

RESEARCH ARTICLE

Effects of resistance training combined with creatine hydrochloride or creatine monohydrate supplementation on oxidative stress-antioxidant markers in trained women: a double-blind randomized placebo-controlled trial

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Received 15 October 2024 | Accepted 9 April 2025 | Published online 11 June 2025

Abstract

This study evaluated the effects of resistance training (RT) combined with creatine hydrochloride (CrHCL) and creatine monohydrate (CrM) supplementation on oxidative stress-antioxidant markers in trained women. In this study, 48 young trained women were selected and randomly assigned to four groups via a double-blind method: Group 1: RT + Creatine hydrochloride (Cr-HCL) supplementation ($n = 12$), Group 2: RT + Creatine monohydrate-loading phase (CrM-LP) supplementation ($n = 12$), Group 3: RT + CrM-without loading phase (CrM-WLP) supplementation ($n = 12$), and Group 4: RT + Placebo (PL) supplementation ($n = 12$). The RT program was performed for eight weeks with three sessions per week. Blood samples were obtained before and after training to evaluate serum levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) activity. Statistical analyses were performed using the Mixed ANOVA and Bonferroni post-hoc tests at a significance level of 0.05. Following the intervention, the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups indicated significant decreases and increases in the blood levels of MDA and SOD, respectively, compared to the RT + PL supplementation group ($P < 0.05$). Likewise, compared with the RT + PL supplementation group, the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups showed a greater decrease and increase in the levels of serum 8-OHdG (effect size [ES] = 1.11, 0.75, 0.66 vs 0.40) and serum GPX (ES = 0.46, 0.71, 0.56 vs 0.08), respectively ($P < 0.05$). However, CAT did not change significantly after eight weeks of training in any group. In general, the results showed that Cr-HCL and CrM, along with RT can positively affect oxidative stress-antioxidant indices in trained women. According to the results, Cr-HCL does not cause more effects than CrM.

The study was conducted based on the Declaration of Helsinki and was approved by the ethics committee of the Sport Sciences Research Institute (IR.SSRC.REC.1399.138).

Keywords

oxidative stress – antioxidant – resistance training – creatine hydrochloride – creatine monohydrate

1 Introduction

Oxidative stress can be defined as an imbalance between the generation of harmful free radicals and their removal by the antioxidant defence system. Highly reactive unstable free radicals are composed of many compounds. However, the most common are ROS (superoxide, hydroxide, alcoxide, peroxide, hydrogen peroxide) and reactive nitrogen species (RNS) (nitric oxide, nitrogen dioxide, peroxynitrite); collectively called reactive oxygen and nitrogen species (RONS) (Burton and Jauniaux, 2011). Free radicals are very reactive atoms or molecules that have one or more unpaired electrons in their outer shell and can be formed by the interaction of oxygen with specific molecules (Chandrasekaran, Idelchik, and Melendez, 2017). Although in small amounts these radicals are necessary for various beneficial physiological actions, an abundance of RONS can cause irreversible damage to biomolecules, proteins, carbohydrates, lipids, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), underlying the development of many pathologies (Liguori *et al.*, 2018).

The varied evidence supporting the efficacy of creatine supplementation as a performance-enhancing substance has made creatine a very popular ergogenic aid in recent decades (Butts, Jacobs, and Silvis, 2018). There are various antioxidant supplements, among which creatine can be mentioned (Bagchi, Nair, and Sen, 2018). Among the common nutritional supplements, creatine is the most commonly used supplement in the sports community, especially among strength and speed athletes (Beck *et al.*, 2007; Cooper, Naclerio, Allgrove, and Jimenez, 2012).

Creatine (C₄H₉N₃O₂) is a molecule synthesised endogenously in the kidneys, liver, and pancreas at a rate of ~1 g/day from the synthesis of two essential amino acids, i.e. arginine and methionine; and one non-essential amino acid, i.e. glycine; and two enzymes, arginine glycine amidinotransferase (AGAT) and guanidinoacetate acid-methyltransferase (GAMT) (Barcelos, Stefanello, Mauriz, Gonzalez-Gallego, and Soares, 2016; Bonilla *et al.*, 2021; Clarke, Kim, Meza, Ormsbee, and Hickner, 2020; Wax *et al.*, 2021). Most of the body's pool of creatine is in tissues with high energy demands, such as the muscle and brain, where it plays a critical role in recycling adenosine triphosphate (ATP). Recycling of ATP is achieved by converting adenosine diphosphate (ADP) back to ATP via the donation of phosphate groups. Creatine itself can be phosphorylated by creatine kinase to phosphocreatine (PCr), which is used as an energy store in skeletal muscles and the brain.

The critical role of creatine in rapid energy production results in approximately 95% of the body's creatine being stored in skeletal muscle (Balsom, Söderlund, and Ekblom, 1994). Within the muscle, approximately 40% of creatine is in the free form and the remaining 60% is in the phosphorylated form as PCr (Fazio, Elder, and Harris, 2022). In addition to endogenous synthesis, creatine can also be obtained exogenously through the consumption of certain foods. Creatine can be ingested through foods such as meat and fish (Brosnan and Brosnan, 2016).

This supplement reduces cytoplasmic calcium levels, reactive oxygen species (ROS) production, proinflammatory cytokine content, muscle cell apoptosis, and The antioxidant effects of creatine may be due to various functional mechanisms, such as indirect mechanisms involved in the cell membrane stabilisation and improvement of cellular energy capacity and its direct antioxidant properties (RNA, mtDNA) or related to creatine compounds (arginine, methionine, glycine) (Grune, Reinheckel, and Davies, 1997; Guidi *et al.*, 2008; Reid, 2001).

CrM is the most commonly used type of creatine (CrM combines creatine and a water molecule). It is generally stable, degrading slowly even at high temperatures and low pH. Intestinal absorption of CrM is close to 100%, and it has a very high purity of creatine (more than 90%) (Gufford *et al.*, 2010). In addition to creatine monohydrate (CrM), which is known as the most common type of creatine and this type of creatine is generally used (Jäger, Purpura, Shao, Inoue, and Kreider, 2011). A new form of creatine called creatine hydrochloride (CrHCl) has been introduced. Creatine hydrochloride (Cr-HCl) has been marketed as a more bioavailable source of creatine than CrM. Cr-HCl is a salt of HCl and creatine molecule. Like other creatine salts, adding hydrochloric acid to creatine would be expected to decrease pH and improve solubility (Stoppani, 2021) and is claimed to have an aqueous solubility of approximately 700 mg/ml (Gufford *et al.*, 2010). By forming Cr-HCl salt, Cr-HCl creates a change in the molecule and can become 40 times more soluble compared to CrM (Alraddadi, Lillico, Vennerstrom, Lakowski, and Miller, 2018).

