EFFECTS OF CREATINE MONOHYDRATE SUPPLEMENTATION ON OXIDATIVE DNA DAMAGE AND LIPID PEROXIDATION INDUCED BY ACUTE INCREMENTAL EXERCISE TO EXHAUSTION IN WRESTLERS

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Abstract:

The purpose of the study was to examine the effects of a seven-day creatine monohydrate (CrM) supplementation on oxidative DNA damage and lipid peroxidation after incremental exercise to exhaustion in wrestlers. Thirty-one college-aged male wrestlers (age 19.52±2.75 years, body mass 79.24±16.13 kg, height 173±6.49 cm, and body fat 16.37±5.92%) volunteered to participate in this double-blind, placebo controlled study and were randomly placed into either the placebo (PL; 4×5 g·day^{-1} of maltodextrine powder; n=16) or the creatine monohydrate (CrM: 4×5 g·day^{-1} CrM, n=15) group. Prior and following the supplementation period, participants performed an incremental cycling ergometer test to exhaustion. Urine samples were collected before and after the supplementation period at before (Pre), after (Post) and 24 hours after (24h Post) the exercise tests to determine the oxidative DNA damage and lipid peroxidation as measured by urinary excretion of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-Isoprostane (8-iso $PGF_{2\alpha}$). Our finding demonstrates that the urinary 8-OHdG level significantly increased at 24h Post to exhaustion by 13.36% in CrM and 24.08% in PL before supplementation ($p<.05$). In contrast to DNA damage, we observed a non-significant trend toward a small increase in urine 8-iso $PGF_{2\alpha}$ by 0.43% in CrM and 3.06% in PL group after exercise to exhaustion in athletes ($p>.05$). In addition, urinary 8-OHdG concentrations at 24h Post significantly decreased by 32.65% in CrM group after supplementation compared with before supplementation. After supplementation, urinary 8-OHdG concentrations were significantly lower in CrM group compared with PL at 24h Post ($p<.05$). These results suggest that CrM supplementation can attenuate oxidative DNA damage induced by acute exercise to exhaustion in wrestlers.

Key words: *incremental exercise to exhaustion, creatine, oxidative stress, 8-OHdG, 8-iso $PGF_{2\alpha}$*

Introduction

It has been shown that exhaustive exercise can increase the generation of reactive oxygen species (ROS) (Davies, Quintanilha, Brooks, & Packer, 1982). These ROS are neutralized by an elaborate antioxidant defense system including enzymatic and non-enzymatic antioxidants (Sen, Packer, & Hannine, 2000). Exhaustive exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress (Alessio, et al., 2000). Oxidative stress can cause lipid, protein and DNA damage (Bloomer, Goldfarb, & McKenzie, 2006; Sen, et al., 2000). Oxidative stress-induced DNA damage may play a significant role in processes that cause many chronic diseases including cancer, cardiovascular disease and aging (Wu, Chiou, Chang, & Wu, 2004). The DNA adduct, 8-hydroxy-2-deoxyguanosine (8-OHdG) is a reliable marker of oxidative DNA damage that can be measured in body fluids such as plasma and urine (De Zwart, Meerman, Commandeur, & Vermeulen, 1999). In addition, urinary 8-OHdG excretion, as a reliable non-invasive marker to assess oxidative stress, is of interest in the field of redox biology.

In terms of urinary 8-OHdG excretion after exercise, there are conflicting results with some demonstrating increased 8-OHdG excretion following aerobic exercise in an intensity- and duration-dependent fashion (Radak, Pucsok, Boros, Josfai, & Taylor, 2000), others showing no change in 8-OHdG excretion after short duration trails (Sumida, Doi, Sakurai, Yoshioka, & Okamura,

1997, Sumida, et al., 1997b). Besides oxidative DNA damage, oxidative stress can be measured by lipid peroxidation products which include expired pentane, malondialdehydes (MDA), lipid hydroperoxides, isoprostanes, and conjugated dienes (Sen, et al., 2000). MDA have been frequently used as markers of oxidative stress in response to exercise (Bloomer, et al. 2006; Cakir-Atabek, Demir, Pinarbasili, & Gunduz, 2010; Dixon, et al., 2006; Rahimi, 2011). Nonetheless, isoprostanes have been used for detecting radical-initiated lipid peroxidation, since discovered in 1990 (Morrow, et al., 1990) which is produced when arachidonic acid is subjected to peroxidation in vivo (Roberts & Morrow, 2002). Recently, isoprostanes such as F_2-isoprostane (8-iso $PGF_{2\alpha}$ or $iPF_{2\alpha}$-III) have attracted more attention for measuring lipid peroxidation due to their higher stability and detectability at very low levels (Nikolaidis, Kyparos, & Vrabas, 2011) compared to MDA and alkenes (Sachdev & Davies, 2008).

