60.34^{Ac}$	$1{,}865.44 \pm 100.87^{Ab}$	$2{,}851.08 \pm 201.30^{Aa}$	$2{,}377.94 \pm 476.53^{Aa}$

*Different letters in columns (A, B, C) and in rows (a, b, c, d) denote significant differences ($p < 0.05$).

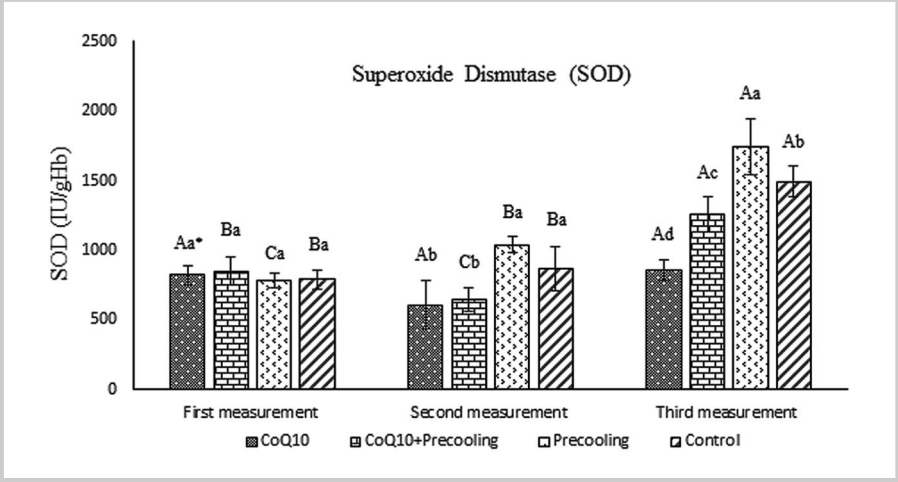

Figure 2. Mean ± *SD* of superoxide dismutase (SOD) ($n = 9$). *Different letters among the 3 measurements (A, B, C) and among the groups (a, b, c, d) denote significant differences ($p < 0.05$).

summer. Training variables (volume, intensity, and frequency) were kept constant every week, and the training distance for every session was considered to be 5 km (23). Performances of the swimmers were measured by recording freestyle swimming at 800, 200, and 50 m with the maximum power and heart rate control following Leelarungrayub et al. (23) and similar to the matches with a 10- to15-minute active rest between the trainings. Training sessions were conducted in 2 shifts, 1 in the morning and 1 in the evening, without also working with weights and 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 International Swimming Federation. Ambient pool temperature was 32 ± 1° C with the relative humidity of 60–70%.

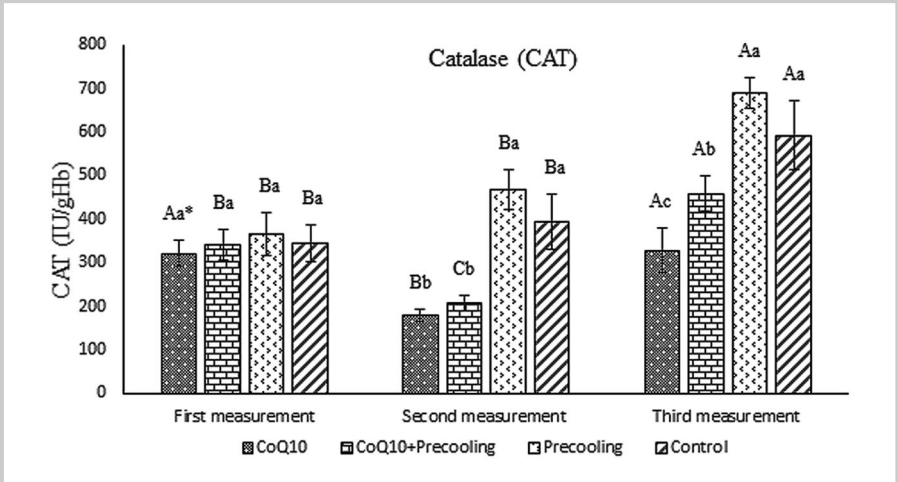

Figure 3. Mean ± *SD* of catalase (CAT) ($n = 9$). *Different letters among the 3 measurements (A, B, C) and among the groups (a, b, c) denote significant differences ($p < 0.05$).