In addition, Cr-HCl dissolves more easily than monohydrates. This greater solubility indicates the ability of Cr-HCl to be more easily absorbed. It seems that greater solubility and permeability can reduce the amount of creatine needed to increase muscle creatine reserves (Dash, Miller, Huai-Yan, Carnazzo, and Stout, 2001). This means more absorption and less excretion of creatine.

Marketing claims indicate that Cr-HCl has a 38 times greater bioavailability than CrM (Stoppani, 2021). The basis for this claim appears to come from a report from Gufford and colleagues who conducted physiochemical characterisation studies on several N-methylguanidinium salts, including creatine Cr-HCl. They reported that Cr-HCl contains about 78% creatine by molecular weight and that Cr-HCl was 37.9 times more soluble in water than CrM at 25 °C (Gufford *et al.*, 2010). Also, Tuckfield (2015) reported that Cr-HCl supplementation (for two years) significantly reduced brain atrophy and in patients with Huntington's disease compared to CrM provided better molecular absorption.

Regarding the effect of creatine hydrochloride on performance, the research of Tayebi and Arazi (2019), DeFrance *et al.* (2015) and McDonough (2017) can be mentioned, who showed a positive effect of Cr-HCl on performance in their research. The results of Tayebi and Arazi (2019) show that multi-day (7 days) supplementation of creatine hydrochloride does not have a significant effect on performance compared to creatine monohydrate. DeFrance *et al.* (2015) stated in their study that creatine hydrochloride and creatine monohydrate improved upper and lower body muscle strength after four weeks of resistance training in recreational weightlifters. Also, the results of McDonough (2017) show that creatine hydrochloride supplementation (4 g for 7 days) in healthy resistance-trained men significantly increases the number of repetitions of the bench press and VJCM and body weight.

No research has been done on the effects of Cr-HCl on oxidative-anti-oxidative stress indicators. Contradictory findings have been reported in the studies conducted on the antioxidant effects of CrM supplementation. Accordingly, Stefani *et al.* (2014) stated that receiving creatine monohydrate supplements and RT in rats could reduce oxidative stress and lipoperoxidation. However, creatine supplementation did not affect CAT activity (Stefani *et al.*, 2014). Also, Araújo *et al.* (2013) reported that creatine supplementation exerts antioxidant activity in the liver of rats and increases the activity of antioxidant enzymes GPX and CAT. Examining the short-term use of creatine supplements during resistance exercise, Rahimi (2011) demonstrated that creatine supplements can reduce DNA oxidative damage and lipid peroxidation. In contrast, Deminice *et al.* (2013) evaluated short-term creatine intake plasma oxidative stress, and no antioxidant effect was observed.

The literature has investigated the effect of CrM on men; however, the effectiveness of CrHCL has not been investigated yet. In addition, it is not clear which type

of creatine among the types of creatine made and used by athletes can be more effective in improving oxidative status. Based on the literature, no research has been done on the medium-term effects of Cr-HCl and CrM on oxidative-antioxidant stress indices in trained women. Also, its effectiveness compared to CrM has not yet been proven. Additional research on the effect of this new type of creatine and comparing its effect with CrM is needed. Therefore, this research is the first study on the effect of RT and CrHCL supplementation and its comparison with CrM. Accordingly, the present study aims to investigate the effects of RT combined with supplementation with two forms of CrHCL and CrM on oxidative-anti-oxidative stress indicators in trained women.

2 Materials and methods

Participants

After the calls in the sports centres of Shiraz City, 63 individuals volunteered to participate in the research. To be included in the final analysis, the subjects must complete all the measurement practice sessions and have the criteria to enter the study.

The criteria for entering the research were having at least 6 to 12 months of RT experience; absence of neurological, musculoskeletal, orthopedic, and cardiovascular diseases, cancers, liver diseases, kidney diseases, and respiratory diseases; no consumption of drugs or supplements affecting the research results for six months before the start of the study and during the study period; enjoyment of mental health and motivation to participate in research; failure to participate in other competitive sports activities other than RT during the intervention period; and enjoyment of a normal menstrual cycle. Exclusion criteria also included food supplementation; medication consumption; occurrence of disease and metabolic disorder, including known electrolyte abnormalities; any absolute prohibition of exercise according to ACSM and physician instructions; absence of more than three sessions of the subject in the protocol implementation process; and the absence of the subject in the trial stages after the training period (Ives *et al.*, 2017).

Before the start of the training and supplementation course, the objectives of the research possible benefits and harms, were explained to the participants, and the participants were asked to complete the consent form, medical records, physical activity questionnaire and menstrual cycle questionnaire.