Recently, Kürkçü, Tekin, Özda, and Akçakoyun, (2010) demonstrated that regular bouts of wrestling cause an imbalance in the redox status by increased oxidative stress in young wrestlers when compared to the untrained healthy controls of a similar age and sex. Another study of the female wrestlers found an increase in oxidative stress with exercise and fall in antioxidant capacity (Kahraman, et al., 2003). Therefore, to reduce oxidative stress in athletes, supplementation with antioxidant nutrients may provide protection against the harmful effects of ROS caused by exercise (Arent, Pellegrino, Williams, DiFabio, & Greenwood, 2010). Evidence of the effect of exercise and antioxidant supplementation on oxidative DNA damage has not been definitively established. Results from previous studies of antioxidant supplementation on the 8-OHdG level after exercise have been conflicting, with some demonstrating decreased 8-OHdG level after vitamin E supplementation (Hartmann, Niess, Grunert-Fuchs, Poch, & Speit, 1995), vitamin C plus vitamin E (Mastaloudis, et al., 2004) and supplementation with Funciona™ (Munoz, et al. 2010), and others showing no protective effect after the intake of vegetable juice powder concentrate for two weeks (Bloomer, et al., 2006) or after high-dose vitamin E or C supplementation for two months (Huang, Helzlsouer, & Appel, 2000).

Creatine monohydrate (methyl guanidine-acetic acid; CrM) is the most popular form of supplement used by athletes to increase muscle mass, strength and sports performance (Kraemer & Volek, 1999; Rahimi, Faraji, Sheikholeslami-Vatani, & Qaderi, 2010; Rawson & Persky, 2007). Apart from its use in sports, the positive therapeutic benefits of CrM in various oxidative stress-associated diseases have been reported in literature (Sestili, et al., 2011). Additionally, CrM has been shown to exert direct antioxidant effects (Lawler, Barnes, Wu, & Song, 2002).

For the first time Lawler et al. (2002) demonstrated that CrM has the potential to act as a direct antioxidant in non-cellular systems. This study showed that CrM significantly reduced levels of superoxide radical (O_2^-), peroxynitrite (OON^-) and $ABTS^+$. Later, Sestili et al. (2006) demonstrated that CrM supplementation has a direct antioxidant effect via scavenger mechanism on three different oxidative agents including H_2O_2, $OONO^-$ and tB-OOH in cultured cells. Recently, in vivo studies on rats, Deminice, Portari, Vannucchi, and Jordao (2009) demonstrated that CrM supplementation reduced homocysteine levels and consequently cell toxicity and concluded that these effects may be considered as an indirect antioxidant action of CrM. Furthermore, Guimaraes-Ferreira, et al. (2012) demonstrated a direct scavenger effect of CrM on superoxide radical production by the slow- and fast-twitch skeletal muscle.

Several studies demonstrated other indirect antioxidant mechanisms such as the normalization of energy status of the cell, membrane stabilization and augmentation of antioxidant capacity in cells (Wyss & Schulze, 2002; Lenz, et al., 2005, Young, et al., 2010). In vitro study by Young et al. (2010) demonstrated that CrM supplementation exerts an indirect antioxidant effect via the up-regulation of two thioredoxin reductases located in the mitochondria and cytoplasm, which consist of peroxiredoxin-4 and thioredoxin-dependent peroxide reductase.

However, few studies exist demonstrating the *in vivo* antioxidant effects of CrM on oxidative stress induced by exercise in athletes (Basta, Skarpańska-Stejnborn, & Pilaczyńska-Szcześniak, 2006; Rahimi, 2011). Moreover, there is no information regarding the CrM supplementation on oxidative DNA damage and lipid peroxidation as measured by urinary 8-OHdG and 8-iso $PGF_{2\alpha}$ levels after an acute bout of incremental exercise to exhaustion in wrestlers. Since strenuous exercise to exhaustion is closely related to the production of free radicals and oxidative damage to lipids and DNA, we aimed at investigating the effect of CrM supplementation on oxidative DNA damage and lipid peroxidation in wrestlers.

Methods

Participants

Thirty-one college-aged male wrestlers (age 19.52±2.75 years, body mass 79.24±16.13 kg, height 173±6.49 cm, and body fat 16.37±5.92%), who had at least six years experience in wrestling and were among the top ten in national championships, participated voluntarily in this study. Each of the wrestlers provided a written informed consent after they were fully informed of the nature, potential risks, discomforts, and benefits involved in

this investigation. All experimental procedures were performed in accordance with the policy statement of the University of Guilan on research with human participants and were approved by the Ethics Committee of the Department of Exercise Physiology. The participants' characteristics are presented in Table 1. Exclusion criteria included: consuming any supplementation, alcohol, or tobacco products. Inclusion criteria for participants included: age 17–21 years, well-trained athletes with a training status of at least six years of experience, and having at least one medal in the national championships. After the baseline testing, CrM was administered to half of the wrestlers in randomized double-blind fashion while the other half received the placebo (PL) made up of maltodextrine. All participants completed two aerobic exercise tests until exhaustion on the cycle ergometer before and after the supplementation period (Figure 1).

