Creatine Supplementation Decreases Oxidative DNA Damage and Lipid Peroxidation Induced by a Single Bout of Resistance Exercise

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Abstract
Rahimi, R. Creatine supplementation decreases oxidative DNA damage and lipid peroxidation induced by a single bout of resistance exercise. J Strength Cond Res 25(12): 3448–3455, 2011—Creatine (Cr), or methyl guanidine–acetic acid, can be either ingested from exogenous sources, such as fish or meat, or produced endogenously by the body, primarily in the liver. It is used as an ergogenic aid to improve muscle mass, strength, and endurance. Heretofore, Cr’s positive therapeutic benefits in various oxidative stress-associated diseases have been reported in the literature and, recently, Cr has also been shown to exert direct antioxidant effects. Therefore, the purpose of this study was to investigate the effects of an acute bout of resistance exercise (RE) on oxidative stress response and oxidative DNA damage in male athletes and whether supplementation with Cr could negate any observed differences. Twenty-seven resistance-trained men were randomly divided into a Cr supplementation group (the Cr group [21.6 ± 3.6 years], taking 4 × 5 g Cr monohydrate per day) or a placebo (PL) supplementation group (the PL group [21.2 ± 3.2 years], taking 4 × 5 g maltodextrin per day). A double-blind research design was employed for a 7-day supplementation period. Before and after the seventh day of supplementation, the subjects performed an RE protocol (7 sets of 4 exercises using 60–90 1 repetition maximum) in the flat pyramid loading pattern. Blood and urine samples taken before, immediately, and 24-hour postexercise were analyzed for plasma malondialdehyde (MDA) and urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) excretion. Before the supplementation period, a significant increase in the urinary 8-OHdG excretion and plasma MDA. These results indicate that Cr supplementation reduced oxidative DNA damage and lipid peroxidation induced by a single bout of RE.

Key Words Cr monohydrate, 8-hydroxy-2-deoxyguanosine, malondialdehyde

Introduction
Free radicals and reactive oxygen species (ROS) are highly reactive molecules, which are produced pursuant to normal cellular metabolism and appear to be increased under conditions of both psychological and physical stress (37). These free radicals are generally neutralized by antioxidant defense systems that are composed of both endogenous and exogenous antioxidants (14).

Oxidative stress has been defined as a situation in which an increased level of ROS generation overwhelms the physiological capacity of the antioxidant’s system, resulting in oxidative damage to lipids, proteins, and DNA (15). Although regular physical exercise is recommended for reducing the risk of cancer and cardiovascular disease, and for its other beneficial effect (39), acute bouts of exercise can result in activation of several distinct systems of radical generation (5). For example, aerobic exercise causes whole-body oxygen consumption increases 10- to 20-fold over the resting state and can result in elevated levels of free radicals (20). Also, in relation to resistance exercises (REs), despite the lower oxygen demands compared to those of aerobic exercise, generation of free radicals through other mechanisms is possible: (a) xanthine-xanthine oxidase pathway, (b) respiratory burst of neutrophils, (c) catecholamine autooxidation, (d) local muscle ischemia-hypoxia, and (e) conversion of the weak superoxide to the strong hydroxyl radical by lactic acid (18). These ROS cause extensive DNA damage, including single-strand breaks and the formation of modified bases (16). One of the most abundant forms of oxidized...
DNA (9), 8-hydroxy-2-deoxyguanosine (8-OHdG), has been extensively investigated because it can be measured with high sensitivity (19), induces mutation (41,44), and is found frequently in tumor-related genes (43). For these reasons, 8-OHdG levels were measured in response to acute RE in trained men.

