Supplementing With Which Form of Creatine (Hydrochloride or Monohydrate) Alongside Resistance Training Can Have More Impacts on Anabolic/Catabolic Hormones, Strength and Body Composition?

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Received January 12, 2024
Accepted April 23, 2024

Summary

The purpose of this study was to determine the effects of resistance training (RT) alongside creatine-hydrochloride (Cr-HCl) or creatine monohydrate (CrM) supplementation on anabolic/catabolic hormones, strength, and body composition. Forty participants with an age range of 18-25 years were randomly divided into four groups (n=10): RT+Cr-HCl (0.03 $g.kg^{-1}$ of body mass), RT+CrM-loading phase (CrM-LP) (0.3 $g.kg^{-1}$ of body mass for five days (loading) and 0.03 $g.kg^{-1}$ body mass for 51 days (maintenance)), RT+CrM-without loading phase (CrM-WLP) (0.03 $g.kg^{-1}$ body mass), and RT+placebo (PL). The participants consumed supplements and performed RT with an intensity of 70-85 % 1RM for eight weeks. Before and after the training and supplementation period, strength (1RM), body composition (percent body fat (PBF), skeletal muscle mass (SMM), muscular cross-sectional area (MCSA)) and serum levels of testosterone, growth hormone (GH), insulin-like growth factor-1 (IGF-1), cortisol, adrenocorticotropic hormone (ACTH), follistatin and myostatin were measured. The results showed that in the supplementation groups, strength, arm and thigh MCSA, and SMM significantly increased, and PBF significantly decreased ($P \leq 0.05$); this change was significant compared to the PL group ($P \leq 0.05$). In addition, the results showed a significant increase in GH, IGF-1 levels, the ratio of follistatin/myostatin, testosterone/cortisol ($P \leq 0.05$), and a significant decrease in cortisol and ACTH levels ($P \leq 0.05$) in the supplementation groups. Hormonal changes in GH, IGF-1, testosterone/cortisol, cortisol, and ACTH levels in the supplementation groups were significant compared to the PL group ($P \leq 0.05$). The results showed that CrM and Cr-HCl significantly enhanced the beneficial effects of RT on strength, hypertrophy, and hormonal responses, with Cr-HCl showing no benefit over CrM.

Key words

Resistance training • Supplementation • Creatine • Hormonal adaptation • Strength

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Introduction

Ninety-five percent of the body's creatine stores are found in skeletal muscle, and the remaining 5 % is distributed in the brain, liver, kidneys, and testes [1]. Creatine is synthesized endogenously from two essential amino acids, arginine, and methionine, and a nonessential amino acid, glycine, in the kidneys and liver [2]. Creatine monohydrate (CrM) supplementation affects skeletal muscles by increasing strength, fatigue resistance, muscle phosphocreatine (PCr) content, and markers of protein synthesis [2,3]. This supplement reduces cytoplasmic calcium levels, reactive oxygen species (ROS) production, proinflammatory cytokine content, muscle cell apoptosis, and activation of satellite cells [4,5]. The primary role of creatine is to combine with a phosphate group to form PCr through the enzymatic reaction of creatine kinase [6]. PCr is then used by the muscle cell to rapidly regenerate ATP for further muscle contractions. Creatine supplementation can increase muscle creatine and PCr concentrations by 20-40 %, and this increase has significant effects, as the accumulation of phosphate and other metabolites is responsible for muscle fatigue [6].

Creatine is associated with muscle anabolism due to a considerable increase in muscle volume when consumed during resistance training (RT) [7]. There are several hypotheses about increased muscle fibers *via* supplementation, one of which is proposed to prevent protein breakdown by supplementation. Creatine supplementation with RT increases protein synthesis and insulin-like growth factor-1 (IGF-1) to a greater extent than RT in men and women and helps to increase muscle hypertrophy further [8]. Increased muscle mass appears to result from improving the ability to perform high-intensity training by increasing access to PCr and increasing adenosine triphosphate (ATP) synthesis. In this way, athletes can develop more significant training stimulus and more muscle hypertrophy by increasing myosin heavy chain expression, possibly due to increased myogenic regulatory factors (MRFs), myogenin, and MRF-4 [9].

There are many forms of creatine on the market, including creatine nitrate, creatine citrate, creatine ethyl ester and buffered forms of creatine, which are not bioavailable sources of creatine and are less effective [10] or more expensive than CrM [11]. 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 [12]. Intestinal absorption of CrM is close to 100 %, and it has a very high purity of creatine (more than 90 %) [12]. One of the forms of creatine salt is hydrochloride (HCl), which is claimed to have an aqueous solubility of approximately 700 mg/ml [13]. The considerable solubility of HCl relative to CrM suggests that better absorption may be possible. Physiological pharmacokinetic simulations and modeling showed a significant increase in blood and tissue creatine levels following creatine hydrochloride (Cr-HCl) supplementation compared to CrM [14]. By forming Cr-HCl salt, one can create a change in the molecule, and Cr-HCl becomes more soluble than CrM. The basis for this claim seems to be the report of Gufford *et al.* [13], who stated that Cr-HCl contains approximately 78 % creatine by molecular weight and is 37.9 times more soluble than CrM in water at 25 °C. Higher solubility and permeability appear to reduce the amount of creatine required [15]. Marketing claims suggest that Cr-HCl is more bioavailable than CrM [16]. These claims are also based on a report by Alradadi *et al.* [14] who used a simulated prediction model to estimate how Cr-HCl affects tissue creatine retention based on differences in solubility. However, their review was only a theoretical modeling study, and the researchers did not directly compare Cr-HCl with CrM intake on plasma or tissue creatine content [17]. Therefore, the results of this research cannot be directly generalized to humans. There is limited information on the effect of Cr-HCl and there is a need for more research in this field. There have been few studies on the effects of Cr-HCl; the results of Tayebi and Arazi [18] showed that Cr-HCl supplementation did not have a significant effect on performance and hormonal response compared to CrM [18,19]. Additionally, de Franca *et al.* [19] stated that Cr-HCl and CrM improved muscle strength after four weeks of RT, but only Cr-HCl changed body composition in recreational weightlifters [19]. In addition, Arazi *et al.* showed that acute CrM supplementation and RT positively affected testosterone and cortisol concentrations [20].