Procedure

A randomized double-blind independent group design was used to determine the effect of short-term CrM supplementation on oxidative DNA damage and lipid peroxidation, as measured by 8-OHdG and 8-iso $PGF_{2\alpha}$, after an acute incremental cycle ergometer test to exhaustion in wrestlers. Thirty-one college-aged male wrestlers in randomized double-blind design received CrM (n=15) and the placebo (n=16). The participants were familiarized with the experimental testing procedures during a control day about one week before the actual measurements. Participants performed the incremental cycle ergometer test to exhaustion before (test 1) and after seven days of supplementation (test 2) (Figure 1). Urine samples for the measurement of 8-OHdG and 8-iso PGF2α were collected before (Pre), after (Post) and 24 hours after (24h Post) the acute performance tests.

Anthropometric and nutritional data

Participants were invited to come to the Exercise Physiology Laboratory for the collection of anthropometric data. Standing height was measured to the nearest 0.1 cm (Seca). Weight, body fat and body fat percentage were measured by electric bioimpedance (Body, InBody 3.0, Biospace, Korea). The participants were instructed to follow their habitual diet throughout the study period and to fill out a food recall form for three days before the

Table 1. Participants' physical characteristics

	Group	Mean±SD
Age (year)	CrM	19.07±2.72
	PL	20.00±2.82
Height (cm)	CrM	173±5.82
	PL	174±7.38
Weight (kg)	CrM	73.00±13.09
	PL	86.00±16.88
% body fat	CrM	13.99±4.68
	PL	18.95±6.21
BMI ($kg \cdot m^{-2}$)	CrM	24.14±3.46
	PL	28.19±4.35
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	CrM	49.88±8.09
	PL	47.68±7.33

Table 2. Dietary intake assessed during 3 days prior to each testing session

	Group	Mean±SD
Energy intake (kcal)	CrM	2723.5±47.37
	PL	2668±83.43
Protein (g)	CrM	101.5±28.99
	PL	74.41±9.46
Carbohydrate (g)	CrM	252.25±140.5
	PL	354.55±13.5
Fat (g)	CrM	148.55±50.84
	PL	112.50±0.14
Vitamin E (mg)	CrM	40.02±8.40
	PL	27.82±0.05
Vitamin C (mg)	CrM	187.15±95.38
	PL	128.90±11.17
Vitamin A (RE)	CrM	1247.8±937.90
	PL	1977±91.92

No significant difference between groups (p>.05).

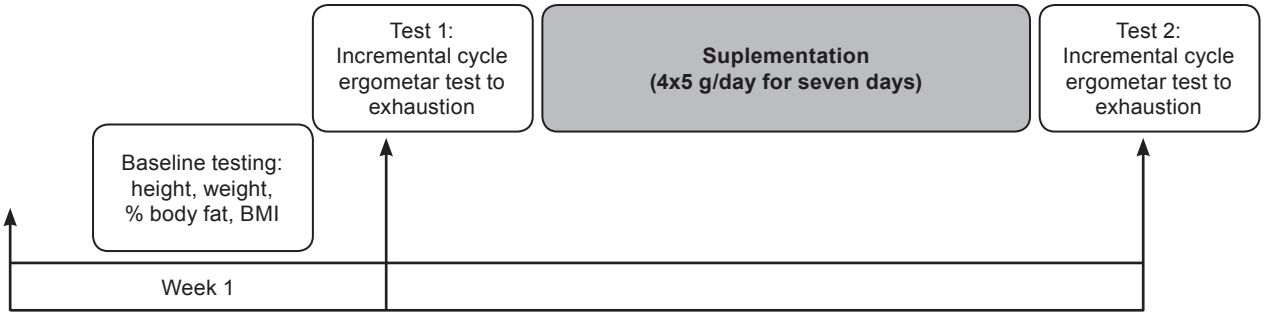

Figure 1. Experimental design

first testing session and three days before the last testing session. The participants completed a six-day diet record, beginning on the third day before the first testing session and ending three days before the last testing session, to determine their total calories intake, carbohydrates, proteins, lipids, and vitamins A, C, and E using the Nutritionist IV computer program (Diet analysis model 3.5.2, the Hearst Corporation, San Bruno, CA 94066) (Table 2).

