Asian J Sports Med. 2016 March; 7(1): e26843. DOI: 10.5812/asjsm.26843

Published online 2016 March 1. **Research Article**

Repeated Supramaximal Exercise-Induced Oxidative Stress: Effect of β-Alanine Plus Creatine Supplementation

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Received: January 10, 2015; **Accepted:** January 25, 2015

Background: Carnosine is a dipeptide formed from the β-alanine and histidine amino acids and found in mainly in the brain and muscle, especially fast twitch muscle. Carnosine and creatine has an antioxidant effect and carnosine accounts for about 10% of the muscle's ability to buffer the H^+ ions produced by exercise.
Objectives: The aim of the study was to investigate the effects of beta alanine and/or creatine supplementation on oxidant and antioxidant status during repeated Wingate tests (WTs).
Patients and Methods: Forty four sedentary males participated in the study. Participants performed three 30s WTs with 2 minutes rest between exercise bouts. After the first exercise session, the subjects were assigned to one of four groups: Placebo, Creatine, Beta-alanine and Beta-alanine plus creatine. Participants ingested twice per day for 22 consecutive days, then four times per day for the following 6 days. After the supplementation period the second exercise session was applied. Blood samples were taken before and immediately after the each exercise session for the analysis of oxidative stress and antioxidant markers.
Results: Malondialdehyde levels and superoxide dismutase activities were affected by neither supplementation nor exercise. During the pre-supplementation session, protein carbonyl reduced and oxidized glutathione (GSH and GSSG) levels increased immediately after the exercise. However, during the post-supplementation session GSH and GSSG levels increased in beta-alanine and beta-alanine plus creatine groups immediately after the exercise compared to pre-exercise. In addition, during the post-supplementation session total antioxidant capacity increased in beta-alanine group immediately after the exercise.
Conclusions: Beta-alanine supplementation has limited antioxidant effect during the repeated WTs.

Keywords: Beta-Alanine; Creatine; Circuit-Based Exercise; Antioxidant; Oxidative Stress

1. Background

Oxidative stress was first defined by Sies et al. (1) as a disturbance between the pro-oxidant and antioxidant balance in favor of the pro-oxidants. In some situations such as exercise, an excessive production of pro-oxidants or a suppression of antioxidant defenses causes an imbalance (2, 3). Exercise induced increase in production of free radicals may be an underlying cause of exercise-induced disturbances in muscle redox status and contributed to muscle fatigue and exercise-induced muscle damage (4).

Recently, antioxidant supplementation has become a common practice among both the professional and amateur athletes (5). Antioxidant supplementation prevents the deleterious effects of exercise-induced oxidative stress, accelerates recovery of muscle function and improves performance. Indeed, there is now an enormous range of vitamins, minerals and extracts such as vitamin E, vitamin C, β-carotene, coenzyme Q10, α-lipoic acid, quercetin, resveratrol, grape seed extract, taurine, carnosine, creatin and β-alanine used as antioxidant supplement in exercise studies in both human and animal subjects (6).

It has been demonstrated that anaerobic exercise, such as 30-second supramaximal Wingate test (WT), strongly stimulates both the adenosine triphosphate-phosphocreatine and glycolytic systems (7), and thus activates purine catabolism and lactic acid production (8). Therefore it may induce oxidative damage to proteins, lipids, and DNA (9).

β-alanine is a nonessential and nonproteogenic amino acid that is synthesized in the liver (10). The ergogenic properties of β-alanine by itself appear to be very limited, but when combined with histine to form carnosine in the skeletal muscle, it does appear to have ergogenic effects (11). A number of explanations have been suggested for such an effect, such as H^+ buffering, an increase in the efficiency of electromechanical coupling, stimulation of ATPase activity of contractile proteins and activation of ATP producing enzymes. It is also a powerful antioxidant and protects cell membranes and other cell structures (12). In many studies it has been clearly demonstrated that, 2-4 weeks of β-alanine supplementation (1.6 - 6.4 $g.d^{-1}$) increases carnosine concentrations in muscle tissue (13).