According to previous claims, the hypothesis of supplementation with Cr-HCl instead of CrM was proposed. Although this information suggests that Cr-HCl is a beneficial ergogenic aid, more information is needed. Considering the high costs of buying Cr-HCl compared to CrM [11], it should be observed that its effects are much higher than those of CrM or that it was only a marketing claim. Therefore, the present study attempted to answer the following question: what are the effects of RT alongside Cr-HCl or CrM supplementation on anabolic/catabolic hormones, strength and body composition in young beginners? We hypothesized that Cr-HCl supplementation can affect hormonal adaptation, strength and body composition more than CrM supplementation.

Materials and Methods

Study subjects

In this study, 40 young beginner athletes in RT aged 20-25 years participated. The inclusion criteria were as follows: having a history of 6-12 months of RT; no injuries in the body causing defects in the performance of exercises; no neurological, musculoskeletal or cardiovascular diseases; no use of sports and medication supplements affecting the results during and six months before entering the study; and having mental health and motivation to participate in the study (Fig. 1). After explaining how to conduct the research and determining the researchers' goals, benefits, possible harms, and requests from the subjects, the subjects completed the consent form, medical records, and physical activity questionnaire. Subjects were asked to have the same

bedtime. After the pretest, the subjects were randomly (www.randomization.com) divided into four groups (n=10): 1 – RT+Cr-HCl, 2 – RT+CrM-loading phase (CrM-LP), 3 – RT+CrM-without loading phase (CrM-WLP), and 4 – RT+placebo (PL). The research protocol was approved by the Research Ethics Committee of the Sports Science Research Institute (IR.SSRC.REC.1400.011) and was conducted in accordance with the Helsinki Declaration.

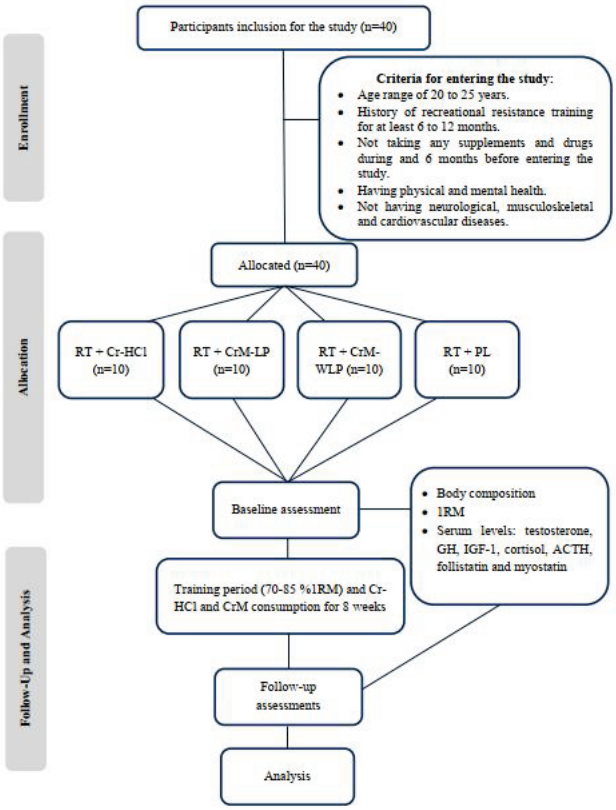

Fig. 1. Flow chart of the study. Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL), One repetition maximum (1RM), Insulin-like growth factor-1 (IGF-1), Growth hormone (GH), Adrenocorticotropic (ACTH).

Research design

In the first session, after explaining the researchers' purpose, steps and expectations, the subjects were asked to fill out a questionnaire related to personal information, physical activity and medical health. Additionally, in this stage, the participants were familiar with the tests and steps of the training and measured stature, body mass, estimated body fat percentage and body circumference. In the second session, 1RM (one maximum repetition) was conducted in the bench press, cable pull down, machine leg extension, and calf raise; during the third session (the interval between sessions was 24 h), 1RM was conducted in leg press, incline bench press, cable row, cable curl; the fourth session measured 1RM for barbell shoulder press, machine lying leg curl, butterfly press, cable push-down; the fifth session, 1RM was measured for dumbbell shoulder press, T-bar row, dumbbell curl, and barbell skull crush. Subjects performed their first RT session four days after the fifth session. Blood samples were taken 48 h before and after the training period; the other tests were repeated. In each session, the subjects performed RT with an intensity of 70-85 % 1RM [21,22] at certain hours of the day (10:00-13:00 in the morning) in a constant environment (same temperature and humidity) for eight weeks. Subjects were asked to refrain from other sports activities during the study.

Table 1. Descriptive characteristics of participants.

	RT + Cr-HCl	RT + CrM-LP	RT + CrM-WLP	RT + PL
Age (years)	22.42 ± 2.84	24.13 ± 2.30	23.54 ± 2.64	21.95 ± 1.82
Stature (cm)	179.10 ± 3.41	177.90 ± 5.34	179.90 ± 4.06	178.00 ± 5.16
Body mass (kg)	69.50 ± 2.99	72.10 ± 3.21	71.95 ± 3.13	73.01 ± 3.51

Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL).

Sample size

The number of subjects was selected using G*Power version 3.1 software with an α value of 0.05, β value of 0.20 (80 % power) and reflected related research [23-25].

Training protocol

The training program in each session included 10 min of warm-up, 70-80 min of RT, and 10 min of cool down. The training program consisted of 8 weeks of training and three sessions per week. The training protocol consisted of 2 RT programs repeated alternately. The plan of the first session consisted of performing nine exercises: leg press, machine lying leg curl, machine leg extension, calf raise, cable pull down, cable row, T-bar row, back extension and crunches. The plan for the second session consisted of nine

moves: bench press, incline bench press, chest fly machine, barbell shoulder press, dumbbell shoulder press, cable curl, dumbbell curl, cable push-down and barbell skull crush. The movements were performed in three sets with an intensity of 70-85 % 1RM with 6-12 repetitions (the rest interval between sets was 60-90 s and between movements, 2-3 min) [21,22]. Training intensity increased by five percent every two weeks (Fig. 2). The researcher monitored subjects' performances in training sessions and all training variables such as training intensity, rest and tempo were carefully controlled (tempo was controlled by a metronome (soundbrenner app)).