Aerobic exercise test

The participants completed an incremental cycle test to exhaustion on a calibrated electronic cycle ergometer (*Tunturi* E433) before and after seven days of the supplementation period. Before each incremental cycle test the seat height of the cycle ergometer was adjusted for near full extension of the participants' legs while pedaling. Participants were instructed not to train or to be involved in strenuous exercise for 72 hours before each exercise session and 24 hours after completion of the exercise bout. To avoid circadian rhythm effects, testing sessions began on the same day of the week and at the same time of the day. The incremental exercise test to exhaustion began at 50 watts (W) for five minutes, and the power output was increased by 30 W every three minutes until voluntary exhaustion or until the subject could no longer maintain a pedal cadence of 60 rpm despite strong verbal encouragement. The power output at exhaustion was recorded to measure the endurance capacity by Storer, Davis, and Caiozzeo (1990) formula: VO_{2max} (ml/min) = [10.51(W, max)]+[6.35(wt, kg)]-[10.49(age, year)]+519.3

Supplementation protocol

The study was carried out using a randomized double-blind independent group design with a CrM group and a PL group. CrM group was supplemented for seven days with 20 $g \cdot day^{-1}$ of CrM (Mass Global Nutration/ 5460 Yonge St., Suite 1505, Toronto, ON., M2N 6K7, Canada) at 4×5 g doses. PL group was supplemented for seven days with maltodextrine (Mass Global Nutration/ 5460 Yonge St., Suite 1505, Toronto, ON., M2N 6K7, Canada) in the same dosage as CrM group. The powders were identical in taste and shape and were dissolved in 200 mL of water and ingested four times per day for seven consecutive days. Participants were instructed to avoid caffeine consumption for 72 hours prior to testing.

Biochemical analysis

Urine samples were collected before (Pre), after (Post), and 24 hours after (24h Post) each bout of incremental aerobic exercise to exhaustion in both groups. After collection, urine samples were stored at -20°C until analysis. Oxidative DNA damage was measured by urinary 8-OHdG level using the 8-OHdG EIA kit (Cayman Chemical, Catalog No. 589320, USA) which has high sensitivity and specificity. At the time of analysis, urine samples were thawed at 4°C and centrifuged at 2000 g for 10–15 minutes to remove the particulate matter.

At room temperature, the 8-OHdG standards were prepared based on the kit instruction which included 10.3, 23.1, 52, 117.1, 263.4, 592.6, 1333, 3000 pg/ml. Then 50 µL of each standard were added to two rows of plates. 50 µL of each urine sample were added to plates in duplicate. 50 µL of 8-OHdG AChE tracer was added to each well except the Total activity (TA) and the Blank (Blk) wells. 50 µL of the primary antibody was added to 50 µL aliquot of each sample and standard in microtiter plates pre-coated with 8-OHdG. Plates were covered by plastic film and incubated 18 hours at 4°C. After that, plates were emptied and rinsed five times with wash buffer. 200 µL of Ellman's reagent was added to each well and 5 µL of tracer was added to the total activity wells. Plates were covered with plastic film and put on the flat of the orbital shaker in the dark for 90–120 minutes. The bottom of the plate was then wiped with a clean tissue, the plastic film was removed and the plate was read at a wavelength between 405 and 420 nm, based on the kit instructions.

Lipid peroxidation was measured by urinary 8-iso $PGF_{2\alpha}$ using the 8-iso $PGF_{2\alpha}$ EIA kit (Cayman Chemical, Catalog No. 516351, USA) based on the kit instructions. The 8-iso $PGF_{2\alpha}$ standards were prepared based on the kit instructions which included 0.8, 2.0, 5.1, 12.8, 32, 80, 200, and 500 pg/ml. The assay protocol for measuring urinary 8-iso $PGF_{2\alpha}$ was similar to the 8-OHdG protocol as presented above.

Statistical analyses

Statistical evaluation was performed using the SPSS software for Windows, version 16.0 (SPSS, Chicago, IL, USA). Pre-supplementation and post-supplementation values for urinary 8-OHdG and 8-iso $PGF_{2\alpha}$ concentrations were analyzed using 2×6 (group × time) ANOVA with repeated measures. Box's test and Mauchly's test were used to check for the equality of variance and sphericity levels, respectively. The Greenhouse-Geisser adjustment was used to correct any violation of sphericity assumption. Effect sizes (ES) were computed to compare the magnitude of a *post–pre* change in the CrM and PL groups using a modified Cohen effect size scale: trivial 0.0-0.2, small 0.2-0.6, moderate 0.6-1.2, large 1.2-2.0, very large 2.0-4.0 and extremely large >4 (Hopkins, Marshall, Batterham, & Hanin, 2009).

Results

The results of oxidative DNA damage, as measured by 8-OHdG, are presented in Figure 2. Results from 2×6 (group × time) ANOVA with repeated measures revealed a significant group × time interaction for urinary 8-OHdG concentrations (p=.015). Before the supplementation period, urinary 8-OHdG concentrations significantly increased by 13.36% in CrM group and 24.08% in PL group at 24h Post compared with Pre (ES=1.23 and 1.35, respectively). However, there were no significant changes in urinary 8-OHdG concentrations at Pre and Post in between or within the groups (p>.05).

