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The Effect of Creatine Supplementation on Some Oxidative Stress Indices and the Glutathione Ratio (GSSG/GSH) After Repeated Wingate Tests in Wrestlers

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Abstract

Introduction: The purpose of this study was to investigate the effects of creatine supplementation on malondialdehyde (MDA), oxidized glutathione (GSSG), reduced glutathione (GSH), and the GSSG/GSH ratio following repeated Wingate tests in wrestlers.

Methods: This randomized, double-blind, placebo-controlled crossover study included 10 male wrestlers (mean age, 22.5 ± 3 years). The exercise protocol consisted of four bouts separated by 15 min of passive recovery; each bout included three 10-s leg Wingate tests and three 10-s arm Wingate tests performed alternately, with 1 min of active recovery between tests. In each experimental session, participants consumed 0.1 g/kg body mass of creatine or placebo 1 h before the protocol. The second dose was administered after the second bout and the third dose after completion of the fourth bout. Blood samples were obtained before the first dose, immediately after completion of the exercise protocol, and after 1 h of recovery.

Results: Creatine supplementation, compared with placebo, attenuated the increase in GSSG (time × condition interaction, P = 0.008) and MDA (time × condition interaction, P = 0.001). No significant time × condition interactions were observed for GSH (P = 0.21), the GSSG/GSH ratio (P = 0.20), or blood lactate (P = 0.71).

Conclusions: Acute creatine supplementation may attenuate selected oxidative stress responses, particularly increases in GSSG and MDA, in wrestlers performing repeated high-intensity exercise. However, further research is warranted because other oxidative and antioxidant indices were not significantly affected.

Key Words: Creatine; oxidative stress; glutathione; malondialdehyde; Wingate test; wrestler.

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1. Introduction

Oxidative stress is defined as a disruption of the balance between pro-oxidants and antioxidants in favor of pro-oxidants. Under certain conditions, such as exercise, this imbalance may result from excessive production of pro-oxidants or suppression of antioxidant defenses (1). One of the common physiological conditions associated with increased oxidative stress is exercise (2). Exercise may exert both beneficial and detrimental effects on oxidative stress. High-intensity exercise and physical activity can lead to a transient imbalance between the production and elimination of reactive oxygen/nitrogen species (ROS/RNS), thereby resulting in oxidative stress (3). Although exercise-induced ROS are required for normal force production in skeletal muscle, excessive ROS levels appear to impair contractile function (4). Exercise-induced ROS production is also important for exercise-induced mitochondrial biogenesis, as ROS act as signaling molecules that activate redox-sensitive signaling pathways (5).

Evidence indicates that exercise intensity and duration are associated with oxidative stress in humans, a finding supported by several studies (6, 7). Vigorous exercise in untrained individuals is associated with greater increases in oxidative stress compared with moderate and regular aerobic exercise (8). Furthermore, prolonged regular exercise may enhance certain antioxidant defense mechanisms and, consequently, may limit oxidative damage to mitochondria (9).

On the other hand, many athletes use ergogenic aids as part of long-term exercise programs to maintain physical fitness, improve recovery, and promote physiological adaptations. Therefore, the effects of ergogenic aids have received considerable attention, and many researchers have attempted to combine exercise programs with ergogenic supplementation to enhance the benefits of exercise (10). The use of antioxidant supplements in conjunction with physical activity may reduce the detrimental effects of exercise-induced oxidative stress, enhance exercise-related antioxidant defenses, and augment the beneficial effects of physical activity (11).

Creatine is one of the most popular supplements among athletes. Creatine (Cr) is a naturally occurring amino acid-like compound in humans. Approximately 95% of the body's creatine is stored in skeletal muscle, with about 40% present in free form and 60% as phosphocreatine (PCr) (12). Creatine supplementation is believed to help maintain high-energy phosphate stores during exercise. In addition, several mechanisms by which creatine supplementation may improve exercise performance have been identified (13). Creatine is predominantly used in anaerobic sports to facilitate ATP resynthesis and increase anaerobic power (14). However, there is still uncertainty regarding the effects of creatine on oxidative stress and the underlying mechanisms involved. The antioxidant effects of creatine may be attributable to several mechanisms, including indirect mechanisms involved in stabilizing cell membranes and improving cellular energy capacity, as well as its direct antioxidant properties (13).