The effects of creatine supplementation on muscle creatine phosphate concentration and exercise performance

have been extensively investigated by many studies (14-16). Creatine supplementation also has an antioxidant potential (16). It has been claimed that creatine and β-alanine work synergistically in muscle tissue and reduce intramuscular lactate accumulation and buffer H^+ during incremental exercise test (17). In the present study it has been hypothesized that creatine and β-alanine supplementation may have antioxidant effect during the repeated bouts of supramaximal exercise. Because, during the supramaximal exercises both lactate accumulation and H^+ production are the main sources of the exercise-induced oxidative stress. To our knowledge, only two studies investigated the effects of β-alanine supplementation on exercise-induced oxidative stress, but no study to date has investigated antioxidant effect of β-alanine plus creatine supplementation. Dawson et al. (18) reported that β-alanine supplementation did not affect exercise-induced oxidative stress in muscle tissue of rats. In a recent study (19), it has been reported that β-alanine supplementation have limited antioxidant effect on aerobic exercise-induced oxidative stress in moderately trained women.

While numerous studies (10, 13) demonstrated role of β-alanine and creatine as an ergogenic aid, the potential of oral β-alanine and creatine intake to improve antioxidant capacity especially during the repeated high-intensity activities has yet to be fully quantified. It is thus important to measure, oxidative stress and antioxidant defense markers. Therefore, in the present study we aimed to investigate the effects of β-alanine and creatine supplementation on oxidative stress and antioxidant defense systems during the repeated bouts of supramaximal exercises in sedentary men.

2. Objectives

The aim of the study was to investigate the effects of beta alanine and/or creatine supplementation on oxidant and antioxidant status during repeated WTs.

3. Patients and Methods

3.1. Subjects

Forty four healthy, nonsmoker and sedentary men (aged 21.7 ± 1.9 years, height 175.9 ± 5.9 cm, and body weight 70.9 ± 7.9 kg) participated in the present study. Subjects completed a medical history, diet and supplementation history, and physical activity questionnaire to determine eligibility. No subject was a vegetarian or a smoker, nor did they use anti-inflammatory drugs or any other antioxidant supplements before (for a minimum of 6 months) or during the study period. Individuals did not participate in any regular form of aerobic and/or anaerobic exercise for at least the past year.

The study protocol was approved by the local ethical committee of Meram Faculty of Medicine, Selcuk University. Written informed consent was obtained from all study participants after they were informed about the purpose, procedures, and possible risks of the study. A randomized, placebo controlled design was used in the study. Each participant visited the laboratory twice to undergo pre- and post- testing.

In the baseline exercise session, exercise tests were performed on all participants. After the baseline exercise session, the participants were randomly allocated to one of the four experimental groups. At the end of 28 days of the supplementation period, a second exercise session the same as the baseline exercise session was administrated. Blood samples were taken before and immediately after the each exercise session. The choice of 11 subjects in each group was based on practical consideration and previously published studies (17, 19-21). This sample size was determined to permit a power of 80%.

3.2. Supplementation Protocol

After the pre-supplementation exercise session, the subjects were randomly assigned one of the four treatment conditions using a double blind design: a) Placebo (PL; 10 g maltodextrose; n = 11), b) Creatine (Cr; 5 g creatine plus 5 g maltodextrose; n = 11), c) Beta-alanine (β-ALA; 1.6 g beta alanine plus 8.4 g maltodextrose; n = 11) and d) Beta-alanine plus creatine (β-ALA+Cr; 1.6 g beta alanine plus 5 g creatine plus 3.4 g maltodextrose; n = 11).

The beta-alanine and creatine powders were purchased from General Nutrition Center (GNC, Pittsburgh, PA, USA). All supplements were in the same color and taste. Participants ingested the supplements twice per day for 22 consecutive days, then four times per day for the following 6 days. This dosage and supplementation regimen were chosen since previous studies (17, 19) have demonstrated the effectiveness of this supplementation protocol.