Urinary 8-OHdG concentrations at 24h Post significantly decreased by 32.65% in CrM group after the supplementation period compared with the before-supplementation period (p=.001, ES=-3.38). However, after the supplementation period, urinary 8-OHdG concentrations decreased by 6.73% and 16.93% in CrM group at 24h Post compared with Pre and Post (p=.067 and .04; ES=0.50 and -3.125, respectively). In addition, after the supplementation period, urinary 8-OHdG concentrations were significantly lower in CrM group compared with PL at 24h Post (p=.001, ES=-3.13).

The results of lipid peroxidation, as measured by urinary 8-iso $PGF_{2\alpha}$, are presented in Figure 3. No significant group × time interaction in urinary 8-iso $PGF_{2\alpha}$ was observed as a function of supplementation and incremental cycle ergometer exercise to exhaustion (CrM : ES at pre-supplementation and post-supplementation = -0.021, 0.019 and 0.20,

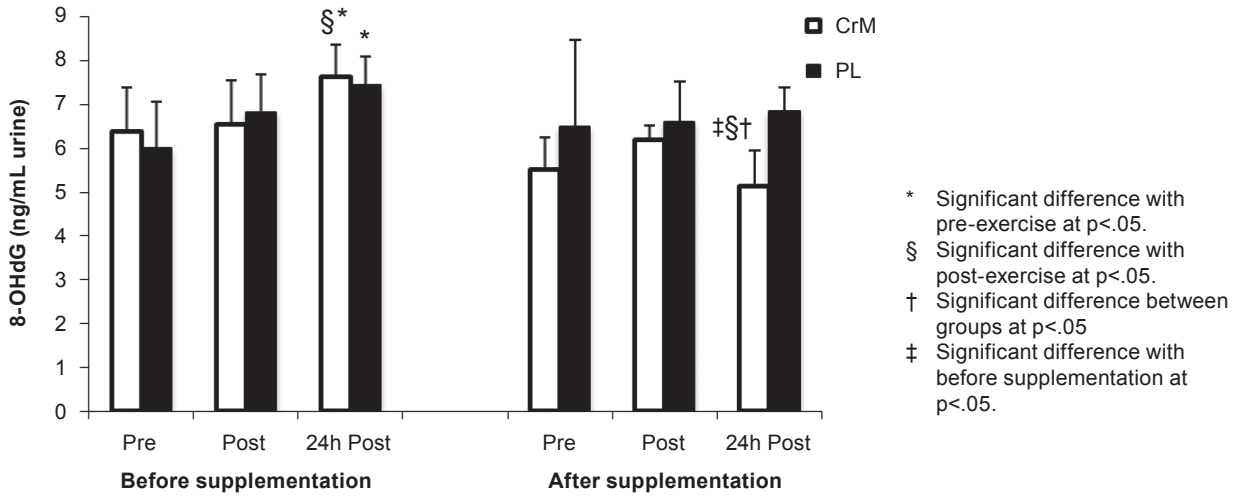

Figure 2. Urinary 8-OHdG concentration at pre-, post and 24 hours post exercise in CrM and PL groups before and after supplementation.

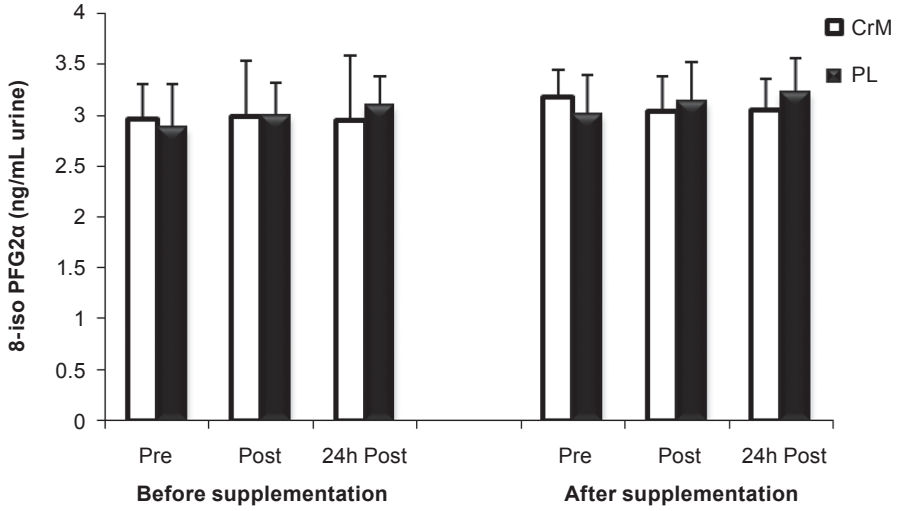

Figure 3. Urinary 8-iso PGF2α concentration at pre-, post and 24 hours post exercise in CrM and PL groups before and after supplementation.