Oxidative stress can impair muscle strength and physical performance (13). Mechanistically, reactive oxygen species may accelerate skeletal muscle fatigue by reducing calcium sensitivity and decreasing the maximal force generated in response to calcium activation (15). Several studies have investigated the potential antioxidant properties of creatine. For example, Lawler et al. (2002) demonstrated that creatine significantly reduced the levels of superoxide anion and peroxynitrite, which are important free radicals, under in vitro conditions (16). In contrast, Deminice et al. (2013) reported that creatine supplementation had no effect on antioxidant parameters and was insufficient to attenuate exercise-induced oxidative stress following repeated acute sprint exercise. They suggested that further studies are required to confirm the antioxidant effects of creatine supplementation in humans (17).

Based on the available evidence, creatine appears to possess antioxidant properties and may exert its effects through both direct and indirect mechanisms. Creatine may preserve mitochondrial integrity, increase PCr availability, act as a cellular energy buffer, and protect

mitochondrial DNA (mtDNA) and RNA against oxidative damage (13). Furthermore, the antioxidant properties of creatine may be related to its constituent amino acids, including arginine, glycine, and methionine (18). Some studies have attributed the antioxidant properties of creatine to the presence of arginine in its molecular structure. Arginine serves as a substrate for the nitric oxide synthase family and may increase nitric oxide production; moreover, a protective role of arginine against oxidative stress has been demonstrated in endothelial cells. Arginine may also be capable of scavenging free radicals such as superoxide anions. In addition, creatine has been shown to influence glutathione (GSH) levels and the glutathione redox ratio (13).

In contrast, strenuous exercise may alter this balance in the opposite direction. Acute, high-intensity exercise, such as the Wingate test, has been shown to decrease GSH and increase oxidized glutathione (GSSG) levels, consequently altering the GSSG/GSH ratio (19). Creatine supplementation may potentially exert a synergistic effect with exercise; however, exercise intensity and duration, as well as the duration of supplementation, may play important roles in determining its antioxidant activity. To date, relatively few studies have investigated the effects of creatine supplementation combined with short-duration, single-session exercise on oxidative stress. Therefore, further research is needed to provide more definitive conclusions.

Given the limited evidence regarding the acute antioxidant effects of creatine supplementation during a single bout of anaerobic exercise, the present study was designed to investigate the effects of acute creatine supplementation before, during, and after repeated Wingate tests on the glutathione redox ratio (GSSG/GSH) and malondialdehyde (MDA) levels in wrestlers. Specifically, this study aimed to determine whether acute creatine supplementation could attenuate or counteract the oxidative stress induced by repeated bouts of high-intensity exercise.

2. Methods

2.1. Participants

The inclusion criteria were professional or semi-professional male wrestlers (with at least a provincial-level ranking), aged 18–30 years, with a minimum of 5 years of wrestling training experience. Participants' health status was confirmed using the Physical Activity Readiness Questionnaire (PAR-Q). Individuals who had not used supplements, including creatine or antioxidants, for at least 4 weeks before the study were eligible for participation. The exclusion criteria included acute or chronic diseases, recent acute injuries, use of medications that could affect metabolism, smoking or alcohol consumption, unusual dietary practices, or inability to complete the Wingate protocol. Participants were also required to refrain from strenuous exercise for at least 48 h before each experimental session. Accordingly, 10 male wrestlers from Tehran, all of whom had achieved at least a provincial-level ranking and had a history of regular wrestling training (7 ± 2 years), voluntarily participated in the study. Their mean age was 22.5 ± 3 years, and their mean BMI was 23 ± 3.7 kg/m².

2.2. Study design

This study was conducted using a randomized, double-blind, placebo-controlled crossover design. Participants visited the laboratory on three separate occasions. During the first visit, height, body mass, and BMI were measured. The second and third visits were experimental sessions conducted one week apart using a randomized crossover design.