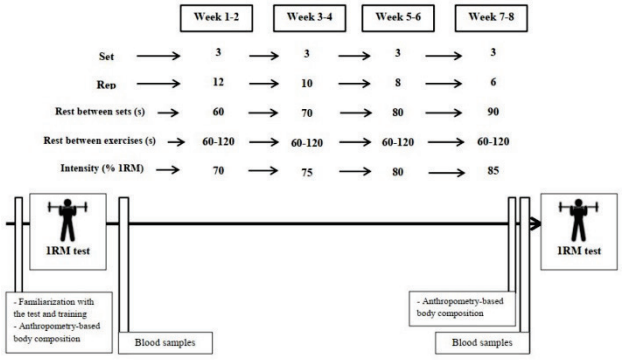

Fig. 2. Training program. One repetition maximum (1RM), repetitions (Rep).

Body composition

A Seca stadiometer (Seca, Hamburg, Germany) was used to measure stature, and a Seca digital scale was used to measure body mass (Seca, Hamburg, Germany). In addition, body composition (estimated percent body fat (PBF) and skeletal muscle mass (SMM)) was calculated using a multi-frequency impedance body composition analyzer (Mediana, Seoul, South Korea). The arm and thigh circumference were measured by a tape and skinfold on the triceps and anterior midline of the thigh by a caliper (Lafayette Instruments, Lafayette, IN, USA). A caliper (Mitutoyo China Corporation, model 533-404, Shanghai, China) was used to measure the distance between the medial and lateral epicondyles of the femur. The test-retest of this assessment at the intraclass correlation coefficient (ICC) of these tests was above 0.88, and the coefficient of variation (CV) was less than 4.1 %.

Assessment of muscular cross-sectional area (MCSA)

The extent of hypertrophic changes in participants was measured using the model proposed by Heymsfield *et al.* and Knapik *et al.* who proposed two formulas for measuring the amounts of hypertrophic changes in the arm (upper body) and thigh (lower body) [26,27]. In this method, the following formula was used for the MCSA of the arm [26]:

$$\text{Arm MCSA } (cm^2) = \pi ((CA/2\,\pi) - (TA/2))^2 - 5.5$$

CA: circumference arm (cm), π: 3.14, TA: triceps skinfold arm (cm), r=0.97 (strongly correlated with measures derived by computed tomography scans) [26].

The following formula was used for the MCSA of the thigh [27]:

$$\text{Thigh MCSA } (cm^2) = 0.649 \times ((CT/\pi - SQ)^2 - (0.3 \times dE)^2)$$

CT: circumference thigh (cm), π: 3.14, SQ: skinfold quadriceps (cm), dE: distance epicondyles (cm), r=0.96, SEE=10.1 cm^2 (correlated highly with cross-sectional area (CSA) determined by magnetic resonance imaging) [27].

Muscle strength (1RM)

The methods of Tibana *et al.* and Brown and Weir were used to evaluate the 1RM [28,29]. In this method, the subjects performed 5-minute low-intensity walks and then had eight repetitions with an estimated intensity of 50 % 1RM. After one minute of rest, they performed three repetitions with an estimated intensity of 70 % 1RM. Subjects rested for three minutes, and 5 % was gradually added to each load until they could perform a complete repetition of three to five attempts for 1RM and 3 to 5-minute rest between attempts [28,29]. The ICC and CV of the leg press were 0.93 and 3.2 %, respectively, and those of the bench press were 0.91 and 3.5 %.

Blood sample analysis

Sampling was performed twice, 48 h before the start of the training period and 48 h after the last training session (12 h of fasting and 8 h of sleep) between 8:00 and 9:00 AM. To control the circadian rhythm of the hormonal range, blood sampling time was considered to be 48 h before and after the training period [30]. Sampling from the brachial vein was 10 ml. After placing the samples at room temperature (28 °C) for 30 min, it was centrifuged at 1500 rpm for 10 min. Then, the serum was isolated and frozen at -80 °C to study the desired variables. ELISA kits were used to assess serum levels of testosterone, GH and IGF-1 (testosterone: Abcam, ab108666, Cambridge, MA, USA, sensitivity: 0.07 ng/ml; GH: Abcam, ab190811, Cambridge, MA, USA, sensitivity: 1.6 pg/ml; IGF-1: Abcam, ab100545, Cambridge, MA, USA; sensitivity: <0.2 ng/ml) (intra and inter-assay coefficient of variation (CV) for testosterone was 5.8 and 10.5 %, GH was 3.6 % for both, IGF-1 was

<10 and <12 %). In addition, serum levels of cortisol and adrenocorticotropic hormone (ACTH) were measured using commercially available kits (cortisol: Monobind, 3625-300A, CA, USA, sensitivity: 0.25 µg/dl; ACTH: Monobind, 10625-300, CA, USA, sensitivity: 2.81-3.13 pg/ml) with a CV of less than 6 %. To measure serum levels of myostatin and follistatin (myostatin: CUSABIO, E11300h, Houston, TX, USA; follistatin: CUSABIO, E08506h, Houston, TX, USA), ELISA kits (sensitivity of 0.312 ng/ml for myostatin and 0.250 ng/ml for follistatin, CV<15 %) were used.

Supplementation

In this study, a Cr-HCl supplement (ProMera Sports CON-CRET, USA) was used for the RT+Cr-HCl group. The dose was 0.03 $g.kg^{-1}$ of body mass 30 min before training on the training day and at the same time of training on non-training days. For the CrM-LP and CrM-WLP groups, the Olimp supplement (Olimp, Poland) was used. The dose of the CrM-LP group was 0.3 $g.kg^{-1}$ of body mass for five days (loading), which was consumed in 4 servings during the day; it was followed by 0.03 $g.kg^{-1}$ of body mass for 51 days, which was consumed 30 min before training on a training day or during training hours on non-training days. In the CrM-WLP group, 0.03 $g.kg^{-1}$ of body mass was consumed during the training period, 30 min before training (on training day), or during training hours (non-training days) [3,31,32]. In addition, the PL group was given maltodextrin under the same conditions as the other groups. The supplement package was given to each subject every week, and each subject brought the relevant package to the training site in each training session to ensure that the dose was observed. Also, during eight weeks of supplementation, the researcher reminded and checked the supplement by phone.