0.205; PL: ES at pre-supplementation and post--supplementation = -0.14, -0.27 and -0.05, -0.22; p>.05).

There were no significant differences between the groups in total calories intake, carbohydrates, proteins, lipids, and vitamins A, C, and E at three days before each testing session (p>.05).

Discussion and conclusions

Acute exhaustive exercise-induced oxidative stress, leading to DNA damage and lipid peroxidation, has been well documented over the last decade (Nikolaidis, et al., 2011; Reichhold, Neubauer, Bulmer, Knasmü, & Wagner, 2009). Acute exhaustive exercise-induced ROS production could be attributed to a mitochondrial electron transport chain, ischemia-reperfusion, xanthine oxidase catalyzed reaction, inflammatory mechanism and the production of catecholamine (Hartmann & Niess, 1999). It is known that DNA oxidation, which is generally measured by the levels of 8-OHdG, is formed from hydroxyl radicals and singlet oxygen attack on 2'-deoxyguanosine, resulting in a hydroxyl moiety replacing the hydrogen atom (Wu, et al., 2004). Given the potential involvement of 8-OHdG in mutagenesis and induction of tumors (Valavanidis, Vlachogianni, & Fiotakis, 2009), research has focused on the potential benefits of antioxidant consumption.

In the present investigation, we studied the effect of CrM supplementation on DNA oxidation and lipid peroxidation after acute incremental exercise to exhaustion on a cycle ergometer in wrestlers. Our data indicate that the urinary excretion of 8-OHdG did not change significantly immediately after (Post) acute exercise to exhaustion in wrestlers. This finding is consistent with the report showing that the cycle ergometer test to exhaustion had no effects on urinary 8-OHdG values in untrained men (Sumida, et al., 1997a).

The results of the present study showed that the urinary 8-OHdG levels significantly increased at 24 hours post exercise (24h Post) to exhaustion by 13.36% in CrM and 24.08% in PL before supplementation. Our finding is consistent with the finding of Morillas-Ruiz et al. (2005), who evaluated the effect of cycle ergometer test performed at 70% VO_{2max} for 90 minutes on urinary 8-OHdG levels which were assessed, using HPLDECD, in urine collected for 24-hours periods pre- and post exercise. Also, Orhan et al. (2004) studied 60-minute cycling at 70% VO_{2max} on urinary 8-OHdG levels (excretion post exercise versus pre-exercise; using ELISA) that were collected over 24 hours, one day before and for 72 hours after the exercise. Increased 8-OHdG levels were found in both studies. Furthermore, a significant increase in urinary 8-OHdG levels was observed after exercise at 70% of VO_{2max} in untrained men (Nakajima, Kamohara, Nakano, & Ohno, 2006). In contrast, Bloomer, Goldfarb, Wideman, Mckenzie, and Consitt (2005) did not observe any significant changes in serum 8-OHdG level 24 hours after 30 minutes of continuous cycling at 70% of VO_{2max} in trained men. The discrepancies between results may be related to the time of sampling after exercise, the training status of the participants, as well as the type and duration of exercise studied, which included exercise on a treadmill (Sumida, et al. 1997a), bicycle ergometer (Sumida, et al., 1997b) and long distance running (Radak, et al., 2000).

Results from previous studies of antioxidant supplementation such as vitamin E and C on 8-OHdG level after exercise have been inconsistent until now (Hartmann, et al., 1995; Huang, et al., 2000; Mastaloudis, et al., 2004). To our knowledge, the present investigation is the first to demonstrate that short-term CrM supplementation can attenuate oxidative DNA damage induced by an acute exercise to exhaustion in well-trained wrestlers. Our finding demonstrated that urinary 8-OHdG concentrations at 24 hours post exercise significantly decreased by 32.65% in CrM group after supplementation compared with before supplementation. Furthermore, urinary 8-OHdG levels were significantly lower in CrM group compared with the PL group at 24h Post.

Only two studies have evaluated the CrM supplementation effects on exercise-induced oxidative stress (Basta, et al., 2006; Rahimi, 2011). Corroborating our finding, a recent study that investigated the effects of seven days of CrM supplementation (4×5 $g \cdot d^{-1}$ of CrM) on oxidative DNA damage induced by strenuous resistance exercise in young resistance trained men showed that CrM significantly reduced oxidative DNA damage compared to PL at post and 24 hours post resistance exercise (Rahimi, 2011).

Another measure that has been used to detect oxidative stress is 8-iso $PGF_{2\alpha}$ which in body fluid such as plasma and urine provides a reliable approach to assess oxidative stress *in vivo* (Nikolaidis, et al., 2011; Roberts & Morrow, 2002). Few studies have used this marker of oxidative stress after exercise, especially with reference to acute exercise to exhaustion (Arent, et al., 2010; Mastaloudis, et al., 2004; Rietjens, et al., 2007).