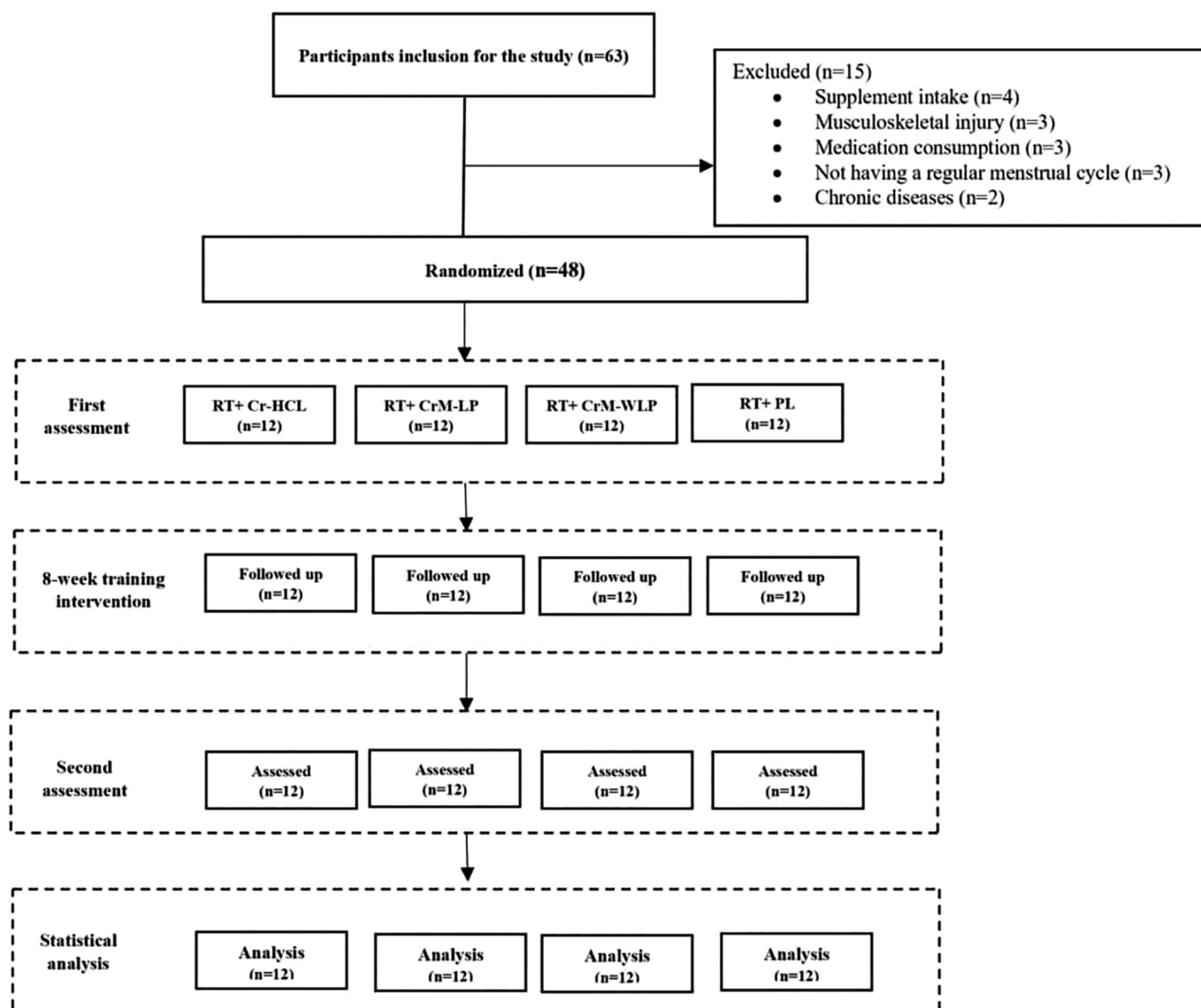


FIGURE 1 Study flow.

During the research period, the participants were asked to have the same sleeping hours and keep their diet the same according to the nutritional recommendations given to them.

As a result of these requirements, 15 individuals were excluded from the research. Therefore, 48 women who had done RT were selected and divided randomly into four groups via a double-blind method: (1) RT + Cr-HCL, (2) RT + CrM-LP, (3) RT + CrM-WLP, and (4) RT + PL (Figure 1).

The study was conducted following the Declaration of Helsinki and was approved by the ethics committee of the Sport Sciences Research Institute (IR.SSRC.REC.1399.138) (Table 1).

Experimental design

This study employed a double-blind, randomised placebo-controlled trial design. In the first session, after getting acquainted and explaining the research pur-

pose and procedure and the researchers' expectations, the subjects were asked to complete the consent form, the physical activity questionnaire, the medical-sports records scale, and the menstrual cycle questionnaire. At this stage, the subjects were introduced to the stages of RT. The subjects' stature, body mass (BM), and anthropometric indices were measured and recorded during the second session. The IRM was measured in the third, fourth and fifth sessions. After four days from the fifth session, the subjects performed the first RT session. Blood sampling was done 48 h before and after the training intervention. Also, the initial tests were repeated.

Measurements

Each subject's standing stature was measured with a stadiometer (SECA 213 Portable Height Measure) with a sensitivity of one millimetre, and their BM was measured with the light clothes they were wearing and with the SECA digital scale with a sensitivity of 100 g (made

TABLE 1 Subjects characteristics (mean $\pm$ SD)

Variable	RT + Cr-HCL (n = 12)		RT + CrM-LP (n = 12)		RT + CrM-WLP (n = 12)		RT + PL (n = 12)	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Age (year)	21.16 $\pm$ 2.58		22.25 $\pm$ 2.30		24.25 $\pm$ 1.05		23.25 $\pm$ 1.95	
Stature (cm)	160.25 $\pm$ 3.60		162.27 $\pm$ 2.66		161.23 $\pm$ 3.53		162.81 $\pm$ 5.27	
BM (kg)	56.03 $\pm$ 7.62	57.27 $\pm$ 7.66	55.45 $\pm$ 4.88	56.55 $\pm$ 4.35	57.10 $\pm$ 4.15	58.19 $\pm$ 4.30	56.23 $\pm$ 6.38	56.65 $\pm$ 6.16
BMI (kg/m ²)	21.86 $\pm$ 2.69	22.34 $\pm$ 2.68	21.14 $\pm$ 2.20	21.56 $\pm$ 2.04	22.01 $\pm$ 1.55	22.43 $\pm$ 1.57	21.20 $\pm$ 1.54	21.36 $\pm$ 1.48
BF (%)	25.29 $\pm$ 3.09	23.99 $\pm$ 2.93	24.32 $\pm$ 2.08	22.94 $\pm$ 1.88	24.97 $\pm$ 1.77	23.46 $\pm$ 1.56	24.61 $\pm$ 1.59	24.12 $\pm$ 1.69

Abbreviations: RT, resistance training; Cr-HCL, creatine hydrochloride; CrM-LP, creatine monohydrate – loading phase; CrM-WLP, CrM – without loading phase; PL, placebo; BM, body mass; BMI, body mass index; BF%, body fat percentage.

in Germany). Then, a body composition analysis device (In Body 720, made in South Korea) was employed to measure and record anthropometric indicators by entering variables such as BM, stature, and gender. The measurement method was based on the protocols adopted by the manufacturing companies.