3.3. Exercise Protocols

Each exercise session started in the same hours (between 9 AM and 11 AM), 2 hours after a carbohydrate-rich light breakfast, and consisted of three 30 -second WTs with a 2 -minute rest between consecutive exercise bouts. Before the tests, each participant's body weight and height were measured. The WT was performed on an Ergometric 894 E Peak Bike (Monark Exercise AB, Varberg, Sweden) against a resistance of 75 $g.kg^{-1}$ body weight (4.41 $J.pedal\ revolution^{-1}.kg^{-1}$ body weight). The participants were allowed to pedal unloaded and instructed to reach a pedaling rate of 100 revolutions per minute. The predetermined load was applied to the flywheel automatically by the Monark Anaerobic Test Software and the participants were verbally encouraged to maintain as high a pedaling rate as possible throughout the test period. The subjects rested on the ergometer during two-minute intervals between subsequent tests. All subjects completed the two of the exercise sessions.

3.4. Blood Sampling

Blood samples were taken before and immediately after the each exercise session. Samples were obtained via va-

cutainers into the EDTA-coated tubes, gently inverted five consecutive times and immediately centrifuged at 3200 rpm for 15 minutes. Plasma samples were stored at -80°C until to the time of analysis.

3.5. Biochemical Analysis

Lipid peroxidation was measured using standardized thiobarbituric acid reactive substances (TBARS) Assay Kit (Cat. no 10009055; Cayman Chemical, Ann Arbor, USA). The measurement of plasma TBARS was based on the formation of malondialdehyde (MDA) according to the manufacturer's instructions. MDA levels were expressed as $nM.L^{-1}$.

Superoxide dismutase (SOD) activity was determined using a commercialized chemical SOD assay kit (Cat. no 706002; Cayman Chemical, Ann Arbor, USA). The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. SOD activity was expressed as $U.mL^{-1}$.

Protein oxidation was measured by Cayman's protein carbonyl (PC) assay kit (Cat. no 10005020; Ann Arbor, USA) as carbonyl content in the samples. This kit is based on the principle which utilizes the 2, 4 -Dinitrophenylhydrazine reaction to measure the PC content in sample. The absorbance was measured at a wavelength of 360 nm by using a plate reader (Powerwave XS, Biotek Inc. USA). PC levels were expressed as $nmol.mL^{-1}$.

Concentrations of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the plasma samples were determined by a Cayman's GSH assay kit (Cat. No 703002; Cayman Chemical, Ann Arbor, USA) using an enzymatic recycling method. In this assay, both GSH and GSSG levels were measured. GSH and GSSG levels were expressed as $\mu M.mL^{-1}$.

Total antioxidant capacity (TAC) was assessed by using the colorimetric 6 -hydroxy- 2, 5, 7, 8 -tetramethylchroman- 2 -carboxylic acid (Trolox)-equivalent antioxidant capacity assay kit (Cat. No 709001; Cayman Chemical, Ann Arbor, USA), which is based on the suppression of the absorbance of radical cations of 2,2' - azino-di- (3 -ethylbenzthiazoline sulphonate) by antioxidants in the sample when ABTS is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide. The absorbance was read at a wavelength of 405 nm using a Micro Plate Reader (Powerwave XS, Biotek Inc. USA). TAC activity was expressed as $mM.mL^{-1}$.

3.6. Statistical Analysis

Statistical analysis was performed with SPSS 15.0 for Windows. All data are presented as mean ± SD. Within-group, one-way repeated measures ANOVA and post hoc pairwise, Bonferroni-corrected comparisons were conducted. Time dependent changes among the groups were examined using the analysis of covariance (ANCOVA). A p value less than 0.05 was considered as statistically significant.

4. Results

MDA levels and SOD activities of the groups are demonstrated in Table 1 and Table 2, respectively. MDA levels and SOD activities were affected by neither supplementation nor exercise ($P > 0.05$). Besides, they were not different among the groups ($P > 0.05$). PC levels significantly increased immediately after the exercise compared to pre-exercise during the pre-supplementation session ($P < 0.05$) (Table 3). However, during the post-supplementation session, PC levels did not change immediately after the exercise compared to pre-exercise ($P > 0.05$). Furthermore, during the post-supplementation session PC levels were not different among the groups ($P > 0.05$). GSH and GSSG levels of the groups during the repeated bouts of supramaximal exercise are demonstrated in Table 4 and Table 5, respectively. During the pre-supplementation exercise session GSH and GSSG levels significantly increased immediately after the exercise compared to pre-exercise ($P < 0.05$). During the post-supplementation session GSH levels were significantly elevated immediately after the exercise in Cr, β-ALA and β-ALA + Cr groups compared to pre-exercise ($P < 0.05$). Additionally, GSSG levels increased in β-ALA and β-ALA + Cr groups immediately after the exercise compared to pre-exercise during the post-supplementation session ($P < 0.05$). However, during the post-supplementation session GSH and GSSG levels were not different among the groups ($P > 0.05$). TAC levels of the groups during the pre- and post- supplementation exercise sessions are demonstrated in Table 6. During the pre-supplementation session TAC levels were not different between the pre- and post- exercise sessions ($P > 0.05$). During the post-supplementation session TAC levels significantly increased in β-ALA group immediately after the exercise compared to pre-exercise ($P < 0.05$). However, there was no statistically significant difference among the groups ($P > 0.05$).