Dietary assessment

A three-day food recall questionnaire (two normal days and one day off) was used to assess calorie intake. Subjects recorded their diet, three days before the start of the training program and three days in the last week of the training program. Calorie intake, carbohydrate, fat, and protein intake were calculated. 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) [33]. In addition, the subjects were instructed to maintain their diet during the training period.

Table 2. Dietary daily intake (mean (SD)).

Variable	Group	Pre-training	Post-training	P
Calories (kcal)	**RT + Cr-HCl**	2607.27 (135.91)	2577.08 (114.98)	0.13
	RT + CrM-LP	2744.58 (105.74)	2669.94 (119.67)	0.25
	RT + CrM-WLP	2713.93 (96.72)	2699.25 (101.58)	0.13
	RT + PL	2773.98 (92.44)	2740.85 (75.62)	0.09
CHO (gr)	**RT + Cr-HCl**	298.55 (15.18)	293.90 (16.56)	0.23
	RT + CrM-LP	291.92 (23.41)	298.49 (15.63)	0.55
	RT + CrM-WLP	305.70 (19.04)	302.91 (14.72)	0.40
	RT + PL	311.48 (26.74)	306.32 (14.85)	0.41
PRO (gr)	**RT + Cr-HCl**	94.55 (8.10)	95.72 (8.78)	0.28
	RT + CrM-LP	104.45 (11.02)	106.50 (8.07)	0.50
	RT + CrM-WLP	103.29 (11.99)	101.25 (11.17)	0.40
	RT + PL	107.59 (13.27)	108.62 (10.99)	0.63
FAT (gr)	**RT + Cr-HCl**	104.74 (12.61)	101.62 (13.30)	0.09
	RT + CrM-LP	120.12 (6.56)	113.44 (8.33)	0.07
	RT + CrM-WLP	112.58 (6.39)	110.98 (5.12)	0.22
	RT + PL	115.23 (9.23)	111.77 (3.50)	0.26

Carbohydrate (CHO), Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL).

The amount of calories consumed and the amount of energy obtained from proteins, fats, and carbohydrates were calculated using the diet analysis program (Master Diet Pro, IRAN) and analysed using paired samples *t*-tests. Based on the results of the *t*-tests, there was no significant difference in the subjects' nutrition during the training period (P>0.05) (Table 2).

Statistical analysis

Descriptive statistics (mean (standard deviation [SD])) for each of the assessed variables were determined (Table 1). The distribution of each variable was examined using the Shapiro-Wilk test to ensure the normality of distribution. After confirming data normality, a one-way ANOVA test was used to detect possible differences in the pretest variables. Student's *t*-tests were used to compare the pre-and post-tests in all groups. Analysis of covariance (ANCOVA) was used to analyze the data (baseline values were used as a covariate to identify potential time group interactions and to eliminate the possible influence of baseline scores). When necessary, multiple comparisons using the Bonferroni procedure were used for *post hoc* analysis. All between-group differences were further analyzed using Hedge's g effect sizes (ES). Interpretations of effect sizes were evaluated at the following levels: trivial <0.20; small 0.20-0.50; moderate 0.50-0.80; large 0.80-1.30; and very large >1.30 [34]. For each variable, a percent change score was calculated ((post - pre)/pre) × 100. Statistical significance was set at P≤0.05 for these analyses (with 95 % confidence intervals (CI)). All analyses were conducted using SPSS version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

Results

Strength and body composition

The results of Table 3 show that in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups, bench press (P<0.001, P<0.001, P<0.001; respectively), leg press (P<0.001, P<0.001, P<0.001; respectively), arm MCSA (P<0.001, P=0.001, P<0.001; respectively), thigh MCSA (P<0.001, P<0.001, P<0.001; respectively), and SMM (P<0.001, P<0.001, P<0.001; respectively) increased significantly and PBF (P=0.001, P=0.002, P=0.001; respectively) decreased significantly. In addition, BMI did not show significant differences in all groups (P=0.12, P=0.11, P=0.11; respectively). Changes in the RT+PL group showed that bench press, leg press, arm MCSA, thigh MCSA and SMM increased and PBF and BMI decreased (Fig. 3B), but only changes in bench press, leg press and PBF were significant (P<0.001; P<0.001; P=0.01; respectively). The RT+Cr-HCl and RT+CrM-WLP groups showed very large (ES: >1.30) supplementation effects in the 1RM leg press, bench press and SMM. The RT+CrM-LP groups also showed very large effects (ES: >1.30) in the 1RM leg press. PBF in the supplement group showed large effects (ES: 0.80-1.30) and arm MCSA showed large supplementation effects (ES: 0.80-1.30) in the RT+Cr-HCl and RT+CrM-WLP groups following eight weeks of training and supplementation (Table 3).

The results indicated a significant difference between groups in strength, MCSA, SMM and PBF (bench press: F=5.53, P=0.003; leg press: F=10.08, P<0.001; arm MCSA: F=6.82, P=0.001; thigh MCSA: F=5.04, P=0.005; SMM: F=5.33, P=0.004; PBF: F=6.07, P=0.002). The bench press, leg press, arm and thigh MCSA, SMM, BMI and PBF in the RT+Cr-HCl group were not significantly different from those in the RT + CrM-LP and RT+CrM-WLP groups (P>0.05) (Table 3). However, changes in bench press, leg press, arm and thigh MCSA, SMM and PBF in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups were significant compared to the RT+PL group (bench press: P=0.04, P=0.003, P=0.04; leg press: P<0.001, P<0.001, P=0.01; arm MCSA: P=0.03, P=0.007, P=0.001; thigh MCSA: P=0.01, P=0.03, P=0.01; SMM: P=0.004, P=0.04, P=0.03; PBF: P=0.006, P=0.007, P=0.01; respectively), but BMI did not change significantly (F=1.14, P=0.34). In addition, no significant changes were observed between the RT+CrM-LP and RT+CrM-WLP groups (P>0.05) (Table 3).