Swimming speed was controlled through counting stroke rate and stroke length by a digital chronometer and a Polar watch. Before the main training, swimmers performed a warm-up outside the water (general and dynamic) and warm-up inside the water. Also, after the main training, a cool-down inside water was performed for 15–20 minutes (a total of 45–60 minutes). Intensity of the trainings was set according to the Karvonen Formula or target heart rate (THR) based on the equations below (equation 1). Main trainings were practiced for 2 hours using different drills of 4 styles swimming in the intervals and repetitive sets, focused on the speed (SP1-3) and endurance (En1-3) swim (31).

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, 800–1,500 m), En2 (60–70% THR, 400–800 m), and En3 (70–80% THR, 200–400 m).

$$\begin{aligned} &\text{maxHR} = 220 - \text{age} \\ &\text{THR} = \text{restHR} + \%\ \text{intensity} \times (\text{maxHR} - \text{restHR}) \\ &\text{V} = \text{SL} \times \text{SR}. \end{aligned} \quad (1)$$

Preparation of Blood Samples

Blood sampling was performed 24 hours before and after supplementation (the highest level of effectiveness of CoQ_{10} in blood) in 3 stages (26). Every time, approximately 5 ml of blood was sampled from an antecubital vein of the right arms of the subjects before noon. Then, 2 ml of collected blood was poured and thoroughly stirred in test tubes with anticoagulant substances (K2EDTA) for blood cell count. Three milliliter of the remaining blood was immediately transferred into gel tubes specific for serum without addition of an anticoagulant material for serum preparation and was centrifuged after clotting by centrifugation tool for 10 minutes and 300 rounds per minute. Participants avoided any physical activity 24 hours

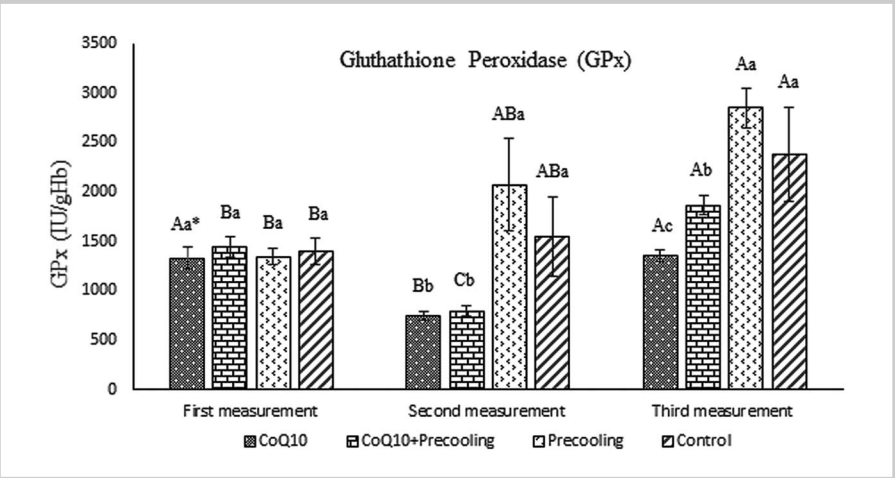

Figure 4. Mean ± *SD* of glutathione peroxidase (GPx) ($n = 9$). *Different letters among the 3 measurements (A, B, C) and among the groups (a, b, c).

before blood sampling and recording. The initial results of complete blood count testing and urinalysis showed normal levels.