2.3. Exercise and Supplementation Protocol

At 8:00 AM, participants consumed the first dose of either creatine or placebo. The creatine condition consisted of 0.1 g/kg body mass of creatine (NutraBio, USA) (20), whereas the placebo condition consisted of 0.1 g/kg body mass of an inert aspartame-flavored placebo dissolved in 250 mL of water (21). Participants then rested for 1 h. Following the rest period, the experimental protocol began with a 5-min warm-up consisting of cycling at 50 rpm followed by stretching exercises. The main exercise protocol consisted of four identical bouts, with 15 min of passive recovery between bouts. Each bout included three 10-s leg Wingate tests and three 10-s arm Wingate tests performed alternately, with 1 min of active recovery between tests. Following the warm-up, participants first performed the leg Wingate test on a cycle ergometer against a resistance of approximately 75 g/kg body mass. Immediately after

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the test, the resistance was removed and participants cycled without resistance at 50–60 rpm for 30 s. They then immediately transferred to the arm ergometer and completed another 30 s of active recovery without resistance at 50–60 rpm. The arm Wingate test was then performed against a resistance of approximately 45 g/kg body mass. Following the arm Wingate test, participants completed 30 s of active recovery on the arm ergometer followed by 30 s on the cycle ergometer. This sequence continued until three 10-s leg Wingate tests and three 10-s arm Wingate tests had been completed. Participants had 15 min of passive recovery between each of the four exercise bouts. The second dose of creatine or placebo was administered after completion of the second exercise bout, and the third dose was administered immediately after completion of all four bouts. Blood samples were collected at three time points: (1) before consumption of the first dose; (2) immediately after completion of the fourth exercise bout; and (3) after a 1-h recovery period (Figure 1). All participants completed both the creatine and placebo conditions in a counterbalanced order. Leg Wingate tests were performed using a Monark cycle ergometer (Model 894E), whereas arm Wingate tests were performed using a Monark arm-crank ergometer (Model 891E).

2.4. Dietary control and physical activity standardization

To standardize dietary intake, participants were instructed to replicate their dietary intake exactly during the 48-h preceding each experimental session using a detailed food diary. The diary included all foods and beverages consumed, including type, quantity, and time of consumption, to minimize the potential influence of dietary differences on the study outcomes. Caffeine-containing products, including coffee, tea, and energy drinks, as well as antioxidant-rich foods, were completely prohibited during this 48-h period. A list of antioxidant-rich foods was provided to the participants. On the morning of each experimental session, all participants received a standardized breakfast containing 500 kcal, consisting of 60% carbohydrate, 20% protein, and 20% fat, to minimize dietary variability.

2.5. Biochemical Analyses

GSSG, GSH, and MDA were analyzed using colorimetric assays with commercially available kits manufactured by Cayman Chemical (USA). The coefficients of variation were 3.6% for GSSG, 1.6% for GSH, and 2.3% for MDA.

2.6. Statistical analysis

Data were analyzed using SPSS software, version 26 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to assess the normality of data distributions. Because the data were normally distributed, parametric statistical methods were applied. A two-way mixed repeated-measures analysis of variance (ANOVA) was used to examine the effects of condition and time. Repeated-measures ANOVA was also used to compare time points within each experimental condition, followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Primary outcomes

Statistical analysis revealed that the oxidized glutathione (GSSG) response to repeated high-intensity exercise was dependent on supplementation condition, as indicated by a significant time × condition interaction ($P = 0.008$). Within-condition analysis showed that this difference was attributable to an increase in GSSG during the placebo condition ($P = 0.01$). Post hoc analysis further demonstrated that GSSG levels were significantly higher at the second and third time points than at the first time point ($P < 0.05$).

Similarly, the malondialdehyde (MDA) response to repeated high-intensity exercise was dependent on supplementation condition, with a significant time × condition interaction ($P < 0.001$). Within-condition analysis indicated that the observed difference was attributable to an increase in MDA during the placebo condition ($P < 0.001$). Post hoc analysis showed that MDA levels at the third time point were significantly higher than those at both the first and second time points ($P < 0.05$), (Table 1, Figure1).