Most of the experimental studies that have investigated the association between exercise-induced oxidative stress and its acute effects on oxidative DNA damage and lipid peroxidation have used endurance exercise (28,36,40). Only a few studies that used RE to investigate this matter have been published (4,6,32). An increase in blood malondialdehyde (MDA), a marker of lipid peroxidation, was reported in the 2 days and immediately after a full-body RE protocol (25,32), whereas no change was reported in MDA 6 minutes after the performance of 20 maximal eccentric-concentric actions with the knee extensors and after circuit RE (8 exercises, 3 sets × 10 repetitions with 10 repetition maximum [10RM]) (10,42). Specific to DNA oxidation, only a study, to date, has assessed the 8-OHdG response, a marker of DNA oxidation, to isotonic RE (6). An increase in 8-OHdG of the quadriceps muscle was reported at the 24 hours after 200 eccentric actions with the knee extensors (29), whereas no change was reported in blood 8-OHdG after 30-minute dumbbell squat with 70% of 1RM (6). Collectively, this evidence demonstrated that acute RE can lead to acute oxidative stress (5). Furthermore, antioxidant supplement among athletes is well documented (20,28). However, there is little information regarding creatine (Cr) supplementation protection against the negative health consequences of ROS such as oxidative DNA damage and lipid peroxidation caused by RE in trained men.

Creatine monohydrate is a popular dietary supplement that is used by athletes to increase muscle mass and strength and improve sports performance (1,8,11,24). The effects of Cr on exercise performance, strength, and body composition have been described in many studies, with the majority reporting an ergogenic effect (33). In addition, heretofore, Cr’s positive therapeutic benefits in various oxidative stress-associated diseases have been reported in the literature and, recently, Cr has also been shown to exert direct antioxidant effects (3,23,45). Lawler et al. (23), for the first time, reported that Cr is capable of directly quenching aqueous radical and reactive species ions (ABTS+, O2•- and OONO-2) in vitro, and a more recent study showed that Cr exerts direct antioxidant activity via a scavenging mechanism in oxidatively injured cultured mammalian cells (38).

There is little or no information, however, regarding the Cr supplementation on lipid peroxidation and oxidative DNA damage after an acute bout of RE in resistance-trained men. Therefore, the purpose of this study was to investigate the effects of an acute bout of RE on lipid peroxidation and oxidative DNA damage in resistance-trained men and whether 7-day supplementation with Cr could negate any observed differences. We hypothesized that acute high intensity RE results in postexercise elevation in oxidative DNA damage and lipid peroxidation biomarkers and that the response would be lower after short-term Cr supplementation possibly because of antioxidant activity, as previously demonstrated for DNA oxidation.

**METHODS**

**Experimental Approach to the Problem**

To compare the effects of a 7-day Cr supplementation on oxidative modification to DNA and lipids after an acute bout of RE, biomarkers of DNA and lipid oxidations were studied with 27 recreationally resistance-trained men in a within-treatment, randomized, double-blind, placebo (PL) controlled protocol, with attempts made to control for exercise intensity and muscle-group recruitment.
Subjects
Twenty-seven experienced resistance-trained college-age men volunteered to participate as subjects. The subjects signed an informed consent document before participating in the study. The subjects were regularly performing RE, 3 d·wk⁻¹ for 1 year. The experimental procedure was explained in detail to all the subjects. These participants were randomly divided into either a Cr supplementation (age: 21.6 ± 3.6 years, height: 174 ± 8 cm, weight: 71.9 ± 7.8 kg) or a PL (age: 21.2 ± 3.2 years, height: 171 ± 6 cm, weight: 69.1 ± 10.4 kg) group. There were no significant differences between groups in physical characteristics. All subjects were healthy, with no major chronic diseases such as diabetes, cardiovascular disease, atherosclerosis, hypertension, or dyslipidemia. The subjects were on their ordinary diet, not permitted to use nutritional supplementation, and did not consume anabolic steroids or any other anabolic agents known to increase performance. Furthermore, the mean daily calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake during the 3 days preceding the Cr supplementation period was measured. Participants who consumed Cr supplements for at least 5 months before the start of this study or had a body mass index ≥24 kg·m⁻² were excluded. The study protocol was approved by the Ethics Committee of the Department of Sport Sciences, University of Kurdistan. Each subject was currently resistance trained for a minimum of a year using standard multiset; multieexercise training protocols typical of health or fitness RE programs directed at developing muscle strength, size, and power and reported training at least 3 times per week.