In relation to lipid peroxidation, our finding demonstrates no significant change in urinary 8-iso $PGF_{2\alpha}$ level induced by acute exercise to exhaustion before and after supplementation. These findings are consistent with those of McAnulty et al. (2007) who did not report any effect of a 160-km ultramarathon, lasting approximately 26 hours on F_2-IsoP levels in urine collected 5–15 minutes after the race. Furthermore, McAnulty et al. (2005b) reported that exhaustive resistance exercise did not result in increased F2-isoprostane. In contrast, Nieman et al. (2004) determined the response of F_2-IsoP

levels after a triathlon race lasting approximately 12 hours, collecting urine samples shortly after exercise and not 12-24 hours after the race, which is the usual time window in similar cases. F-IsoP markedly increased by 89% in 5–10 minutes post race and by 107% at 1.5 hours post race. Also, Rietjens et al. (2007) reported that the levels of 8-isoprostane in urine collected 24 hours after a single session of resistance exercise increased by 40% compared to the pre-exercise baseline values. The contradiction in the findings may be attributed to the difference in exercise protocols, for example long distance running vs. resistance exercise, the training status of participants, the time of sampling and the type of samples and assays.

Moreover, the findings of previous research regarding the effect of antioxidant supplementation on 8-iso $PGF_{2\alpha}$ level after exercise reported inconsistent results, with some demonstrating increased 8-iso $PGF_{2\alpha}$ level after α-tocopherol (542 mg per day) (McAnulty, et al., 2005a), decreased after α-tocopherol (300mg per day) and ascorbic acid (1,000 mg per day) (Mastaloudis, et al., 2004) and decreased after supplementation with Resurgex® following a graded treadmill test to exhaustion (Arent, et al., 2010).

In contrast to DNA damage, we observed a non-significant trend toward a small increase in urine 8-iso $PGF_{2\alpha}$ by 0.43% in CrM and 3.06% in PL group after exercise to exhaustion in wrestlers. Thus, it is possible that the intensity and/or the duration of exercise performed in the current study were not great enough to produce a significant increase in urinary 8-iso $PGF_{2\alpha}$ level. However, our finding demonstrates that incremental exercise to exhaustion does not significantly increase the urinary 8-iso $PGF_{2\alpha}$ level. It should be noted that this does not exclude the possibility that changes in 8-iso $PGF_{2\alpha}$ could be present in active muscle as showed by Karamouzis et al. (2004) who reported a significant increase in the levels of 8-iso $PGF_{2\alpha}$ in the intracellular fluids of the vastus lateralis muscle after 30 minutes of exercise on the cycle ergometer at 150 watts.

Considering the potentially important health effects of oxidative damage to DNA (Wu, et al., 2004), athletes may desire to consider antioxidant supplementation to decrease the harmful effect of DNA oxidation. Our findings demonstrate a protective effect of CrM on oxidative DNA damage induced by incremental exercise to exhaustion in wrestlers. The mechanism(s) behind the decreased oxidative damage to DNA induced by CrM supplementation after acute exercise to exhaustion still remains unclear. However, it is important to note that the protective effect of CrM may derive from different mechanisms such as direct scavenging of radical species (Lawler, et al., 2002; Sestili, et al., 2006), stabilizing biological membrane and amelioration of energy metabolism. (Young, et al., 2010). Recently, Young et al. (2010) examined for the first time the biochemical effect of Cr supplementation at the protein and metabolite level of myotubes by integrating the sensitive explorative techniques, protomics and high-resolution 1H nuclear magnetic resonance (NMR) spectroscopy. The findings of this study demonstrated that Cr supplementation increased the muscle cell energy metabolism as well as the up-regulation of two cellular antioxidant systems including peroxiredoxin-4 which is located in the mitochondria and thioredoxin dependent peroxide reductase located in cytoplasm (Young, et al., 2010). Based on these findings it could be speculated that CrM supplementation, apart from direct scavenging of radical species (Lawler, et al., 2002; Sestili, et al., 2006), exerts its effects by the up-regulation cellular antioxidant systems (Young, et al., 2010).

In conclusion, our findings showed that incremental exercise to exhaustion on a bicycle ergometer induces an oxidative stress demonstrated by an increase in the urinary 8-OHdG level and non-significant trend toward a small increase in urinary 8-iso PGF2α level.

Furthermore, CrM supplementation significantly attenuates oxidative DNA damage induced by incremental exercise to exhaustion in wrestlers. Further studies will, however, be required in order to determine the effects of short-term CrM supplementation on oxidative stress biomarkers, the up-regulation of various antioxidant systems and direct scavenging of radical species after exercise to exhaustion in athletes.