In contrast, no significant time × condition interactions were observed for reduced glutathione (GSH) ($P = 0.21$), the GSSG/GSH ratio ($P = 0.20$), or blood lactate ($P = 0.71$). However, within-condition analysis followed by post hoc comparisons revealed a significant decrease in GSH from the first to the third time point in the creatine condition ($P = 0.005$). For the GSSG/GSH ratio, significant changes between the first and third time points were observed in both the creatine and placebo conditions ($P < 0.05$). Regarding blood lactate, the second time point differed significantly from both the first and third time points in both experimental conditions ($P < 0.05$).

Table 1 Results of repeated-measures ANOVA within and between the creatine and placebo conditions

Variable	Supplement	Pre-exercise (M±SD)	Post-exercise (M±SD)	Recovery (M±SD)	Within-session changes		Time effect		Session effect		Time × Session interaction	
					F	p	F	p	F	p	F	p
GSSG (µM)	Creatine	16.5±1.5	17.7±2.4	19.5±5.5	2.7	0.08	6.83	0.006*	7.54	0.02*	6.4	0.008*
	Placebo	16.4±3.7	25±8.1	22.5±3.5	5.9	0.01*						
GSH (µM)	Creatine	4.18±1.79	3.09±1.69	2.15±1.72	7.1	0.005*	5.9	0.01*	0.72	0.41	0.80	0.21
	Placebo	4.11±2.78	3.24±2.7	2.12±1.62	2.3	0.12						
GSSG/GSH	Creatine	0.26±0.11	0.18±0.09	0.11±0.10	9.9	0.001*	14.52	0.001*	0.08	0.77	1.7	0.20
	Placebo	0.21±0.13	0.11±0.09	0.09±0.06	6.7	0.006*						
MDA (µM)	Creatine	1.23±0.10	1.24±0.06	1.24±0.11	0.04	0.96	5.18	0.01*	26.21	0.001*	39.10	0.001*
	Placebo	1.03±0.07	1.07±0.12	1.26±0.05	13.76	0.001*						
Lactate (mg/dL)	Creatine	20.8±5.4	93.9±5.1	25.8±5.3	0.80	0.001*	72	0.001*	0.26	0.61	0.34	0.71
	Placebo	20±10	91.5±7.8	26.4±9.1	0.27	0.001*						

Note: * Indicates a statistically significant difference.