Hormonal changes

The results of hormonal changes are shown in Figure 4. According to the results, The increase in GH, IGF-1 levels, ratio of follistatin/myostatin and testosterone/cortisol and the decrease in cortisol and ACTH levels in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups were significant compared to the pretest levels (GH: P<0.001, P<0.001, P<0.001; IGF-1: P<0.001, P<0.001, P=0.001; cortisol: P=0.003, P=0.001, P=0.026; ACTH: P<0.001, P<0.001, P<0.001; respectively); however, the increase in testosterone (P=0.63, P=0.85, P=0.64; respectively) and follistatin (P=0.07, P=0.07, P=0.09; respectively) and decrease in myostatin (P=0.06, P=0.08, P=0.08; respectively) levels were not significant (Fig. 3 and Fig. 4). In

Table 3. Body composition, muscle strength and hypertrophy to 8-week intervention (mean (SD)).

		Leg press (kg)	Bench press (kg)	SMM (kg)	BF (%)	BMI (kg/m^2)	Thigh MCSA (cm^2)	Arm MCSA (cm^2)
RT + Cr-HCl	**Pre**	114 (13.68)	57.20 (5.49)	34.97 (2.74)	21.40 (2.63)	21.67 (1.06)	119.22 (18.74)	39.78 (6.65)
	Post	133.60 (12.77)*,#	66.20 (4.10)*,#	40.55 (3.91)*,#	18.60 (1.42)*,#	22.02 (0.84)	137.40 (24.43)*,#	49.03 (8.43)*,#
	ES (CI 95 %)	-1.42 (-2.40 to -0.44)	-1.78 (-2.81 to -0.74)	-1.58 (-2.58 to -0.58)	1.27 (0.31 to 2.23)	-0.35 (-1.23 to 0.53)	-0.80 (-1.71 to 0.11)	-1.17 (-2.11 to -0.22)
RT + CrM-LP	**Pre**	119.70 (12.86)	62.60 (8.04)	33.42 (3.93)	20.80 (3.32)	22.81 (1.40)	135.88 (19.79)	47.44 (10.84)
	Post	138.10 (11.81)*,#	71.30 (6.49)*,#	38.70 (3.08)*,#	18.30 (1.76)*,#	23.11 (1.56)	152.85 (21.95)*,#	56.06 (10.19)*,#
	ES (CI 95 %)	-1.43 (-2.41 to -0.45)	-1.14 (-2.09 to -0.20)	-1.16 (-2.11 to -0.21)	0.90 (-0.02 to 1.82)	-0.19 (-1.07 to 0.68)	-0.78 (-1.69 to 0.13)	-0.78 (-1.69 to 0.12)
RT + CrM-WLP	**Pre**	113.20 (10.14)	58.20 (6.44)	32.25 (3.52)	22.20 (3.08)	22.26 (1.51)	134.50 (24.06)	49.58 (10.02)
	Post	130.20 (9.85)*,#	66.90 (6.22)*,#	38.12 (3.03)*,#	19.20 (2.04)*,#	22.71 (1.19)	152.31 (24.52)*,#	58.69 (11.29)*,#
	ES (CI 95 %)	-1.63 (-2.64 to -0.62)	-1.32 (-2.28 to -0.35)	-1.71 (-2.74 to -0.69)	1.10 (0.16 to 2.04)	-0.32 (-1.20 to 0.56)	-0.70 (-1.61 to 0.20)	-0.82 (-1.73 to 0.10)
RT + PL	**Pre**	119.60 (10.58)	60.70 (8.92)	35.58 (4.51)	22.30 (3.23)	22.78 (1.28)	128.05 (26.97)	45.12 (13.63)
	Post	129.70 (9.42)*	65.30 (7.10)*	36.89 (3.39)	20.90 (2.51)*	22.67 (0.92)	134.54 (22.63)	46.37 (8.45)
	ES (CI 95 %)	-0.97 (-1.89 to -0.04)	-0.55 (-1.44 to 0.35)	-0.31 (-1.20 to 0.57)	0.46 (-0.42 to 1.35)	0.09 (-0.78 to 0.97)	-0.25 (-1.13 to 0.63)	-0.11 (-0.98 to 0.77)

Skeletal muscle mass (SMM), Percent body fat (PBF), Body mass index (BMI), Muscular cross-sectional area (MCSA), Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL), Effect size (ES), Confidence intervals (CI). * denotes significant differences from baseline (P≤0.05). # denotes significant differences from PL groups (P≤0.05). Data represent mean (standard error of mean).

addition, changes in the ratios of follistatin/myostatin and testosterone/cortisol in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups were significant (follistatin/myostatin: P=0.02, P=0.01, P=0.01; testosterone/cortisol: P=0.002, P=0.007, P=0.01; respectively). In the RT+PL groups, GH and IGF-1 levels increased significantly compared to the pretest levels (P=0.04, P=0.006; respectively). ACTH levels in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups showed moderate supplementation effects (ES: 0.50-0.80), while the RT+PL groups showed trivial effects (ES: <0.20). In other variables supplementation effects were trivial (ES: <0.20) and small (ES: 0.20-0.50) in all groups (Fig. 4).

The results of the ANCOVA test indicated a significant between-group interaction in GH, IGF-1, testosterone/cortisol, cortisol and ACTH levels (GH: F=7.69, P<0.001; IGF-1: F=8.93, P<0.001; testosterone/cortisol: F=5.93, P=0.002; cortisol: F=7.51, P=0.001; ACTH: F=7.32, P=0.001). Nonetheless in contrast, the testosterone, follistatin and myostatin levels and the ratio of follistatin/myostatin did not significantly change (testosterone: F=1.38, P=0.26; follistatin: F=0.54, P=0.65; myostatin: F=0.16, P=0.91; follistatin/myostatin: F=0.46, P=0.71). The observed changes in the GH, IGF-1, testosterone/cortisol, cortisol and ACTH levels in the RT+Cr-HCl group were not significant compared to