Table 1. Effects of Beta-Alanine and/or Creatine Supplementation on MDA ($nM.L^{-1}$) Levels Before and After the Three Wingate Tests [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	65.03 ± 6.77	65.21 ± 4.24		68.03 ± 7.94	68.38 ± 8.23	0.879
Cr	11	65.12 ± 6.94	65.66 ± 5.55		67.48 ± 8.11	77.65 ± 32.87	0.337
β-ALA	11	64.41 ± 6.58	64.74 ± 6.78		73.83 ± 32.61	70.63 ± 12.53	0.752
β-ALA + Cr	11	64.97 ± 4.63	66.47 ± 4.37		68.47 ± 7.25	74.53 ± 13.24	0.111
Total	44	64.87 ± 6.12	65.48 ± 5.24	0.235			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD.

Table 2. Effects of Beta-Alanine and/or Creatine Supplementation on SOD (U.mL^{-1}) Activities Before and After the Three Wingate Tests [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	28.5 ± 9.6	30.2 ± 8.7		33.4 ± 14.7	33.1 ± 8.6	0.880
Cr	11	27.7 ± 7.8	25.8 ± 9.5		27.0 ± 4.7	30.1 ± 6.6	0.122
β-ALA	11	33.5 ± 12.2	31.8 ± 9.6		32.0 ± 9.3	35.1 ± 14.2	0.077
β-ALA + Cr	11	29.1 ± 7.1	29.0 ± 6.1		25.8 ± 7.4	30.0 ± 8.7	0.123
Total	44	29.8 ± 9.5	29.3 ± 8.7	0.654			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD.

Table 3. Effects of Beta-Alanine and/or Creatine Supplementation on PC Levels (nmol.mL^{-1}) Before and After the Repeated Supramaximal Exercise Tests [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	305.3 ± 167.8	287.9 ± 139.6		288.4 ± 76.7	285.7 ± 159.5	0.956
Cr	11	241.8 ± 157.9	303.1 ± 158.5		259.9 ± 144.8	238.6±112.8	0.734
β-ALA	11	273.8 ± 200.1	342.7 ± 79.1		272.2 ± 230.7	333.7 ± 173.9	0.491
β-ALA + Cr	11	206.4 ± 128.8	303.7 ± 138.0		187.6 ± 101.6	276.5 ± 119.8	0.160
Total	44	256.8 ± 162.7	309.3 ± 128.1 [c]	0.050			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD.
[c] $P < 0.05$ compared to pre-supplementation session pre-exercise.

Table 4. Effects of Beta-Alanine and/or Creatine Supplementation on GSH Levels (μM.mL^{-1}) Before and After the Three Wingate Tests [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	7.61 ± 2.38	9.64 ± 3.25		7.80 ± 2.74	8.66 ± 3.07	0.297
Cr	11	7.46 ± 3.29	11.32 ± 4.86		7.03 ± 2.70	9.03 ± 3.51[b]	0.050
β-ALA	11	8.94 ± 2.85	9.74 ± 3.58		6.88 ± 2.37	9.80 ± 3.55[b]	0.011
β-ALA + Cr	11	7.21 ± 3.44	11.08 ± 3.50		8.19 ± 3.93	12.01 ± 4.05 [c]	0.008
Total	44	7.80 ± 3.00	10.40 ± 3.80 [d]	0.000			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD.
[c] $P < 0.05$ compared to post-supplementation session pre-exercise.
[d] $P < 0.05$ compared to pre-supplementation session pre-exercise.