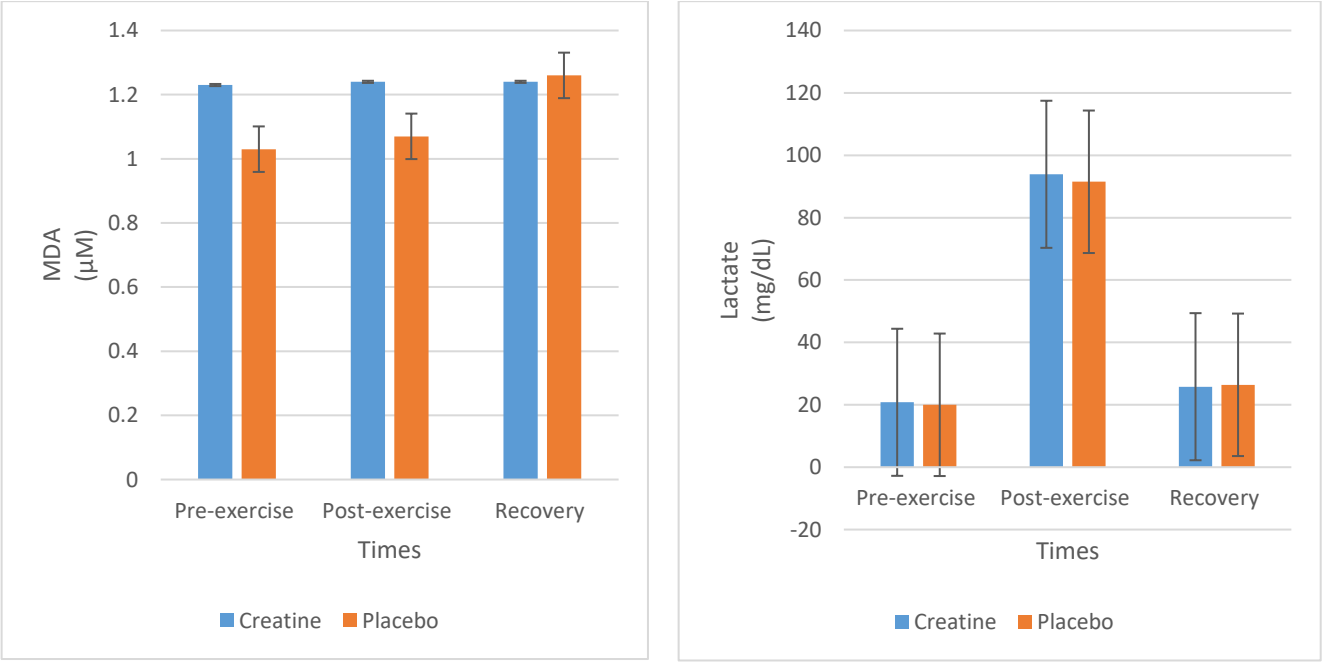

Figure 1. Changes in MDA and lactate levels at different times among different groups.

4. Discussion

In the present study, repeated high-intensity exercise, accompanied by increased lactate production and potential pathways leading to increased free radical generation, altered the measured oxidative stress markers. Specifically, MDA, GSH, GSSG, and the GSSG/GSH ratio changed significantly immediately after exercise compared with baseline in both experimental sessions. However, regarding the effects

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of creatine supplementation, the present findings indicated that creatine supplementation, compared with placebo, prevented the exercise-induced increase in oxidized glutathione and malondialdehyde, whereas no significant effects were observed for the other measured variables.

As described in the Introduction, increased exercise-induced free radical production may be one of the underlying mechanisms responsible for exercise-related disturbances in muscle redox status and may contribute to muscle fatigue and exercise-induced muscle damage (22). Anaerobic exercise, such as the Wingate test, has been shown to strongly stimulate both the adenosine triphosphate–phosphocreatine and glycolytic energy systems (23), thereby activating purine catabolism and lactic acid production (8). Consequently, these processes may contribute to oxidative damage to proteins, lipids, and DNA (24).

The significant increase in lactate observed at the second time point, followed by its return toward baseline at the third time point, was expected given the intensity of the exercise protocol and the limited recovery intervals. Previous research examining the contribution of different energy systems during a 30-s Wingate test demonstrated that approximately 56% of the energy supply is derived from the glycolytic/lactate system, whereas only 28% is provided by the phosphagen system (25). Similarly, the present protocol, which consisted of repeated 10-s Wingate bouts interspersed with 1-min active recovery periods, resulted in an increase in blood lactate following exercise. Consistent with these findings, Davoodian et al. (2012) reported a significant increase in lactate concentration following six 10-s Wingate tests; however, in contrast to the present study, they observed a significant difference in lactate levels between the creatine and placebo groups (26). In the present study, no significant difference in the magnitude of the lactate response was observed between the creatine and placebo conditions, which may be attributable to differences in the dose and duration of supplementation. In the study by Davoodian et al., participants received creatine supplementation over two days, with four doses administered per day. In contrast, the present study employed an acute supplementation model. Their protocol therefore involved a short-term loading strategy, which would be expected to increase total intramuscular creatine stores to a greater extent than acute supplementation. These findings suggest that the potential effects of creatine on lactate metabolism may depend on both the dose and duration of supplementation. Exercise-induced oxidative stress has been shown to decrease GSH and increase MDA and GSSG levels, thereby altering the GSSG/GSH ratio (24). This pattern was also confirmed in the present study, as anaerobic exercise induced these changes irrespective of the supplementation condition. Sastre et al. also reported linear relationships between the GSSG/GSH ratio and the lactate-to-pyruvate ratio before, during, and after exercise (27). Although relationships among these variables were not directly examined in the present study, the changes in lactate were generally consistent with changes in GSH, GSSG, and the GSSG/GSH ratio, providing indirect support for the findings reported by Sastre et al. Regarding the effects of creatine on the measured oxidative stress markers, the present findings demonstrated that creatine significantly prevented the increase in oxidized glutathione at the second blood-sampling time point compared with the placebo condition. Creatine also prevented the increase in MDA observed at the third time point. Creatine is synthesized from three amino acids—arginine, glycine, and methionine—with its synthesis involving several organs, including the liver, pancreas, and kidneys (28). Creatine is one of the most widely used ergogenic supplements among athletes at different performance levels. As a popular supplement in the sports and fitness industry, creatine is believed to help maintain high-energy phosphate stores during exercise. In addition, several mechanisms underlying the beneficial effects of creatine supplementation on exercise performance have been identified (29).