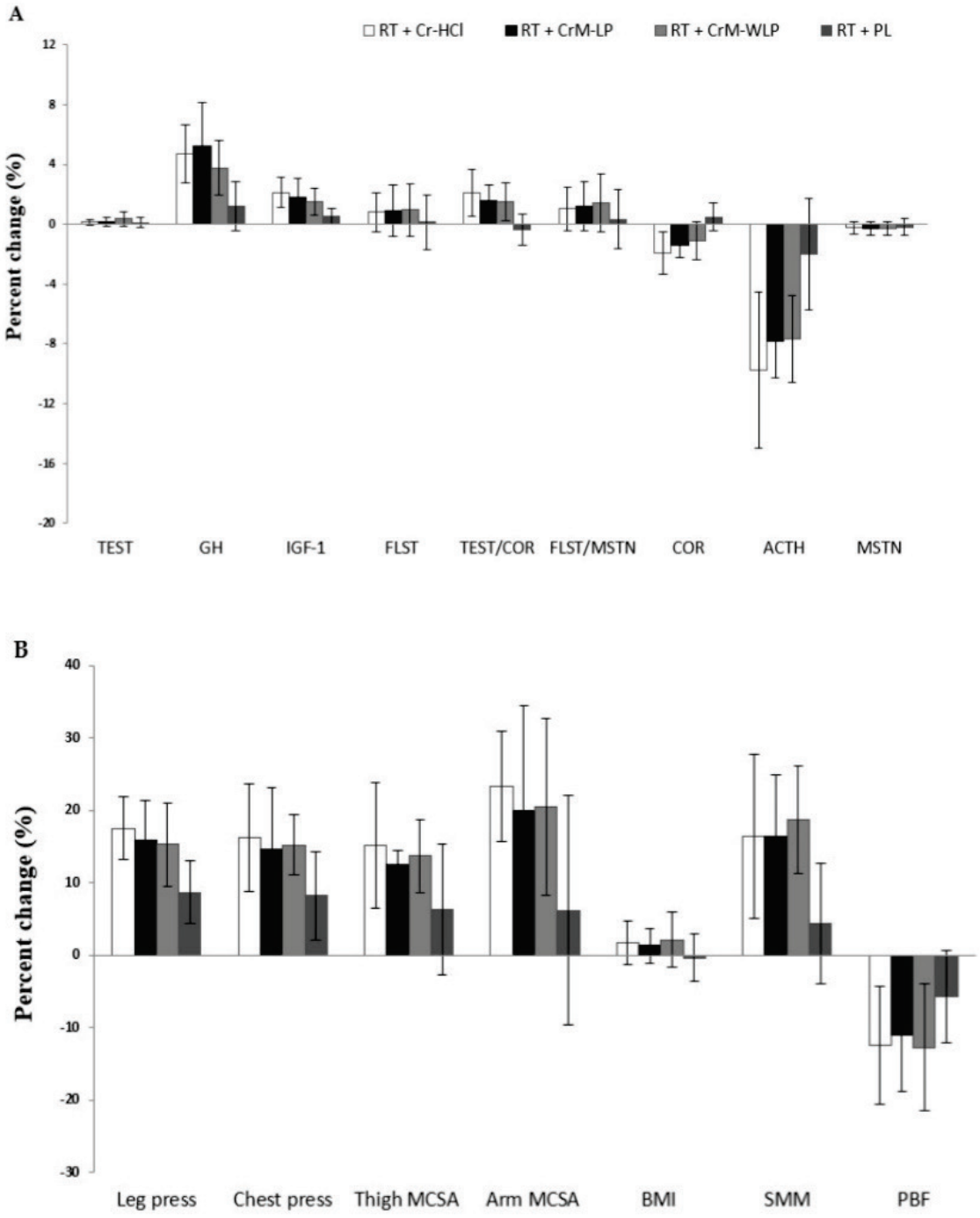

Fig. 3. Percentage changes in the study groups. Hormonal change (**A**); Body composition, muscle strength and hypertrophy change (**B**). Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL), Testosterone (TEST), Insulin-like growth factor-1 (IGF-1), Growth hormone (GH), Cortisol (COR), Adrenocorticotropic (ACTH), Follistatin (FLST), Myostatin (MSTN), Skeletal muscle mass (SMM), Percent body fat (PBF), Body mass index (BMI), Muscular cross-sectional area (MCSA).

those in the RT+CrM-LP and RT+CrM-WLP groups (P>0.05) (Fig. 4). In addition, no significant changes were observed between the RT+CrM-LP and RT+CrM-WLP groups (P>0.05). Changes in the GH, IGF-1, testosterone/cortisol, cortisol and ACTH levels in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups were significant compared to the RT+PL group (GH: P=0.002, P=0.001, P=0.03; IGF-1: P<0.001, P=0.002, P=0.01; testosterone/cortisol: P=0.002, P=0.03, P=0.01; cortisol: P<0.001, P=0.008, P=0.02; ACTH: P=0.001, P=0.01, P=0.01; respectively) (Fig. 4).