Measurement of Blood Indices

Serum enzymes of SOD and GPx were measured based on the biochemical kit of Biorex (Biorex, Co., United Kingdom). Also, serum enzymes of CAT were measured using Goth method (1991), based on the decomposition of H_2O_2 substrate at a wavelength of 240 nm according to the Cayman kit (made in USA), using a spectrophotometer.

Statistical Analyses

In the referential statistics, for evaluating data normality and the data of every index in each group, Kolmogorov-Smirnov test was used. After gaining confidence about data normality, parametric test was used to analyze the mean of the data. For this purpose, repeated measurement in the general linear model and by (post hoc) Bonferroni method was conducted. Also, for the statistical analyses at $p < 0.05$ level to obtain significant difference among the groups and stages, SPSS software version 22 and Excel software were used.

Results

Analysis of Variance indices of SOD, CAT, and GPx were conducted at the first stage of blood sampling with the values of $p = 0.46$, $p = 0.39$, and $p = 0.34$. No significant difference was observed between the groups ($p > 0.05$). At the second and third stages of blood sampling, a significant difference was observed between the groups for all 3 variables ($p < 0.05$). Table 2 shows the mean ± *SD* of some physical features of the swimmers and no significance difference was observed for each variable ($P > 0.05$). Also, Indices of significance results from Bonferroni test are represented in Table 3 and Figures 2–4.

Discussion

The changes in blood sampling results of the second and third stages showed that there was a significant difference in response to the experiment between the study groups ($p < 0.05$). In the supplementation and supplementation with precooling groups, a significant decrease was observed compared with the precooling and control groups. Also, the variables in each group showed a significant increase in the third stage compared with the first and second stages ($p < 0.05$). A previous study showed that light and regular endurance trainings can improve the activity level of total SOD and GPx in actively used skeletal muscles of the athletes; it also found that an improvement in CAT activity level did not occur (29). Concluding the effect of average 4-week sportive activities like swimming (2 sessions of daily trainings at educational level for 1 hour per session), the study of Gonenc et al. (13) showed that the serum level of SOD enzyme had a significant increase ($p < 0.05$) and an increase at the level of GPx which was not significant ($p > 0.05$). As a result, regular swimming training had a positive effect on improving antioxidant system of the healthy children (13). On the other hand, doing heavy and competitive trainings and hyperthermia led to the oxygen shortage, activating anaerobic glycolysis enzymes, imbalance of pro-oxidant and antioxidant response, catecholamine release, and metabolic stresses in the liver and intestine (15). Subsequently, excess ROS production during exercise causes muscular contractile dysfunction; it most likely involves abnormalities in calcium homeostasis and damage or destruction of cellular macromolecules (1,15).

In fact, consistent with the results of this study, serum level of antioxidant enzymes (SOD, CAT, and GPx) and then nonenzymatic increase occurred to cope with free radicals (29). As the results showed, enzymes' level in the supplementation and precooling, and precooling and control groups, showed a significant increase at the third stage compared with the first and second stages ($p < 0.05$), although this result was not found in the supplementation group. Inal et al. (19) found that long-distance (aerobic) and short-distance swimming immediately increases the activity of the antioxidant defense system (CAT, GPx, and GSH) significantly. Moreover, Gul et al. (14) suggested that antioxidant (GPx and SOD) enzyme level of inactive men increases

after repeated short-term supramaximal exercise. Aerobic activity increases oxygen use compared with the resting state, indicating a significant correlation between oxygen uptake levels and production of free radicals (29). Accordingly, 2–5% of intercellular oxygen prefers to take an electron and turn into $O_2^{\bullet -}$ (7). In this respect, as a pro-oxidant, CoQ_{10} plays a significant role in changing its situation into H_2O_2 at the presence of SOD enzyme in complexes I and III of the respiratory chain (7). In fact, oxidation of 1 of 3 structures of CoQ_{10} ($CoQ^{\bullet}$, CoQH, and $CoQH_2$), Ubiquinone or $CoQH_2$ is effective in the cellular antioxidant function of the cell by producing proton (H^+) and combining it with $O_2^{\bullet -}$ to produce H_2O_2 (7).