Blood sampling and analysis

Blood sampling was conducted on two occasions: 48 h before the start of the training period and 48 h after the last training session, after a 12-h fast and after 8 h of sleep between 8-9 a.m. 10 ml of blood samples were drawn from the antecubital vein. The blood was allowed to clot at room temperature for 30 min, they were centrifuged at 1,500 rpm for 10 min. The serum layer was removed and frozen at -80°C for further analyses (Eley, Russell, Baxter, Mukerji, and Tisdale, 2007). Commercially available, enzyme-linked immunosorbent assay (ELISA) kit (ZellBio GmbH: ZB-11436C-H9648, Germany) was used for analysis of serum 8-hydroxy-2-deoxyguanosine (8-OHdG) and intra-assay and inter-assay coefficients of variances was less than 10 and 12%, respectively. Serum oxidative-antioxidant stress markers levels malondialdehyde (MDA) (the intra-assay and inter-assay coefficients of variances 6.7 and 7.2%, respectively), superoxide dismutase (SOD) activity (8 and 7.1%), glutathione peroxidase activity (GPX) activity (7.1 and 8.7%) and catalase (CAT) activity (4.1 and 9.9%) were examined using photometry and commercially available kits (Teb Pazhouhan Razi (TPR) (Tehran, Iran)).

Diet control

To investigate the effect of diet on oxidative stress variables, diet recall questionnaire (two normal days and one day off) was completed for three consecutive days before starting the training period (Wilson *et al.*, 2014). At the end of the training protocol, the participants again recorded their food intake for three consecutive days (before blood sampling). Before starting the training period, the subjects were recommended to adjust their diet based on the recommendations of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine to distribute macronutrients (55-65% of total calories from carbohydrates, approximately 35% of total calories from fats and approximately 10 to 15% of total calories from protein) (Sword, 2012). In addition, the subjects were instructed to maintain their diet during the training period.

Food diaries were analysed. The average intake of daily calories, protein, carbohydrates, fat, vitamins E, C, and A is presented in Table 2.

Based on the results, energy, carbohydrate, lipid, protein, vitamins E, C, and A intakes did not differ between the before and after intervention measurements for groups ($P > 0.05$) (Table 2).

Supplementation

This research used CON-CRET Patented Creatine HCL, produced by CON-CRET®, USA, for the RT + Cr-HCL supplementation group. It consumed 3 g per day, 30 min prior to each exercise session on the training day and in the same hour on non-training days. For the RT + CrM supplementation group, the CrM produced by Olimp

TABLE 2 Dietary intake assessed for the RT + Cr-HCL, RT + CrM-LP, RT + CrM-WLP and RT + PL supplementation groups before and after the training period. Data are presented as means $\pm$ SDs

Variable	RT + Cr-HCL (n = 12)		RT + CrM-LP (n = 12)		RT + CrM-WLP (n = 12)		RT + PL (n = 12)	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Energy intake (kcal)	2,003.31 $\pm$ 63.77	1,979.53 $\pm$ 108.11	2,054.44 $\pm$ 55.67	2,036.28 $\pm$ 38.61	2,018.12 $\pm$ 57.60	1,978.64 $\pm$ 76.07	2,025.10 $\pm$ 79.37	2,011.45 $\pm$ 61.97
Carbohydrate (g)	209.98 $\pm$ 5.37	211.54 $\pm$ 3.34	218.76 $\pm$ 8.88	215.34 $\pm$ 7.80	215.91 $\pm$ 5.72	212.35 $\pm$ 5.00	213.01 $\pm$ 9.16	215.28 $\pm$ 6.06
Fat (g)	92.46 $\pm$ 6.59	88.12 $\pm$ 5.38	91.26 $\pm$ 15.59	86.01 $\pm$ 6.79	80.01 $\pm$ 10.55	78.42 $\pm$ 9.41	78.89 $\pm$ 10.19	75.05 $\pm$ 7.78
Protein (g)	71.72 $\pm$ 10.14	76.50 $\pm$ 6.24	71.81 $\pm$ 7.06	75.95 $\pm$ 4.46	67.30 $\pm$ 5.40	72.90 $\pm$ 7.91	69.24 $\pm$ 6.63	73.83 $\pm$ 5.50
Vitamin E (mg)	5.26 $\pm$ 0.62	5.63 $\pm$ 0.64	5.49 $\pm$ 0.70	5.68 $\pm$ 0.41	5.10 $\pm$ 0.75	5.58 $\pm$ 0.63	5.31 $\pm$ 0.69	5.53 $\pm$ 0.62
Vitamin C (mg)	107.50 $\pm$ 20.19	111.16 $\pm$ 16.43	103.25 $\pm$ 16.13	110.75 $\pm$ 13.47	104.79 $\pm$ 14.37	106.83 $\pm$ 15.48	96.33 $\pm$ 11.87	99.50 $\pm$ 8.52
Vitamin A (RE) ¹	892.33 $\pm$ 62.77	864.50 $\pm$ 40.05	870.83 $\pm$ 55.66	847.91 $\pm$ 60.52	850.58 $\pm$ 50.72	864.16 $\pm$ 54.03	851.08 $\pm$ 59.88	866.0 $\pm$ 59.99

1 Retinol equivalents (1 RE = 1 μ g retinol = 12 μ g β -carotene = 24 μ g α -carotene).

Sport Nutrition*, Poland, was used. The amount of CrM consumed in the RT + CrM-LP supplementation group was 20 g for five days (loading), taken in four servings during the day (Bagchi *et al.*, 2018; Greenwood, Cooke, Ziegenfuss, Kalman, and Antonio, 2015). Then, 3 g were consumed for 51 days, 30 min prior to each exercise session on the training day or during exercise hours on the non-training days. In the RT + CrM-WLP supplementation group, 3 g was consumed during the training period, 30 min prior to each exercise session (on training days) or exercise hours (on non-training days) (Bagchi *et al.*, 2018; Greenwood *et al.*, 2015). In addition, the RT + PL supplementation group consumed maltodextrin under the same conditions as other groups. The supplements were in powder form, dissolved in 250 to 300 ml of water, and then taken by the group.

Sample size

The number of subjects was selected using G*Power software, version 3.0.10 (Franz Faul, Universität Kiel, Germany) (Faul, Erdfelder, Lang, and Buchner, 2007), a priori power analysis was conducted with an alpha level of 0.05. Also, a statistical power of 0.80 was performed to detect the research sample size.

