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The effects of short time monohydrate creatine supplementation on systemic stress homeostasis following repeated maximum swimming in young women

Original Article

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Abstract

Introduction: This study aimed to evaluate the effect of short-term creatine supplementation on oxidative stress and antioxidant enzymes after six bouts of 50-meter sprint swimming.

Methods: This quasi-experimental study recruited eight trained female swimmers with the mean age of 25±4.4 years and body mass index (BMI) of 21.8±4.2 kg/m2 to perform six bouts of 50-meter sprint swimming with a 120-second active recovery in water. Then, subjects consumed Cr supplement (capsules containing 5 g monohydrate creatine), four times a day for six days. Blood samples were taken in resting position after the sixth bout of swimming before and following Cr supplementation period. Protein carbonyl (PC) and superoxide dismutase (SOD) concentration were measured using ELISA method.

Result: Sprint swimming significantly increased PC concentration compared to resting state, but an insignificant increase was detected after Cr supplementation. In addition, sprint swimming led to a significant reduction in SOD levels after creatine supplementation compared to pre-test. Swimmers' records after creatine supplementation in sixth bout of sprint swimming was also lower compared to the first bout.

Conclusion: Cr supplementation can inhibit increased oxidative stress markers induced by high-intensity and short-duration exercise in trained female swimmers.

Introduction

Creatine monohydrate is an endogenous nitrogenous organic acid that is often synthesized from glycine, methionine and arginine amino acids in the liver (1). So far, oral administration of creatine monohydrate has been found to increase creatine plasma concentrations and total creatine concentration, which is often stored in skeletal muscles in the form of creatine phosphate and creates a potential capacity to provide improved performance during high-intensity exercises (1). In addition to the ergogenic features (endogeneity), it is also found that creatine might boost cellular energy supply and improve cellular calcium homeostasis. Since the accumulation of intracellular calcium widely indicates the formation of Reactive Oxygen Species (ROS) and oxidative stress, it was recently raised that creatine supplementation creates a capacity for calcium homeostasis improvement, reduces ROS production, and reduces oxidative damages (2). In this regard, Laweler et al. reported the antioxidant properties of creatine for the first time (3). They suggested that creatine was effective as a scavenger of radicals such as Super Oxide Anion. In this regard, Sestili et al. showed that exogenous creatine was also effective as a scavenger of ROS such as hydroxyl radicals and reactive nitrogen species (RNS) in cultured human cells (4). Rakpongsiri et al. reported that creatine supplementation can be a treatment for protecting heart functions and an antioxidant defense in ovariectomized hamsters (5). In addition, other researchers also examined the effect of creatine supplementation on different markers related to oxidative stress such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase in different tissues of rats and found similar results (6, 7). Therefore, due to limited significant human evidences on the effects of creatine supplementation on oxidative and anti-oxidative homeostasis, particularly following high-intensity intermittent swimming in women, the present study was conducted to determine the effect of short-term creatine monohydrate supplementation on Carbonyl Protein (PC) as a lipid peroxidation marker, and on superoxide dismutase (SOD) as an anti-oxidation marker following 6 bouts of 50-meter high-intensity intermittent swimming in young women.

Material and Methods

This was a quasi-experimental study with a pre-test and post-test design. The population of the study was female swimmers with a mean age of 25±4.4 years in Qaem Shahr, Iran. They were examined at two phases before and after creatine monohydrate supplementation

in line with the following conditions. The inclusion criteria were: at least 6 months of regular swimming exercise, no cardiovascular disease or hypertension (according to medical examination of the subjects), no smoking for at least three months before the start of the study. In addition, due to the antioxidant effect of creatine and in order to avoid the synergistic effect of taking vitamins or multivitamin on the dependent variables, it was tried to enroll people that knowingly refrained the consumption of such material during the period of the investigation. Thus, avoiding the consumption of vitamins such as vitamin E and C, as well as multivitamins and ergogenic supplements in a week before the implementation of the protocol, avoiding consuming more than 300 grams of meat during the period of creatine supplementation, doing no intensive exercise 24 hours before and during the protocol, and consuming no tea and coffee during the study were among other inclusion criteria.