Procedures
A within-treatment, randomized, double-blind, PL-controlled protocol was used to investigate the effects of a 7-day Cr supplementation on oxidative DNA damage and lipid peroxidation. Two familiarization sessions were used to determine the maximal strength test (1RM) 1 week before the study. Participants then reported to the human performance laboratory on 3 separate weeks. During the first week, the participants performed the 1RM with the bench press, leg press, lat pull down, and seated rows (27,30,31). After the 1RM testing, the participants were randomly divided into the Cr (n = 15) or PL (n = 12) group. In the second week, participants reported to the human performance laboratory on 4 separate sessions. In the first and second visits (24 hours between each visit), the participants performed familiarization sessions with the exercise protocol (7 sets of 3–6 repetitions of bench press, leg press, lat pull down, and seated rows with 80–90% of 1RM) to ensure proper technique and reliability of the testing methods. In the third visit (before the start of Cr loading), blood draw was done, and the participants performed the RE protocol; then blood draws were performed immediately after and 24 hours after the RE protocol for measurement of MDA concentration. Urine samples were also taken at the time of blood sample collection for measurement of urinary 8-OHdG excretion. In the fourth visit, the subjects took the supplements (CR or PL) for 7 days. The participants then returned to the human performance laboratory for their final RE session.

![Figure 2. Resistance exercise protocol with a flat pyramid loading pattern.](image-url)
Blood draws were performed in the final acute RE protocol (after 7 days of supplementation) before exercise (Pre), immediately postexercise (Post), and 24 hours postexercise (24 hours Post). All the tests were scheduled at the same time of the day (9:00 hours) to negate confounding influences of diurnal hormonal variations. The experimental design is depicted in Figure 1.

**Resistance Exercise Protocols**
Before RE protocols, all subjects performed a warm-up, which consisted of 3-minute running, 5–10 repetitions at 50% of perceived maximum and stretching period. The RE protocol performed in a flat pyramid loading pattern, which consisted of 7 sets of 3–6 repetitions of bench press, leg press, lat pull down, seated rows with 80–90% of 1RM (Figure 2).

Bompa (7) stated that this type of loading pattern starts with a warm-up lift of, say, 60%, followed by an intermediary set at 80%, then stabilizing the load at 90% for the entire workout. If the instructor wishes to add variety at the end of training, a set of lower loads may be used. The physiological advantage of the flat pyramid is that by using a load of only 1 intensity level, the best neuromuscular adaptation for maximal strength is achieved without “confusing” the body with several intensities.

**Dietary Assessment**
The mean daily calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake during the 3 days preceding the first testing session (supplementation periods) are presented in Table 1. Dietary intake was analyzed by Nutritionist IV diet analysis software. No statistically significant differences were noted between groups for any measured dietary variables.

**Lipid Peroxidation Measurement**
Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is MDA. Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation (12). Plasma MDA was measured by using a spectrophotometric assay for MDA. The assay was carried out in duplicate; intrasay
coefficients of variances were 3.4%, and detection limit was 0.0088 μM, using the manufacturer’s instructions (Bioxytech® MDA-586™, Spectrophotometric Assay for MDA. Catalog Number 21044; OXISResearch, Portland, OR, USA).

Oxidative DNA damage
Oxidative DNA damage, urinary 8-OHdG excretion, was measured by using the enzyme-linked immunosorbent assay (ELISA). The assay was carried out in duplicate using the manufacturer’s instructions (New 8-OHdG Check, ELISA, Japan Institute for the Control of Aging; Catalog No: KO G-2005/E). Urinary creatinine levels were determined using a standard colorimetric ultraviolet spectrophotometric assay. The 8-OHdG was expressed relative to creatinine and expressed in absolute terms.

Statistical Analyses
Data are expressed as mean ± SD. Statistical evaluation was performed with SPSS (SPSS, Chicago, IL, USA) for windows. The 8-OHdG and MDA were analyzed using a 2-factor (group × time) analysis of variance with repeated measures on the within (time) factor. Multiple comparisons with confidence interval adjustment by the Bonferroni method were used as post hoc when necessary. Independent-sample t-tests were performed to determine possible group differences for the following variables: height, body mass, and dietary intake. The significance level was set at \( p \leq 0.05 \).