Table 5. Effects of Beta-Alanine and/or Creatine Supplementation on GSSG Levels (μM.mL^{-1}) Before and After the Three Wingate Tests [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	3.61 ± 1.10	4.61 ± 1.49		3.69 ± 1.22	4.12 ± 1.41	0.276
Cr	11	3.55 ± 1.56	5.39 ± 2.23		3.35 ± 1.31	4.28 ± 1.66	0.076
β -ALA	11	4.28 ± 1.30	4.65 ± 1.65		3.27 ± 1.07	4.66 ± 1.72[b]	0.013
β -ALA + Cr	11	3.44 ± 1.69	5.28 ± 1.65		3.88 ± 1.88	5.61 ± 1.86 [c]	0.009
Total	44	3.74 ± 1.14	4.97 ± 1.75 [d]	0.000			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD.
[c] $P < 0.05$ compared to post-supplementation session pre-exercise.
[d] $P < 0.05$ compared to pre-supplementation session pre-exercise.

Table 6. Effects of Beta-Alanine and/or Creatine Supplementation on TAC Levels ($mM.mL^{-1}$) Before and After the Repeated Supramaximal Exercise Test [a,b]

	n	Pre-Supplementation		P	Post-Supplementation		P
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise	
PL	11	6.01 ± 5.97	7.43 ± 8.07		9.32 ± 9.81	8.28 ± 7.40	0.466
Cr	11	7.63 ± 6.91	9.30 ± 8.57		9.25 ± 7.04	8.98 ± 6,69	0.788
β–ALA	11	8.67 ± 7.35	8.91 ± 5.61		8.96 ± 6.52	11.89 ± 6.51 [c]	0.045
β-ALA + Cr	11	9.64 ± 6.76	9.17 ± 5.39		12.60 ± 8.72	9,92 ±5 .89	0.090
Total	44	7.97 ± 6.68	8.70 ± 6.85	0.365			

[a] Abbreviations: β-ALA, Beta-alanine; β-ALA + Cr, Beta-alanine plus creatine; Cr, Creatine; PL, Placebo.
[b] All values are presented as mean ± SD
[c] $P < 0.05$ compared to post-supplementation session pre-exercise.

5. Discussion

The results of the present study suggest that 28 days of β-alanine supplementation especially in combination with creatine has a slight antioxidant effect during the repeated bouts of supramaximal exercise. For the evaluation of exercise-induced oxidative stress, markers such as MDA, SOD, PC, GSH, GSSG and TAC were chosen.

Although it has been reported that moderate and high intensity aerobic exercise increases the production of reactive oxygen and nitrogen species; exceeding the capacity of antioxidant defense (22-25), there are contradictory reports (8, 26-28) about the effects of anaerobic exercise on blood oxidative stress markers. To our knowledge, limited studies (18, 19) investigated the antioxidant effects of β-alanine.

In the present study neither exercise nor supplementation affected MDA levels. As opposed to this, Marzatico et al. (29) found elevated plasma MDA levels 6 to 48 hours after six sprints (150 m) in sprint athletes. Groussard et al. (8) demonstrated an increase in lipid radical production for 20 minutes after a short-term supramaximal exercise test (Wingate test) using electron spin resonance spectroscopy in male physical education students. Nevertheless, no change was seen at 20 and 40 minutes of post exercise recovery in TBARS and the authors (8) suggested that it was due to post exercise clearance from the plasma. In contrast to these results, Ortenblad et al. (30) found no change in MDA in either muscle or blood in trained and untrained subjects following a strenuous jumping protocol consisting of six 30 s continuous jumping. Cuevas et al. (31) showed that plasma TBARS levels were unchanged after both a single Wingate test and a series of four Wingate tests with the rest intervals of 60 minutes in professional cyclists. The present findings support the results of an earlier study from the same laboratory that MDA levels were not affected immediately after the repeated bouts of supramaximal exercises (5 Wingate tests), but were increased 15 and 60 minutes after the exercise in sedentary males. These findings indicated that the blood sampling time is important for determination of the MDA levels. The differences among the results may depend on the duration and the intensity of the exercise, supplementation protocol and analysis method of MDA.