Training program

The supervised resistance training (RT) protocol consisted of an 8-week program involving 3 sessions per week. Each training session lasted 90 to 100 min, includ-

ing 10 min of standard warm-up, 70 to 80 min of main training and 10 min of cool down. participants performed 3 sets of 6 to 12 repetitions with 70 to 85% of 1RM (squat, leg press, knee extension, knee flexion, lat pull down, seated rows, bench press and pec fly) per session. The 60 to 90 and 120 to 180 s resting intervals were assigned between sets and exercises, respectively. It was added once every two weeks to increase the training load by 5% on the 1RM (Fleck and Kraemer, 2014; Liguori, 2020). To ensure that all training exercise sessions can be performed correctly, they were supervised cautiously, and the training was controlled throughout the whole RT period.

Statistical analyses

All data are presented as mean $\pm$ standard deviation (SD). The normality of the data was confirmed by the Shapiro-Wilk test. The dependent variables were analysed using Mixed ANOVA and Bonferroni post-hoc tests. The calculation of effect sizes (ES) was used to examine the magnitude of any treatment effect. The degree of ES statistics was considered trivial <0.20; small, 0.20-0.50; moderate, 0.5-0.80; large, 0.8-1.30; very large >1.30 (Cohen, 2013). For each measure, a percent change score was calculated ((post-pre)/pre $\times$ 100). All analyses were conducted using SPSS version 26.0, and the statistical significance was set at $P \leq 0.05$.

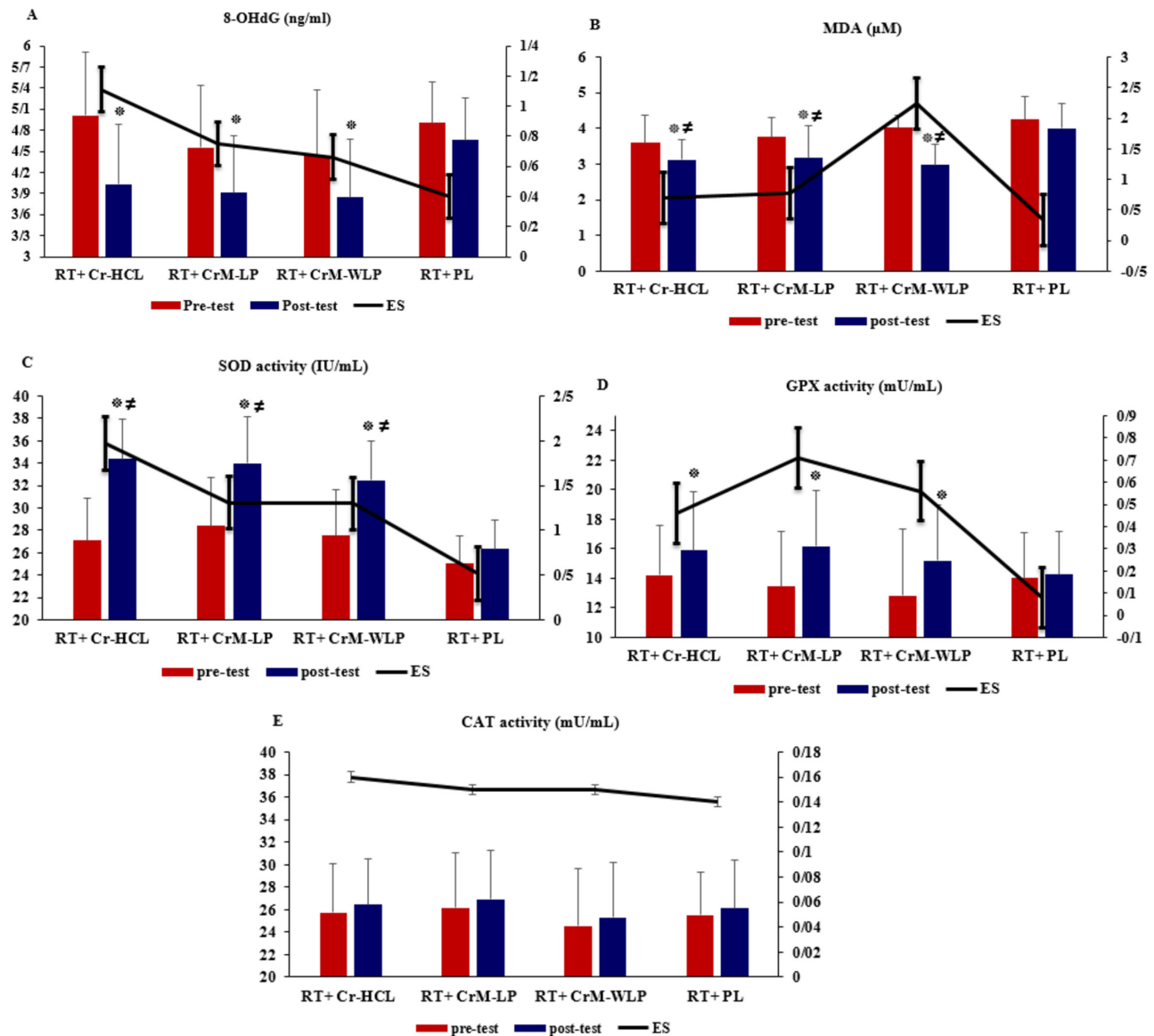


FIGURE 2 Changes in oxidative-antioxidant stress markers following eight weeks of a training intervention (means $\pm$ SDs). *: denotes significant differences between baseline and post training values ($P \leq 0.05$); (pre-test, post-test); †: denotes significant differences between the RT + Cr-HCL, RT + CrM-LP, RT + CrM-WLP and placebo supplementation groups at post-training ($P \leq 0.05$); RT: Resistance training, (A) 8-OHdG: 8-hydroxy-2-deoxyguanosine, Concentrations of (B) MDA: malondialdehyde, (C) SOD activity: superoxide dismutase, (D) GPX activity: glutathione peroxidase and (E) CAT activity: catalase.