H_2O_2 turns into H_2O at the presence of GPx and CAT as a result of which adenosine triphosphate is produced; otherwise, it turns into OH• (7). CoQ turns into $CoQH_2$ in complexes I and II by receiving electron from NADH and $FADH_2$ coenzymes. Then, it plays an important role in the oxidative phosphorylation by transferring electron to complex III (7). Different mechanisms have been suggested in relation to the efficiency of CoQ_{10} by the researchers. Some believe that CoQ_{10} supplementation causes the decrease of lipid peroxidation by increasing antioxidant ability and removing free radicals (33). According to the results, in the second (before the second recording) and third stages (after the second recording), serum enzymes showed a significant decrease in the groups of supplementation and supplementation and precooling compared with precooling and control groups. This result confirmed the improved activity of the antioxidant system after heavy trainings and recording in CoQ_{10} supplementation group.

The study of Cooke et al. (8) is a pioneer in this field whose findings agree with these results; because, according to their reports, a 2-week supplementation of CoQ_{10} (200 $mg \cdot d^{-1}$) increases concentration and decreases serum level of SOD during and after sportive (isokinetic) activities compared with the control group, and time to exhaustion increases during trainings and MDA level. Also, one conclusion can be that for the complexity and defect of some enzymes and regulating proteins in making CoQ_{10} from the amino acid of tyrosine, some shortages may occur in the childhood and adolescence; this shortage is especially higher after the age of 20 when the ability of its synthesis from food decreases (7). Thus, young athletes can use its antioxidant properties by using oral CoQ_{10} supplementation.

In contrast to the results of this study, Díaz-Castro et al. (10) demonstrated that CAT activity, the hydrogen peroxide (H_2O_2)-removing enzyme, increased its activity in the CoQ_{10} supplementation group compared with the placebo group in the strenuous exercise (50 km running), indicating enhanced antioxidant capacity induced by the CoQ_{10} supplement. The reason for such discrepancy could be the subjects being nonathletic (amateur male athletes), the dosage and duration of using supplementation (3 days), and the type of their sportive contracts. Moreover, in a recent study by Okudan et al. (28) in 2011, 6 weeks of intraperitoneal CoQ10 injection on a daily dose of 10 $mg \cdot kg^{-1}$ body mass and exercise training significantly inhibited exhaustive exercise-induced lipid peroxidation and DNA damage, but did not affect GSH levels and SOD activity in the heart tissue of rats. Most likely, the study type, supplementation dosage, experimental method of antioxidant calculations, the tissue sampled, training protocol used to induce oxidative stress, the time of measurement, rats' type (i.e., trained vs. untrained and old vs. young), and other variables cause such contradictory results (3). Some nonathletic and human studies, such as the study by Lee et al. (22), suggested that patients with coronary vessels' diseases increased serum level of CoQ_{10} and activity level of antioxidant enzymes and vitamin E. Meanwhile, the level of inflammatory indices (TNF-α) decreased significantly.

Interest in precooling has increased among the athletes; since, over 3 decades ago, using exposure to the cold weather, icing jackets, immersion in the cold water and ice, using cold drinks at the beginning and during activities has enhanced (32). According to Arngrïmsson et al. (2) and Siegel et al. (35), precooling and percooling decrease the consequences of warm-up stress by increasing body endurance in the heat. Agreeing with the results of this study, Kenz and Periard studied the effect of antioxidant markers and oxidative stress in tennis games in hot (36° C with relative humidity of 35%) and cold (22° C with relative humidity of 70%) environments. Tennis games in a hot environment did not increase oxidative stress but increased antioxidant markers significantly. As a result, having activity in the hot environment may be a necessary signal for regulating the antioxidant enzyme system after which dampening cellular damage occurs (21). Also, like the finding of this study, the reason for the significant increase of antioxidant enzymes level (especially SOD) of the precooling group compared with the control group at the second and third stages of blood sampling could be the different physiological responses of the body to the decrease of body core temperature for immersion in the water at 18 ± 0.5° C. Also, the consequences such as body trembling stimulated by sympathetic neural system, increased blood pressure and heart rate (Tachycardia), rapid and unbalanced breathing (reduction of available oxygen to the tissues and creating hypoxia and acidosis conditions), immune system's decline, and contraction of blood vessels (loss of muscle blood flow) are likely (1).