The precise mechanism underlying the antioxidant effects of creatine remains unclear. However, evidence suggests that creatine may enhance antioxidant enzyme activity and the capacity to scavenge reactive oxygen species (ROS) and reactive oxygen and nitrogen species (RONS). Creatine may also preserve mitochondrial integrity through organelle-directed antioxidant activity (30). Furthermore, creatine supplementation may be particularly effective during short-duration exercise by reducing intracellular calcium accumulation, limiting ROS formation, and attenuating oxidative damage, compared with its effects during prolonged exercise (13). The antioxidant effects of creatine may also be related to the presence of arginine within its molecular structure. Arginine is a substrate for the nitric oxide synthase family and can increase nitric oxide (NO) production, which contributes to the regulation of metabolism, muscle contraction, and glucose uptake in skeletal muscle. Other constituent amino acids, such as glycine and methionine, may also contribute

to antioxidant defense through mechanisms related to their chemical properties and susceptibility to oxidation (13). Araújo et al. reported that creatine supplementation acted synergistically with exercise to increase antioxidant enzyme activity in the liver of rats (31). Their results demonstrated increased glutathione peroxidase activity in both the exercise and exercise-plus-creatine groups compared with the control group. The discrepancy between the findings of the present study and those of previous investigations may primarily be attributable to differences in the supplementation protocol. To the best of our knowledge, the present study is among the few investigations to examine the effects of acute creatine supplementation on oxidative stress responses to repeated high-intensity exercise, whereas most previous studies investigating creatine supplementation have employed multi-day loading protocols. It is therefore possible that a single acute dose of creatine is insufficient to induce the full antioxidant effects expected from creatine supplementation following increased intramuscular creatine stores. Nevertheless, the present findings suggest that acute supplementation may still have a protective effect against increases in MDA and GSSG.

The small sample size represents an important limitation of the present study and may have reduced the statistical power to detect significant changes in all measured variables. Therefore, the generalizability of the findings should be considered with caution, and future studies involving larger sample sizes are warranted. Although some dietary controls were implemented, another limitation was the absence of comprehensive monitoring of all food and fluid intake throughout the study period. Future investigations should therefore include a more comprehensive assessment and control of participants' dietary intake, together with larger sample sizes, to improve interpretation of the findings and provide more robust validation of the observed differences.

5. Conclusion

Overall, the findings suggest that acute creatine supplementation may exert protective effects on selected oxidative and antioxidant markers, particularly oxidized glutathione (GSSG) and malondialdehyde (MDA), during repeated high-intensity exercise. However, given the limited number of studies investigating acute, single-session creatine supplementation and the inconsistencies among existing findings, further well-controlled studies with larger sample sizes and different supplementation protocols are needed to clarify the acute antioxidant effects of creatine supplementation.

6. Highlights

Acute creatine supplementation may help limit increases in oxidative stress markers during repeated high-intensity Wingate exercise.

Creatine supplementation attenuated increases in oxidized glutathione (GSSG) and malondialdehyde (MDA) following repeated Wingate tests.

Statements

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

All authors participated in the design, implementation, and writing of all parts of this study.

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