3 Results

Dietary data were analysed using the Nutritionist IV diet analysis software (1995, First Databank, San Bruno, CA, USA). There was no difference in the intake of energy, carbohydrate, fat, protein, vitamins E, C, and A between the measurements before and after the intervention for the RT + Cr-HCL, RT + CrM-LP and RT + CrM-WLP and the RT + PL supplementation groups (Table 2). Also, there was no significant difference between the groups regarding age, stature, BM, BMI, and body fat percent-age (BF %). Changes in serum (A) 8-OHdG, (B) MDA,

(C) SOD activity, (D) GPX activity and (E) CAT activity are illustrated in Figure 2.

As observed, the serum 8-OHdG levels significantly decreased ($P = 0.001$, $P = 0.008$; $P = 0.014$, respectively) in the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups (from 5.01 ± 0.90 to 4.03 ± 0.85 , 4.55 ± 0.88 to 3.91 ± 0.81 , 4.43 ± 0.95 to 3.84 ± 0.83 ng/ml; % of Change = -19.49 , -11.51 , -11.58 , respectively) after eight weeks of training, while the decrease ($P = 0.300$) was not significant in the RT + PL supplementation group (from 4.90 ± 0.59 to 4.66 ± 0.60 ng/ml; % of Change = -4.27); there was no statistically significant difference between the groups ($P = 0.121$) (Fig-

ure 2, A). All the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups showed a significant decrease ($P = 0.036$, $P = 0.012$; $P = 0.001$, respectively) in the serum levels of MDA (from 3.60 ± 0.77 to 3.12 ± 0.58 , 3.76 ± 0.56 to 3.17 ± 0.92 , 4.02 ± 0.34 to 2.98 ± 0.56 μM ; % of Change = -9.29 , -15.83 , -25.41 , respectively) compared to before training, but there was no significant difference in the RT + PL supplementation group ($P = 0.301$) (from 4.24 ± 0.66 to 4.01 ± 0.67 μM ; % of Change = -4.86). After examining the results, a significant difference was observed between the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups with the RT + PL supplementation group in the serum MDA. ($P = 0.006$, $P = 0.024$, $P = 0.035$, respectively) (Figure 2, B). After eight weeks of RT intervention, the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups showed a significant increase ($P = 0.001$, $P = 0.001$; $P = 0.001$, respectively) in serum SOD activity (from 27.08 ± 3.82 to 34.39 ± 3.57 , 28.38 ± 4.32 to 33.99 ± 4.21 , 27.51 ± 4.07 to 32.48 ± 3.53 IU/ml; % of Change = 28.13 , 22.32 , 20.02 , respectively), while the increase ($P = 0.259$) in the RT + PL supplementation group was not significant (from 25.02 ± 2.53 to 26.36 ± 2.56 IU/ml; % of Change = 5.92). Also, according to the results, a significant difference was observed between the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups and the RT + PL supplementation group ($P = 0.001$, $P = 0.001$; $P = 0.007$, respectively) (Figure 2, C). For the serum GPX activity, a significant increase ($P = 0.044$, $P = 0.002$; $P = 0.007$, respectively) compared to before exercise was observed in the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups (from 14.21 ± 3.37 to 15.90 ± 3.94 , 13.45 ± 3.74 to 16.16 ± 3.80 , 12.85 ± 4.50 to 15.18 ± 3.77 mU/ml; % of Change = 12.88 , 24.33 , 29.13 , respectively), but no significant increase ($P = 0.746$) was observed in the RT + PL supplementation group (from 14.01 ± 3.08 to 14.28 ± 2.93 mU/ml; % of Change = 4.13). In addition, no significant difference was observed between the groups ($P = 0.852$) (Figure 2, D). After separating the statistical results, the RT + Cr-HCL, RT + CrM-LP, RT + CrM-WLP, and RT + PL supplementation groups showed a non-significant increase ($P = 0.611$, $P = 0.582$; $P = 0.569$, 0.661 , respectively) in the CAT activity compared to before training (from 25.76 ± 4.34 to 26.44 ± 4.04 , 26.18 ± 4.82 to 26.91 ± 4.33 , 24.54 ± 5.16 to 25.30 ± 4.87 , 25.54 ± 3.75 to 26.12 ± 4.25 mU/ml; % of Change = 4.23 , 5.88 , 4.61 , 3.01 , respectively). Also, after examining the data, no significant difference was observed between the groups ($P = 0.768$) (Figure 2, E).

4 Discussion

This study aimed to investigate the effects of RT combined with CrHCL and CrM supplementation on oxidative-antioxidant stress markers in trained women. Our findings showed that Cr-HCL did not provide any advantages over CrM.

The research results showed that the following the intervention, the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups indicated significant decreases and increases in the blood levels of MDA (from 3.60 ± 0.77 to 3.12 ± 0.58 , 3.76 ± 0.56 to 3.17 ± 0.92 , 4.02 ± 0.34 to 2.98 ± 0.56 μM ; % of Change = -9.29 , -15.83 , -25.41 , respectively) and SOD activity (from 27.08 ± 3.82 to 34.39 ± 3.57 , 28.38 ± 4.32 to 33.99 ± 4.21 , 27.51 ± 4.07 to 32.48 ± 3.53 IU/ml; % of Change = 28.13 , 22.32 , 20.02 , respectively), respectively, compared to the RT + PL supplementation group (in the serum levels of MDA from 4.24 ± 0.66 to 4.01 ± 0.67 μM ; % of Change = -4.86 and in serum SOD activity from 25.02 ± 2.53 to 26.36 ± 2.56 IU/ml; % of Change = 5.92) ($P < 0.05$).

Likewise, compared with the RT + PL supplementation group (the serum 8-OHdG levels from 4.90 ± 0.59 to 4.66 ± 0.60 ng/ml; % of Change = -4.27 and the serum GPX activity from 14.01 ± 3.08 to 14.28 ± 2.93 mU/ml; % of Change = 4.13), the RT + Cr-HCL, RT + CrM-LP, and RT + CrM-WLP supplementation groups showed a greater decrease and increase in the levels of serum 8-OHdG (effect size [ES] = 1.11 , 0.75 , 0.66 vs 0.40) (from 5.01 ± 0.90 to 4.03 ± 0.85 , 4.55 ± 0.88 to 3.91 ± 0.81 , 4.43 ± 0.95 to 3.84 ± 0.83 ng/ml; % of Change = -19.49 , -11.51 , -11.58 , respectively) and serum GPX (ES = 0.46 , 0.71 , 0.56 vs 0.08) (from 14.21 ± 3.37 to 15.90 ± 3.94 , 13.45 ± 3.74 to 16.16 ± 3.80 , 12.85 ± 4.50 to 15.18 ± 3.77 mU/ml; % of Change = 12.88 , 24.33 , 29.13 , respectively), respectively ($P < 0.05$). Also, CAT activity did not change significantly after eight weeks of training in any of the groups.