Thus, because of the decrease of cellular oxygen, reactive oxygen and nitrogen species leak from mitochondria as a result of which the function of lipids, proteins, and nucleic acids is disrupted. Therefore, the increase of activity levels of antioxidant enzymes and other small antioxidant molecules such as GSH leads to the resistance against free radicals for balancing physiological conditions of the body (maintaining homeostasis) (1). Present results are inconsistent with the findings of Marsh et al.; because, before cooling through

vasoconstriction of the skin, blood flow to active muscles increases. As a result, an increase in the muscular blood flow can improve aerobic metabolism and energy production of the muscle. The present result disagrees with the findings of Marsh et al.; because, precooling increased blood flow to active muscles through vasoconstriction. As a result, increased muscle blood flow can improve aerobic metabolism and production of muscle energy. By lactate acid transfer for oxidation, its accumulation and pH decrease are prevented and fatigue is delayed (25). This disagreement can be related to the constant and low temperature of the pool water (27 ± 1° C) compared with the high temperature of their activity's environment. Also, the type, method, and duration of precooling may cause such discrepancy in the results (37). But, in CoQ_{10} supplementation group and even precooling and supplementation group in the second and third stages of blood sampling, antioxidant enzymes were significantly lower than precooling and control groups.

When the supplementation with precooling group was compared with the supplementation group, a synergistic effect against oxidative stress and improved performance of endogenous antioxidant enzyme system were not observed. The reason for this is that the increase of serum CoQ_{10} leads to the increase of oxidative phosphorylation, acceleration of electron transfer from flavoproteins to cytochromes, less dependence on anaerobic glycolysis path, fuel increase of fatty acids in slow twitch muscle vessels, and lower lactate accumulation and free radicals (7). Also, the activity of GPx, SOD, and CAT is different across muscle fibers, so that in muscle fibers type I (slow twitch), maximum activity and in muscle fibers type II (fast twitch), minimum activity is observed (29). Thus, it is suggested that taking CoQ_{10} improves the activity level of antioxidant enzymes by decreasing their serum level during trainings and recording. Although in this respect, results of Booth et al. (6) disagree with this result. Because according to their reports, muscle metabolism does not change by precooling, and if it is useful for physical performance, it plays a role by decreasing the body temperature and reducing pressure on the cardiovascular system. In fact, precooling does not work in offsetting or decreasing increased level of antioxidant properties after heavy trainings and swimming recording.

Practical Applications

According to studies, the dramatic increase in free radicals or ROS during intense and prolonged physical exercise and sport can damage cell membranes and interfere with excitation contraction coupling, having deleterious effects on skeletal muscle performance. Results of this study showed that taking CoQ_{10} decreased endogenous antioxidant enzymes' levels during heavy trainings and recording (competition stage) for elite swimmers in hot and humid environments. In fact, as an antioxidant and cell signaling and body temperature regulator, CoQ_{10} improves activity level of GPx, SOD, and CAT. Also, CoQ_{10} supplementation prevents the adverse consequences of precooling. The precooling strategy in the swimming did not affect the decrease of serum level or did not improve activity level of GPx, SOD, and CAT enzymes individually. Presumably, precooling decreased the body temperature and increased the time to exhaustion during the swim trainings; however, future research is needed in this area.

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