Eligible subjects were familiarized with the research phases a week before the implementation of the protocol and their written consent was taken. The anthropometric characteristics of the subjects including age (25±4.4 years), height (164.4±4.6 cm), weight (58.7±10.6 kg), body mass index (BMI) (21.8±4.2 kg/m2), and body fat (26.3±5.6%) were measured and recorded using the body composition analysis device. In addition, the anaerobic power of the subjects' hand was obtained as 0.562±0.5524 watts per kilogram using the Wingate test, which was collected for the homogeneity of people in the groups. Then, the details of oxidant and antioxidant enzymes after 6 bouts of 50-meter high-intensity intermittent swimming were collected. A week after the first phase (before creatine supplementation), the very participants repeated the same program of high-intensity intermittent swimming bouts following the creatine supplementation period.

The training program in this study included a set of 50-meter sprint swimming with 6 reps during a week with 120-second rest intervals between the six bouts of 50-meter high-intensity intermittent swimming. Since the recent study used maximum interval training program, the participants performed a set of 50-meter swimming in each training bout with 120-second of active, in-water rest intervals. In this regard, they were instructed to perform the recovery rhythm, which was equal to 60% of the best 100 record for each person (8,9). Therefore, for regulating recovery rhythm, the researcher colleagues were recommended to move by the swimmers alongside the pool and thereby provide feedback about the movement rhythm to the swimmers. In addition, verbal encouragement and material and spiritual gifts were used in this regard. Moreover, all the tests were taken at the same time of the day and following a controlled warm-up program including swimming 600 meters (200 meters of kicking, 200 meters of hand stretching and 200 meters of full front crawl swimming). After 2 minutes, the main activity bouts started and after the completion of the blood sampling program, participants attempted to cool down. All tests used front crawl swimming. The test started from inside the pool and by pushing the pool wall.

Creatine monohydrate supplement (Weider, Canada) was weighed by a medical precision scale and packed in 5-gram packages. Each 24 packages were given to the subjects in a plastic bag. Supplementation started from the next morning of subjects' familiarization with the implementation of the research protocol (basic protocol). The subjects consumed 4 of 24 packs they were given in each day in 4 meals (breakfast, lunch, dinner and late snacks before bed). They were also recommended to dissolve the contents of each pack in 250 ml (a soda bottle) of lukewarm water and then consume it (10, 11). Supplementation was performed in 6 consecutive days (120 grams of creatine in total). The subjects continued their routine diet in the course of supplementation and were recommended to do no additional activity during the six days, and refrain from taking any high caffeine products, or excessive amounts of fish, white or red meat (more than 300 grams per day). In order to control this, some forms were distributed among the subjects so that they write down any kind of food that they took and might have affected the results during the study. Laboratory analysis was performed by protein carbonyl using a special human PC kit (ANOGEN), and superoxide dismutase using a special human SOD kit (SIGMA) with ELISA method. The inter-assay coefficient of variation and sensitivity of measurements were 4.69% and 0.8 nmol/ml for PC, and 8.3% and 3.4 units per ml for SOD, respectively. All the statistical affairs were performed by the SPSS Software Version 21 using repeated measures analysis of variance (rANOVA) to examine the intergroup effect of variables.

Results

Table 1 presents the descriptive information related to female swimmers before and after the implementation of sixth bouts of high-intensity intermittent swimming in two phases of before and after the period of creatine monohydrate supplementation. Although the implementation of 6 bouts of 50-meter high-intensity intermittent swimming significantly increased PC (P=0.002) compared to the resting levels before creatine supplementation period, the one-week creatine monohydrate supplementation resulted in insignificant increase of PC amounts (P=0.087) (Figure 1.) On the other hand, the implementation of high-intensity intermittent swimming before creatine supplementation period led to an insignificant decrease in SOD levels (P=0.075), while creatine supplementation moderated this decline; so that after creatine supplementation SOD showed a 30% difference at the end of the sixth repeat compared to before the first repeat (P=0.004) (Figure 2). Notwithstanding the foregoing, swimming record tracking shows that the mean swimming record after the 6th bout of 50-meter swimming had a significant increase of 16.22% compared to the 1st bout before creatine supplementation (P=0.001), while these values had a non-significant increase of 4.37% after the supplementation period (P=0.167). In other words, these changes indicate 11.38% improvement of swimming records after creatine supplementation period (Figure 3).