Also, after examining the data, the RT + Cr-HCL, RT + CrM-LP, RT + CrM-WLP, and RT + PL supplementation groups showed a non-significant increase in the CAT activity compared to before training (from 25.76 ± 4.34 to 26.44 ± 4.04 , 26.18 ± 4.82 to 26.91 ± 4.33 , 24.54 ± 5.16 to 25.30 ± 4.87 , 25.54 ± 3.75 to 26.12 ± 4.25 mU/ml; % of Change = 4.23 , 5.88 , 4.61 , 3.01 , respectively) and, no significant difference was observed between the groups ($P = 0.768$).

CAT is an enzyme that is highly modulated by physical training, especially by endurance training, where the formation of ROS by the leakage of superoxide radicals in the electron transporter chain is much higher

due to the greater utilisation of the oxidative pathway (Lambertucci, Levada-Pires, Rossoni, Curi, and Pithon-Curi, 2007; Laughlin *et al.*, 1990; Leeuwenburgh, Fiebig, Chandwaney, and Ji, 1994). Since in our results, serum CAT activity increased compared to before training, but this increase was not significant, It seems that not be necessary to increase this antioxidant enzyme (due to the presence of non-enzymatic antioxidants such as creatine) to reduce the serum lipoperoxidation in the groups.

No research has been done on the effect of CrHCl and CrM supplementation on oxidative stress-antioxidant markers in trained women, but few studies have been done on the effect of CrM on oxidative stress in other population groups.

Consistent with results of the present study, Araujo *et al.* (2013) investigated the effects of creatine supplementation on liver oxidative stress indices in rats. Based on the results, glutathione peroxidase (GSH-GPx) activity increased at the end of the training period in the training and training + creatine groups compared to the control group. They found that creatine intake acts additively to exercise to increase antioxidant enzymes in rat liver (Araújo *et al.*, 2013). Regular exercise activates transcription factors, i.e. nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor- κ B (NF- κ B), that are responsible for stimulating various genes, including mitochondrial GSH-GPx (Çakir-Atabek, Demir, PinarbaSili, and Gündüz, 2010). Furthermore, Stefani *et al.* (2014) investigated the effects of creatine monohydrate supplementation and RT on oxidative stress in different tissues of rats. The results indicate that creatine intake combined with resistance exercise can reduce lipid peroxidation in plasma, liver, heart, and gastrocnemius compared to the control group. Also, creatine supplementation had positive effects on SOD activity in all groups. Probably, creatine supplementation has a synergistic effect with RT in modulating SOD activity in the heart (Stefani *et al.*, 2014). It seems that in conditions of progressive chronic stress and RT, supplements have a synergistic effect due to the compatibility of RT with creatine, which includes enzymatic compatibility of cell signalling with SOD in heart tissue. This mechanism happened through activating the NADPH oxidase system, which in a short time modulates the expression of antioxidant enzymes by angiotensin II and inflammatory mediators (Zhao, Bey, Wientjes, and Cathcart, 2002). Rahimi (2011) investigated the effect of short-term creatine monohydrate supplementation on oxidative DNA damage and lipid peroxidation induced by a single bout of resistance exercise using the flat pyra-

mid loading pattern system on male athletes. His results demonstrated that CrM significantly reduced oxidative DNA damage (8-OHdG) and lipid peroxidation (MDA) markers. In addition, the antioxidant effects of CrM may be associated with its compounds (arginine, glycine, and methionine) (Rahimi, 2011).

In contrast, Silva *et al.* (2013) investigated the effect of CrM supplementation (300 mg/kg/day, 15 days, dose of initially: 2 serving/day, dose after six days: 1 serving/day) after an exhaustion eccentric running session, finding that CrM supplementation had no effect on parameters of muscle oxidative stress and inflammation, and the increase in TBARS was independent of creatine supplementation. CrM supplementation may be more effective than long-term training by reducing intracellular calcium accumulation, limiting ROS formation, and reducing oxidative damage in short-term training (Silva *et al.*, 2013). In addition, Percario *et al.* (2012) investigated the effects of CrM supplementation (during the first five days of the protocol, 20 g per day of CrM intake, i.e. 5 g per serving, four servings per day, and in the remaining 27 days, 5 g of CrM per day) along with RT (five weeks of training resistance with an intensity of 50 to 95% 1RM) on muscle strength and oxidative stress profile in healthy athletes. They indicated that CrM supplementation stimulated oxidative stress and decreased the total antioxidant status (TAS). They reported that the TAS values in the CrM + training group were significantly reduced compared to other groups. It is possible that the significant increase in strength of the creatine + training group also enhances the mechanism of energy production due to the high capacity for ATP resynthesis in cells. This situation may be suitable for the manifestation of ischemia-reperfusion syndrome. Enhanced production of uric acid and hydroxyl radicals, causing the mobilisation of antioxidant stores, prevents oxidative stress (Percário *et al.*, 2012). This difference in the research results can be attributed to the subjects' baseline amounts of creatine, form of creatine, creatine dosage, duration of creatine intake, insufficient loading of creatine at the muscle level due to the loading protocol, different biological potentials for creatine absorption in the muscle, training protocol including intensity and duration of exercises, the type of exercises performed, nutrition, and fitness level of participants.

Taking antioxidant supplements combined with physical activity can reduce the adverse effects of oxidative stress induced by exercise, increase the antioxidant defence system associated with exercise, and increase the positive effects of physical activity (Rudrapal *et al.*, 2022). Antioxidant supplements can have beneficial

and pro-oxidant effects (Fisher-Wellman and Bloomer, 2009; He *et al.*, 2016). Creatine is one of the favourite ergogenic supplements among athletes (Silva *et al.*, 2013).