In the present study β-alanine and/or creatine supplementation did not affect MDA levels. Dawson et al. (18) reported that β-alanine supplementation did not affect MDA levels in soleus and triceps muscles of rats in exercise-induced muscle injury induced by downhill treadmill running. Our present findings support the data of Dawson et al. (18).

In the present study, SOD yielded no significant changes from pre- to post- supplementation or between groups. Miyazaki et al. (32) found an increase in resting erythrocyte SOD activity with a 12-week endurance-training program, and unchanged enzyme activity in response to the acute exercise bout either before or after training in sedentary men. Consistent with our findings, in a recent study (27) we observed unchanged SOD activities immediately after the repeated bouts of supramaximal exercises in sedentary males. This may depend on the intensity of the exercise and time of the blood sampling. In the present study β-alanine and/or creatine supplementation did not affect plasma SOD activity. Similar to our findings, Zoeller et al. (19) observed no significant changes in SOD activity with β-alanine supplementation.

The most general indicator and by far the most commonly used marker of protein oxidation is PC content (33). Relatively few data are currently available in relation to PC following the anaerobic exercise and to our knowledge no study to date has investigated the effects of repeated bouts of supramaximal exercises. In some previous studies (26, 34), it has been demonstrated that PC levels tended to increase immediately after the anaerobic exercise, but significantly increase at 6 hours post-exercise. In the present study, during the pre-supplementation exercise session, PC levels significantly increased immediately after the exercise when compared to the pre-exercise. However, during the post-supplementation exercise session, PC levels were affected by neither supplementation nor exercise. Although the lowest PC values have been observed in β-ALA+Cr group, these were

not statistically significant. These findings suggest that blood sampling time is as important as is the duration and the intensity of the exercise.

Acute strenuous bouts of exercise stress and single WT (8) have been shown to cause a decrease in the GSH and an increase in the GSSG levels, thereby decreasing the GSH: GSSG ratio (35-37). Sastre et al. (38) observed linear relationships between GSSG-GSH and lactate to pyruvate ratios before, during, and after the exercise. In the present study, during the pre-supplementation exercise session, both GSH and GSSG levels significantly increased immediately after the exercise when compared to pre-exercise. However, during the post-supplementation exercise session, GSH levels significantly increased in Cr, β-ALA and β-ALA + Cr groups and GSSG levels increased in β-ALA and β-ALA + Cr groups immediately after the exercise when compared to pre-exercise. However, there was no difference among the groups. In consistence with our findings, Rietjens et al. (39) demonstrated that a single session of resistance exercise causes an increase in erythrocyte GSH levels. This situation was attributed to adaptive response of body against exercise induced increase in radical production. In the present study, elevation of both GSH and GSSG levels immediately after the exercise in supplementation with both β-alanine and creatine might be attributed to β-alanine and creatine supplementation stimulating adaptive response to exercise and therefore increase GSH levels against the oxidation of GSH.

In the present study TAC levels increased with β-alanine supplementation immediately after the exercise compared to pre-exercise during the post-supplementation session. TAC represents the ability of serum to neutralize reactive species and is greatly affected by changes in concentration of uric acid and bilirubin (37). It may be difficult to interpret because it may increase as a result of numerous internal antioxidants, as well as to adaptations in nutrition and/or the adaptability to a state of oxidative stress (40, 41). Rietjens et al. (39) and Nikolaidis et al. (37) demonstrated that a single session of resistance exercise causes an increase in plasma TAC levels. Although there was no significant difference among the groups, increase of the TAC levels in β-alanine supplemented group partially supported the antioxidant potential of β-alanine.

In conclusion, the results of the present study demonstrate that beta alanine and/or creatine supplementation, in vivo, yields minimal effects on oxidative stress and antioxidant markers after repeated bouts of short term supramaximal exercise. However, more detailed researches are needed to clarify the exact mechanism of action and synergic effects of β-alanine and creatine.

Acknowledgements

The authors acknowledge Professor Said Bodur for statistical assistance.

Funding/support

This work was supported by Scientific Research and Project Coordinator of Selcuk University (Grant number: 09401101).

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