Table 1. Descriptive information of PC and SOD levels, swimming performance after 6 bouts of maximum 50-meters in the in-water recovery group before and after creatine monohydrate supplementation period

Marker	Supplementation period	Testing period	Mean	SD	Minimum	Maximum	Variation range
PC (nmol/ml)	Before	Pre-test	0.713	0.0835	0.60	0.80	0.2
		Post-test	0.84	0.092	0.70	1.00	0.3
	After	Pre-test	0.74	0.1022	0.56	0.89	0.33
		Post-test	0.87	0.12	0.70	1.00	0.3
SOD (Units/ml)	Before	Pre-test	79.343	7.85	67.54	94.30	26.76
		Post-test	66.424	11.451	45.70	83.30	37.6
	After	Pre-test	83.79	7.064	71.94	92.10	20.16
		Post-test	64.449	10.62	45	76.9	31
Swimming record (Seconds)	Before	Pre-test	62.625	16.0885	54	91	46
		Post-test	74.750	19.7538	50	105	50
	After	Pre-test	62.8750	16.4094	49	90	41
		Post-test	65.750	17.9742	43	95	52

Figure 1. The amounts of PC before and after the implementation of 6 bouts of high-intensity intermittent swimming in two phases of before and after creatine monohydrate supplementation
* marks a significant difference compared to the resting period

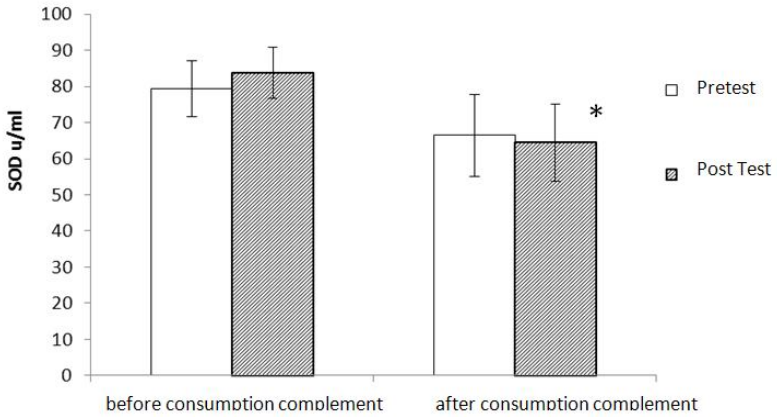

Figure 2. The amounts of SOD before and after the implementation of 6 bouts of high-intensity intermittent swimming in two phases of before and after creatine monohydrate supplementation
* marks a significant difference compared to the resting period

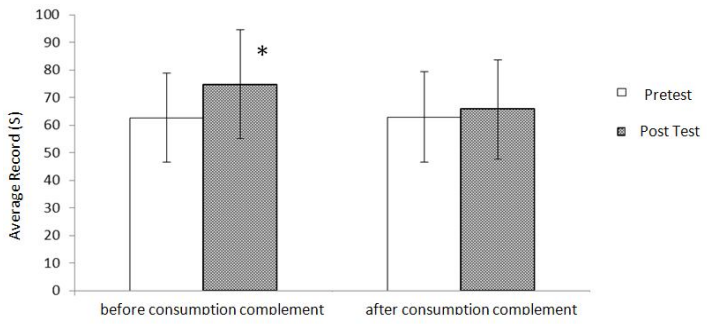

Figure 3. The record values before and after the implementation of 6 bouts of 50-meter high-intensity intermittent swimming in two phases of before and after creatine monohydrate supplementation
* marks a significant difference compared to the resting period

Discussion

The most important finding of the present study was that 6 bouts of 50-meter high-intensity intermittent swimming disturbed the homeostasis of systemic oxidative and antioxidative system in female swimmers. However, although one-week consumption of creatine supplementation did not completely inhibit the disorder in homeostasis system of oxidative and anti-oxidative factors, it moderated these responses. As mentioned in the introduction, one of the limitations of this study was the absence of remarkable human studies on the antioxidant effects of creatine and therefore it is difficult to compare research findings. However, literature review indicated that the results of this study were consistent with the findings of Azizi et al. (12) and inconsistent with the findings of Domenico et al. (13). Notwithstanding the foregoing, there were significant animal studies in this area. Stefani et al. (2014) examined the effects of creatine supplementation associated with 8 weeks of resistance training on oxidative and antioxidative markers in rats. The results suggested that oxidative markers decreased and SOD values increased (7). It is believed that contradictions in the results are caused by the differences in the intensity of training, research protocols, duration, experimental design and other factors such as age, gender, and genetics (14).