RESULTS

Oxidative DNA Damage and Lipid Peroxidation
Urinary 8-OHdG data pre, post, and 3 hours post RE for Cr and PL groups are presented in Figure 3. Before the supplementation period, there were no significant differences for urinary 8-OHdG level, a marker of oxidative DNA damage, between Cr and PL groups at Pre, Post, and 24 hours Post RE \( (p > 0.05) \). However, urinary 8-OHdG level was significantly lower in the Cr group than in the PL group at Post and 24 hours Post RE after 7 days of supplementation (Figure 3; \( p = 0.001 \), Partial Eta Squared = 0.656, Observed Power = 0.995). Urinary 8-OHdG levels were expressed relative to creatinine for the vast majority of DNA degradation products (Figure 4).

The changes in Plasma MDA levels are shown in Figure 5. Before the supplementation period, there was no significant difference between groups in the plasma concentration of MDA at Pre, Post, and 24 hours Post RE \( (p > 0.05) \). However, after the supplementation period, plasma MDA concentrations were significantly greater for the PL group than for the Cr group post RE (Figure 5; \( p = 0.002 \), Partial Eta Squared = 0.541, Observed Power = 0.998).

DISCUSSION

Many studies reported that intense exercise caused oxidative stress because of increased generation of ROS, whereas regular exercise is known as an important factor in preventing many diseases. Oxidative stress-induced cellular damage often appeared as changes in macromolecules such as proteins, lipids, and nucleic acids (DNA). In many studies, 8-OHdG was evaluated as a biomarker of oxidative DNA damage \((6,29,40)\) because it represents 5% of the total oxidized bases in the DNA and is present in quantities that are sufficient to be readily detected \((17)\). This 8-OHdG is a potentially important factor in carcinogenesis because it is prone to induce G-C to T-A transversion during DNA replication, which are frequently found in tumor-relevant genes. Thus, increases in the level of 8-OHdG can have important implications for mutagenesis and the induction of tumors \((35)\).

In this study, urinary 8-OHdG excretion significantly increased immediately and 24 hours post RE in both groups before the Cr supplementation period, which is in accordance with study of Radak et al. \((29)\) who reported that the 8-OHdG level was significantly increased in biopsy samples of the quadriceps at 24 hours after performing 200 eccentric contractions with knee extensors, but our findings are inconsistent with those of Bloomer et al. \((6)\), who did not
observe significant changes in plasma concentrations of 8-OHdG and MDA after 30-minute intermittent dumbbell squat with 70% of 1RM. The possible reasons for the inconsistency in our findings with those of the previous study can imply a difference in the RE protocol. In this study, whole-body RE protocol performed in flat pyramid loading pattern (FPLP), which includes 7 sets of 3–6 repetitions with 80–90% of 1RM. Flat pyramid loading pattern is the most effective method to gain maximal strength, and the physiological advantage of this method is that using a load of only 1 intensity level, the best neuromuscular adaptation for maximal strength is achieved without confusing the body with several intensities (7), which in comparison to the protocol used in the previous study (6) possess a higher intensity and volume. In this case, it has been previously reported that training intensity may be the main factor in producing free radicals (5,6,10).

In addition to oxidative DNA damage, free radicals may damage cellular compartments specially lipids and lead to initiation of chain reactions that are known as lipid peroxidation, the most important consequence of oxidative stress. In this study, before the Cr supplementation period, MDA, a biomarker of lipid peroxidation, is significantly increased immediately and 24 hours post RE in both groups, which is in accordance with the finding of Ramel et al. (32) who reported a significant increase in plasma MDA concentration after a circuit RE bout (18 minutes of RE with 75% of 1RM in 10 exercises) in trained and untrained subjects (3); an increase in plasma MDA concentration after 2 days of whole-body RE as well has been reported (25).

However, our findings are inconsistent with those of previous studies (4,10,42), which did not observe significant changes in MDA immediately and 24 hours after RE. The possible reasons for inconsistency, as previously mentioned, can allude to the type of RE protocols and exercise intensity.