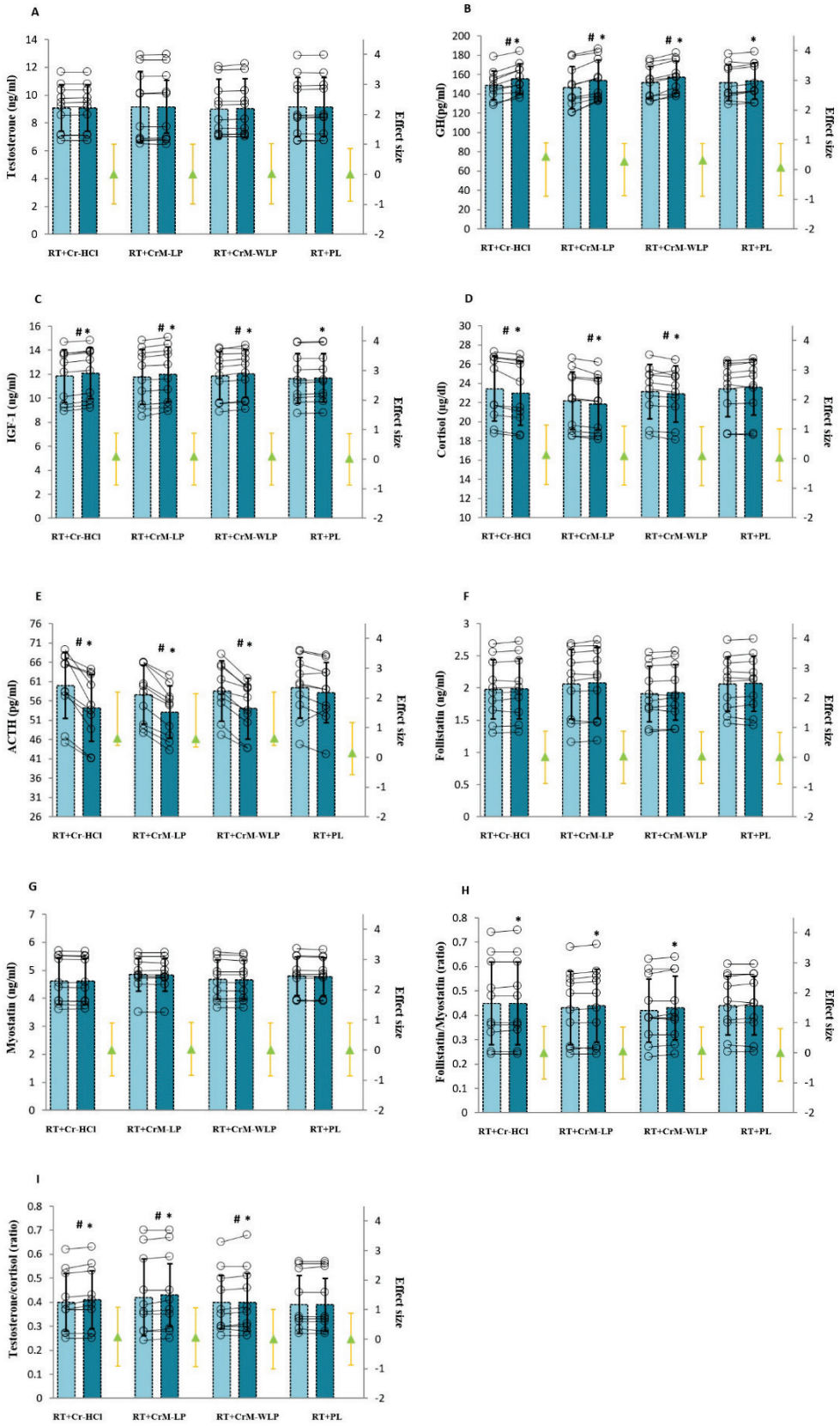

Fig. 4. Serum concentrations of anabolic and catabolic hormonal markers from pre- to post-intervention. (Testosterone (**A**), GH (**B**), IGF-1 (**C**), Cortisol (**D**), ACTH (**E**), Follistatin (**F**), Myostatin (**G**), Follistatin/Myostatin (**H**), Testosterone/Cortisol (**I**)). Resistance training (RT), Creatine hydrochloride (Cr-HCl), Creatine monohydrate – loading phase (CrM-LP), CrM – without loading phase (CrM-WLP), Placebo (PL), Insulin-like growth factor-1 (IGF-1), Growth hormone (GH), Adrenocorticotropic (ACTH). The effect size is shown with confidence intervals of 95 %. * denotes significant differences between baseline and post-testing values (P≤0.05), # denotes significant differences from PL group (P≤0.05). Pre-test ●, Post-test ●, Effect size ▲

Discussion

This study attempted to determine the effects of RT alongside Cr-HCl or CrM supplementation on hormonal compatibility, muscle strength, muscle hypertrophy, and body composition and compare these two types of supplementation. Our findings showed that Cr-HCl did not provide any advantages over CrM.

The results showed that RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP increased GH, IGF-1 levels and the follistatin/myostatin, testosterone/cortisol ratio and decreased cortisol and ACTH levels. Hormonal changes in the RT+Cr-HCl group were insignificant compared to the RT+CrM-LP and RT+CrM-WLP groups. Changes in the GH, IGF-1, testosterone/cortisol, cortisol and ACTH levels in the RT+Cr-HCl, RT+CrM-LP and RT+CrM-WLP groups were significant compared to the RT+PL group, but changes in the testosterone, follistatin, myostatin levels and the ratio of follistatin/myostatin were not significant. It seems that testosterone requires long-term intervention for meaningful changes [35]. More research needs to be done on the hormonal compatibility of Cr-HCl supplementation. Only a few studies [18,36] have examined the effects of this type of creatine supplementation on testosterone and cortisol levels. In this regard, Tayebi and Arazi showed that multiday supplementation (7 days) of Cr-HCl (3 gr) compared with CrM (3 gr) did not have a significant effect on the hormone levels of cortisol and testosterone [18]. Moreover, Tayebi *et al.* stated that Cr-HCl supplementation (3 gr) for two weeks caused significant changes in soldiers' testosterone and cortisol levels [36]. Regarding the effects of CrM supplementation, the results of Arazi *et al.* showed that CrM supplementation (4×5 gr.d^{-1}) for more than five days along with RT (3×10 rep of 9 exercises, 75-85 % 1RM) was sufficient to increase testosterone concentration and decrease cortisol concentration [20]. Burke *et al.* [8] showed a significant increase in intramuscular IGF-1 concentration in creatine-receiving athletes (0.25 g.kg dry mass in 7 days and 0.06 g.kg dry mass in 49 days) after an 8-week RT program. The researchers suggested that creatine-induced cellular swelling may be the starting point for anabolic signals [37]. In contrast to the results of the present study regarding myostatin and follistatin, Saremi *et al.* concluded that creatine supplementation combined with RT (8 weeks) resulted in a further reduction in serum myostatin. They suggested that the effects of RT on serum myostatin levels may explain the increased muscle mass induced by creatine supplementation [24]. Mobley *et al.* stated that creatine prevents or reverses myostatin-induced muscle fiber atrophy [38]. They suggested that creatine may directly affect myostatin levels by increasing the expression of myostatin-related genes such as Akirin-1/Mighty, which negatively regulates myostatin [38]. Anabolic and catabolic hormones affect muscle growth and strength. Two major signaling pathways control protein synthesis. One of pathways is the IGF1-Akt-mTOR, which acts as a positive regulator. The other major signaling pathway that controls skeletal muscle growth involves myostatin. Myostatin is produced by muscles and acts as a negative regulator of muscle growth [39]. Muscle hypertrophy may also be induced by extracellular binding inhibitory proteins, such as follistatin, which have even greater effects than reducing myostatin [39]. Androgens (steroid hormones) strongly stimulate muscle growth. The testosterone/cortisol ratio indicates an anabolic/catabolic environment due to their role in protein synthesis and protein degradation. Testosterone increases androgenic receptors in muscle cells and related myonucleoli and satellite cells. Testosterone also has an increasing effect on IGF-1 and GH and causes the promotion of anabolism by increasing protein synthesis and inhibiting protein degradation [40]. It seems that the reason for the difference between the results is related to the dosage, the type of exercises, the intensity of the exercises, and the training level of the subjects.