In this study, the 6 bouts of 50-meter high-intensity intermittent swimming significantly increased PC values and decreased SOD values. In line with this study, Kingsley et al. demonstrated that short-term use of creatine (20 g per day for 5 days) had no effects in reducing oxidative stress in plasma in acute cycling (13). In this/that study, creatine supplementation along 50-meter bouts of high-intensity intermittent swimming made a significant increase in PC values and reduced SOD levels significantly. The mean record in the 1st and 6th bout of 50- meter swimming before creatine supplementation period was 62.625 and 74.750 seconds, respectively, indicating a 16.19% increase. These values after one week of creatine monohydrate supplementation were 62.875 and 65.750, respectively, indicating an increase of 4.37%. Therefore, it is clear that one week of creatine monohydrate supplementation led to an 11.83% improvement in record values of high-intensity 50-meter swimming. Thus, changes related to the homeostasis of systemic stress can be attributed to changes induced by swimming record, i.e. speed enhancement. Hence, changes in PC and SOD values were despite the significant improvement observed in swimmers' performance. In other words, the speed of swimming bouts has increased, while no similar changes were observed in the values of oxidative and antioxidant markers which indicates the effect of creatine monohydrate supplementation. ROS attacks on amino acid releases Carbonyl group and forms PC, which is a protein oxidation marker that does not increase significantly at rest conditions before exercise. The carbonyl group might be formed by Protein through secondary reactions with aldehydes and then produce lipid peroxidation which leads to oxidative damage. Therefore, the homeostasis of the body requires increased antioxidant and antioxidant enzymes (e.g. SOD, GPx, ACT). The mitochondrial SOD is important in oxygen metabolism and struggles against oxidative stress, which reduces ROS (12, 15, 16).

Researchers reported that creatine plays a vital role in reducing the toxic effects of internal production of oxygen species that occurs in the short-term high-speed exercises. Creatine supplementation increases PCr in muscles and preventing ATP decline, produces energy for intense muscle activity. Increased energy forms hypoxanthine and then frees radicals resulting in the use of antioxidant reserves (6, 17). In this study, the blood lactate in the 6th bout after taking creatine had a significant increase compared to the 6th bout before taking creatine (P=0.006) and as described above, this increase was probably due to increased speed and reduced record after taking creatine. Blood lactate concentration after acute exercise can approve the increase in levels of oxidative stress markers and antioxidant changes. Increasing the buffering capacity, creatine supplementation reduces intracellular calcium reserves and ROS, weakens oxidative damages, prevents increased LDH in fast exercises, and increase lactate threshold, when it is used to prevent lipid peroxidation and cell damages. In addition, it increases the hydration and membrane stability and has a protective effect against damaged mitochondrial DNA and destructive RNA (1, 13). Hence, it is reported that the use of creatine supplementation causes cellular stimulation of young genes to synthesize antioxidants (6). Mazloom attributed reduced MDA to indirect antioxidative effects of creatine including the presence of arginine in the structure of creatine and production of NO through sulfur components of creatine (cysteine and methionine) that both are sensitive to free radicals (11). Brites et al. showed that the mitochondrial activity of SOD increased in trained volleyball players (12). Rahimi (2012) suggested that creatine supplementation reduced oxidative damages and lipid peroxidation levels which are consistent with the findings of this study (18).

In summary, the results showed that 6 bouts of 50-meter high-intensity intermittent swimming were associated with disorders in systemic anti-stress and stress marker levels. In addition, based on the findings of this study, taking creatine monohydrate supplementation for one week improved the performance of sprint swimming in women and also moderated this homeostasis response. Further studies are needed to approve the findings of this study in human subjects.

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