The 8-OHdG and 8-iso $PGF_{2\alpha}$ are useful biomarkers, since they are available by non-invasive sampling techniques and specific for the effect of oxidative free radical damage to DNA and lipids. The results of the present study indicate that acute exercise to exhaustion significantly increases oxidative damage to DNA, and a non-significant trend toward a small increase in damage to lipids was observed as measured by urinary 8-OHdG and 8-iso $PGF_{2\alpha}$ concentrations in well-trained wrestlers. These findings indicate that, despite the high training status of the wrestlers participating in the present study, oxidative damage to DNA could not be prevented. In light of the potentially important health effects of oxidative damage to DNA (Shibutani, Takeshita, & Grollman, 1990; Toyokuni, Okamoto, Yodoi, & Hiai, 1995; Valavanidis, et al., 2009), athletes may wish to consider antioxidant supplementation to quench free radicals and increase total antioxidant capacity. Our findings indicate that short-term CrM supplementation has a beneficial effect on the oxidation of DNA induced by acute strenuous exercise to exhaustion in well-trained wrestlers. As a decrease in urinary 8-OHdG concentrations indicates a decrease in

oxidative damage to DNA, it could be speculated that short-term CrM supplementation may exert its protective effects via the antioxidant properties as shown by Lawler et al. (2002). However, further research is necessary to confirm the real beneficial effects of CrM supplementation on exercise-induced oxidative stress. The exact mechanisms by which CrM supplementation attenuates oxidative damage to DNA following strenuous exercise are unknown. Because urinary 8-OHdG concentrations reflect the integrated rate of oxidative DNA damage and the repair of DNA in the whole body, further studies are necessary to elucidate the effects of CrM supplementation on DNA repair enzymes following strenuous exercise.

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UČINCI SUPLEMENTACIJE KREATIN MONOHIDRATOM NA OKSIDATIVNO OŠTEĆENJE DNK I PEROKSIDACIJU LIPIDA IZAZVANU AKUTNIM PROGRESIVNIM TESTOM OPTEREĆENJA DO OTKAZA U HRVAČA

Cilj ovog istraživanja bio je utvrditi učinke sedmodnevne suplementacije kreatin monohidratom (CrM) na oksidativno oštećenje DNK i peroksidaciju lipida nakon progresivnog testa opterećenja do otkaza u hrvača. Trideset i jedan hrvač studentske dobi (19,52±2,75 godina, tjelesne težine 79,24±16,13 kg, tjelesne visine 173±6,49 cm sa postotkom tjelesne masti 16,37±5,92%) dobrovoljno je pristupio dvostruko slijepom placebo kontroliranom eksperimentu u kojem su slučajnim odabirom bili selektirani u grupu ispitanika koji su uzimali placebo (PL; 4 x 5 g·dan^{-1} maltodekstrin praha; n=16) ili u eksperimentalnu grupu s uzimanjem kreatin monohidrata (CrM: 4 x 5 g·dan^{-1} CrM, n=15). Prije i nakon razdoblja suplementacije ispitanici su bili podvrgnuti progresivnom testu opterećenja do otkaza. Uzorci urina prikupljani su prije i poslije razdoblja suplementacije i to prije (Pre), poslije (Post) i 24 sata nakon (24h Post) testova kako bi se utvrdilo oksidativno oštećenje DNK i peroksidacije lipida mjerene pomoću 8-hidroksi-2-deoksigvanozina (8-OHdG) iz urina i 8-izoprostana (8-iso $PGF_{2\alpha}$). Rezultati su pokazali da su 8-OHdG vrijednosti dobivene iz urina značajno povećane 24 sata nakon testa do otkaza za 13,36% ispitanika u CrM grupi kao i za 24,08% ispitanika u placebo grupi u odnosu na vrijednosti prije suplementacije. Suprotno oštećenju DNK, u sportaša je zabilježen neznačajan trend malom povećanju 8-izo $PGF_{2\alpha}$, dobivenog iz urina, za 0,43% u CrM i 3,06% u placebo grupi nakon testa do otkaza ($p>0,05$). Koncentracije 8-OHdG iz urina uzetog 24 sata nakon testa značajno su niže (za 32,65%) u CrM grupi nakon suplementacije u odnosu na mjerenje prije suplementacije. Nakon suplementacije, koncentracije 8-OHdG u urinu su bile statistički značajno niže u CrM grupi u usporedbi s placebo grupom 24 sata nakon testa ($p<0,05$). Rezultati sugeriraju da suplementacija kreatin monohidratom može smanjiti akutno oksidativno oštećenje DNK izazvano vježbanjem do otkaza u hrvača.

Ključne riječi: *progresivni test opterećenja do otkaza, kreatin, oksidativni stres, 8-OHdG, 8-izo* $PGF_{2\alpha}$