It has been shown that endogenous antioxidant substances could not completely prevent oxidative damage under physiological and pathological conditions, such as strenuous exercise and exercise at altitude, and many diseases. It is possible that these situations disturb endogenous antioxidant balance, which could not neutralize the oxidant effects. During these situations, dietary antioxidants have the most important roles owing to enhancement in the ability of body antioxidant systems. Recently, Cr has been introduced as an antioxidant (23). Therefore, in this study, the effect of 7-day Cr supplementation (4 doses of 5 g.d⁻¹) on MDA and 8-OHdG, which are biomarkers of lipids peroxidation and oxidative DNA damage, is examined after a single bout of RE.

To our knowledge, this was the first investigation to compare Cr supplementation on biomarkers of DNA and lipid oxidation after acute RE. The primary findings of our study were that urinary 8-OHdG concentrations were significantly decreased in Cr group compared to in the PL group immediately and 24 hours after RE. These changes are in accordance with the finding of Basta et al. (3), who reported that Cr supplementation led to a significant decrease in plasma MDA concentration after an exhaustive intense race. As previously mentioned, lower concentration of MDA in the Cr group compared to in the PL group immediately and 24 hours after the RE protocol are in accordance with the findings of Basta et al. (3), who reported that Cr supplementation led to the reinforcement of the antioxidant system (superoxide dismutase [SOD], GPx) in rowers’ blood, confirmed by a significantly lower concentration of lipid peroxidation products upon a 24-hour recovery period and by a lower postexercise activity of glutathione peroxidase. As mentioned above, these changes may be because of the antioxidative properties of Cr. Also, reduction in glutathione peroxidase activity because of Cr supplementation could be the reason for the lower concentration of MDA, lipid peroxidation products, in the Cr group.

In addition, the attenuated oxidative DNA damage and lipid peroxidation responses are consistent with the findings of other studies using high antioxidant diets in athletes (2,26). For example, Arent et al. (2) found that, compared with PL, supplementing with Resurgex® decreased lipid peroxidation (8-isoprostane, lipid hydroperoxide) and creatine kinase (CK) in college soccer players. Also, our findings are consistent with those of the study using isolated Glisodin® supplementation that has reported a protective effect on DNA and reduced 8-isoprostane levels (26).

Although, our subjects were recreationally resistance trained, they were unaccustomed to the format and intensity
of the RE protocol used in this study. The unfamiliarity of the subjects with FPLP most likely is the reason for increasing oxidative stress and oxidative DNA damage before Cr loading. In summary, acute the RE with FPLP method induced oxidative DNA damage and lipid peroxidation in trained subjects, but short-term Cr supplementation decrease these effects, which may be because of increasing the activity of antioxidant enzymes and reducing oxidant production.

**Practical Applications**

A single bout of RE which is performed using the FPLP method increases oxidative DNA damage and lipid peroxidation in athletes. However, Cr supplementation for a short period decreased urinary 8-OHdG excretion and plasma MDA levels after acute RE with the FPLP method, which suggests a positive effect of the Cr supplementation as a strategy in reducing oxidative DNA damage and lipid peroxidation after a strenuous RE protocol. The exact mechanisms by which Cr supplementation exert its antioxidant actions remain to be elucidated, however, increased activity of antioxidant enzymes and ability to remove ROS and reactive oxygen and nitrogen species (RONS) have been implicated, as previously demonstrated (23,38). Also, 90% of the total Cr in the body is stored in skeletal muscles and mitochondria is one important source of ROS that includes H$_2$O$_2$, O$_2^-$, and possibly OH$^-$ and peroxynitrite, in skeletal muscles. Therefore, in this case, further studies on animal or human models will be required to examine short-term Cr supplementation on mitochondrial antioxidant enzymes and components such as mitochondrial genome. The coach and athlete should consider that in current form and dosage, Cr supplementation has ergogenic benefit, so we recommended its use in resistance-trained men seeking to enhance performance and to reduce oxidative DNA damage and lipid peroxidation.

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**References**