Literature demonstrates that creatine supplementation can have antioxidant properties. The exact mechanism of the antioxidant action of creatine is not known. Nevertheless, it has played a role in increasing the activity of antioxidant enzymes and the ability to remove ROS and RONS (Nakajima, Kamohara, Nakano, and Ohno, 2006; Sestili *et al.*, 2006).

Creatine protects two different and important cellular targets, i.e. mitochondrial deoxyribonucleic acid (mtDNA) and RNA, against oxidative damage. In addition, creatine has been found to induce other related effects contributing to cell survival and function under oxidative stress conditions. Creatine probably maintains mitochondrial integrity via organelle-directed antioxidant activity, which causes sufficient mitochondrialogenesis, provides a significant amount of thiols intracellularly, protects RNA from oxidative damage in conditions that require the use of robust messenger ribonucleic acid (mRNA); thus, it has antioxidant effects (Guidi *et al.*, 2008; Sestili *et al.*, 2006). Mitochondria and mtDNA are essential targets for oxidative damage. Mitochondrial antioxidants have been proposed as a valuable tool to protect mitochondria against pathological and toxicological changes (Reddy, 2008). Studies show that creatine significantly protects mtDNA from oxidative damage (Guidi *et al.*, 2008). Creatine may prevent damage through direct antioxidant activity. Therefore, creatine supplementation may play an essential role in genome stability, which can normalise mitochondrial mutagenesis and prevent its functional consequences, such as reduced oxygen consumption, ATP content, cell survival, and mitochondrial membrane potential (Guidi *et al.*, 2008). RNA molecules intervene in all stages of gene expression and many other biological activities. RNA damage may also affect the balance between protein breakdown and synthesis and repair and regeneration processes in skeletal muscles, ultimately determining muscle mass (Hermann and Westhof, 2000). RNA damage can be attributed to exposure to xenobiotics, various compounds capable of damaging RNA. The protective effect of creatine against doxorubicin activity that causes RNA damage can be partially attributed to creating PCr sources that increase ATP regeneration. The protective activity of creatine against radicals also points to the role of creatine as an antioxidant (Fimognari, Sestili, Lenzi, Cantelli-Forti, and Hrelia, 2009).

Creatine also enhances the expression of myogenic transcriptional regulators (MRFs) and insulin-like growth factor-1 (IGF-1) mRNAs and increases creatine phosphate (CrP) sources (Louis, Van Beneden, Dehoux, Thissen, and Francaux, 2004). Increased demand for ATP used during exercise increases ROS levels (Silva *et al.*, 2013); oxidative phosphorylation (OXPHOS) is the main source of ATP production in the cell. Changes in the process of increasing ROS production lead to oxidative damage (Silva *et al.*, 2013). Approximately 2.5% of the molecular oxygen consumed in OXPHOS during exercise is converted into a group of potentially harmful oxygen derivatives known as ROS (Di Meo and Venditti, 2001). CrM increases intracellular CrP; an increase in intracellular CrP can act as a cellular energy buffer, thereby reducing the dependence on OXPHOS during high-intensity and short-duration training (Silva *et al.*, 2013).

In addition, the antioxidant properties of creatine may be related to the presence of arginine in its molecule. Arginine is a substrate of the nitric oxide synthase (NOS) family and can increase the production of nitric oxide (NO) (a free radical that modulates glucose metabolism, contraction, and uptake in skeletal muscle) (Reid, 2001). Other amino acids such as glycine and methionine may be particularly sensitive to free radical oxidation due to the presence of sulfhydryl groups (Grune *et al.*, 1997). Furthermore, another possible mechanism of leading to the increase in ROS level due to creatine supplementation is the control of maintaining intracellular calcium homeostasis. Literature has shown that disturbance in calcium homeostasis is one of the sources of ROS production during exercise (Sen, Packer, and Hiinnincn, 2000). Consuming creatine supplements can improve the calcium buffering capacity by fuelling the calcium pump of the sarcoplasmic reticulum. As a result, the concentration of intracellular calcium and the activation of destructive pathways such as calpain will decrease. The decrease in calpain activity leads to a decrease in the destruction and disintegration of structural proteins as well as the structures of microplates (Proske and Morgan, 2001), as a result of which inflammation and ROS production are reduced (Sen *et al.*, 2000).

According to claims, the solubility of Cr-HCl is higher than CrM, but based on studies that have been conducted, solubility does not affect the bioavailability of creatine, because CrM has 100% bioavailability (Deldicque *et al.*, 2008; Persky, Brazeau, and Hochhaus, 2003). According to the results of the present study, Cr-HCl does not have a greater effect than CrM and due

to its very high price compared to CrM, its use is not economical.

There were limitations in this study that may affect our findings. One of the limitations of this research was the lack of measurement of muscle creatine content. Another limitation of the present study, was the control of participants' nutrition during the training and supplementation period. In this study, a food recall questionnaire was used to control the effects of participants' nutrition. Participants tried to follow a similar eating pattern throughout the study period with the guidance and advice provided at the beginning of the study, so that the effects of nutrition on the desired variables would be reduced. In addition, the level of motivation and the level of stress of the subjects to perform the tests were among the limitations of the present study.

5 Conclusion

The results of the present study showed that there was no significant difference in the indicators of oxidative stress between the two groups of Cr-HCl and CrM, and the claims made about the greater effectiveness of Cr-HCl are not confirmed. Despite claims of increased solubility, bioavailability, and superior absorption mechanisms, there is currently no evidence to support the use of Cr-HCl instead of CrM. Although Cr-HCl effects on oxidative-antioxidant stress indices, due to its high price compared to CrM, its use is not economical and it cannot replace CrM. Considering the very few studies in this regard, for more accurate conclusions, more studies are needed in different age groups and athletes of different fields, at different levels of sports and with longer supplementation periods.

Acknowledgements

The authors would like to thank the athletes who participated in this study and the Human Performance Laboratory team for their technical support.

Authors' contribution

S.S.D. contributed to project administration, conceptualisation, methodology, visualisation, investigation and writing of the manuscript. H.A. supervised the project, methodology and data analysis and contributed to conceptualisation, reviewing, and editing of the

manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Funding

This study (Research Project Code: 99026748) was funded by the Iran National Science Foundation (INSF) from the vice-presidency for science and technology of Iran.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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