The results of the present study showed no difference between the hormonal adaptations caused by supplementation with Cr-HCl and CrM. Cr-HCl has been marketed as a more bioavailable source of creatine than CrM. According to the research, if a bioavailable source of creatine is consumed in physiologically effective doses, it is not broken down during digestion. The creatine content of blood and tissue can be increased to physiologically significant amounts (20 to 40 %). It does not matter which form of creatine has better mixing properties and/or is more soluble [12,41,42]. In addition, Tuckfield suggested that creatine salts with improved aqueous solubility and oral absorption characteristics can provide improvements over CM in therapeutic applications requiring high doses of creatine [43]. It seems that contrary to the claims made, Cr-HCl does not have a greater effect than CrM, and it seems that it only has better solubility. Creatine's solubility depends on

temperature and pH (the lower the pH, the more excellent the solubility). Hence, the common recommendation to dissolve creatine in acidic juice, such as orange juice, was made [6]. Based on the Fazio *et al.* study, market research showed that CrM is the cheapest form of creatine [11]. Therefore, according to the current research results and the effects of Cr-HCl compared to CrM, it is not economical to use and has no more effects.

The results showed that HCl and CrM supplementation along with RT increased muscle strength (bench press, leg press), arm and thigh MCSA, SMM and reduced PBF, but the changes observed between Cr-HCl, RT+CrM-LP and RT + CrM-WLP were not significant. Changes in muscle strength, arm and thigh MCSA, SMM and PBF were meaningful in the supplementary groups compared to the control group. Regarding the effects of Cr-HCl on performance, we can refer to studies by Tayebi and Arazi [18], de Franca *et al.* [19], and Tayebi *et al.* [36]. de França *et al.* stated that Cr-HCl (5 $gr.day^{-1}$ and 1.5 $gr.day^{-1}$) and CrM (5 $gr.day^{-1}$) improved upper and lower body muscle strength after four weeks of RT (4 sets, 10-12 rep, 80-90 % 1RM), but only Cr-HCl changed body composition (fat mass and lean mass) in recreational weightlifters. There was no statistically significant difference between groups [19]. Tayebi *et al.* stated that Cr-HCl supplementation for two weeks improved the performance of soldiers (winging test, vertical jump, and squat) [36]. In contrast, the results of Tayebi and Arazi showed that seven days of Cr-HCl supplementation did not significantly affect performance compared to CrM in sedentary individuals [18]. It seems that the dosage of creatine, supplementation period, training period and training intensity were the reasons for the different results in these studies. Regarding the effects of CrM, Lanhers *et al.* [44,45] in their systematic review stated that creatine supplementation was effective on upper and lower body strength performance for exercises lasting less than 3 min, regardless of population characteristics, training protocols and dose and duration of use. Moreover, Wu *et al.*, in their review article, stated that creatine is an effective supplement to increase muscle strength and increase athletes' performance [46]. The results of Mills *et al.* showed that creatine supplementation (0.1 $gr.kg^{-1}$) during six weeks of RT was a safe and effective strategy for increasing muscle strength in young active adults [47].

Glycogen decreases during RT and creatine can affect muscle glycogen stores (glycogen increases ATP resynthesis during RT sessions) [48]. Creatine also increases intramuscular PCr levels, which may accelerate ATP resynthesis and/or PCr recovery after each set; over time, these factors may help increase strength. In addition, creatine supplementation increases calcium reabsorption into the sarcoplasmic reticulum, leading to faster actin-myosin cross-bridge cycling during repetitive muscle contractions [49] and ultimately improving muscle strength. This supplement can increase glucose disposal and decrease glycogen stores during exercise sessions by increasing mobility and glucose transporter type 4 (GLUT-4) content in people who do RT [47]. Additionally, creatine supplementation can stimulate signaling pathways including IGF-1 and predominantly phosphoinositide 3-kinase (PI3K)/Akt-PKB/mechanistic target of rapamycin complex (mTOR), which plays an important role in the regulation of muscle hypertrophy [50]. In addition, the increase in lean mass following creatine supplementation has been attributed, at least in part, to water retention in muscle tissue [51]. In general, studies on creatine supplementation have reported an increase in intracellular volume without changes in extracellular volume [20,37], possibly due to the high osmotic load associated with increased creatine and Na^+ in the cytosol. Indeed, cell swelling has been characterized as an anabolic signal [6], which can trigger the activation of osmotic molecules as G protein-coupled receptors in the mitogen-activated protein kinase (MAPK) and sphingosine kinase 1 (SPHK1) pathways stimulate and create positive feedback [37]. Regarding the effects of creatine on fat tissue, there is direct evidence that creatine affects certain aspects of fat and adipose tissue metabolism and triglyceride synthesis in cell types. Creatine stimulates mitochondrial ATP turnover in adipose tissue, increasing the metabolic rate of subcutaneous and brown adipose tissues [52]. Lee *et al.* also reported that creatine inhibited cytoplasmic triglyceride formation in adipogenic cell culture models in a dose-dependent manner [53]. Additionally, creatine metabolism is important in fat bioenergetics, and creatine supplementation positively affects energy expenditure [52]. Also, increased lean tissue mass due to creatine supplementation, potentially increasing resting metabolic rate and total daily energy expenditure (through increased participation in physical activity) [54,55]; it can explain the decrease in fat mass and body fat percentage.

Considering the effects of supplementation on hormonal compatibility, strength, hypertrophy and body composition as mentioned above and the results of this study, Cr-HCl, like CrM, has an effect on hormonal

compatibility, strength, hypertrophy and body composition and does not seem to have higher effects than CrM. CrM does not have a high solubility, but Cr-HCl has a high solubility; this is the only difference between the two supplements. According to the available information, solubility does not affect creatine's bioavailability; creatine monohydrate is 100 % bioavailable [12,41,42]. As Kreider *et al.* stated in a systematic review, the claims that Cr-HCl is more bioavailable and more effective than CrM are not supported [10], which is consistent with the results of the present study.

There were limitations in this study that may affect our findings. The first limitation of the present study was the lack of measurement of muscle creatine content. The second limitation was the study of subjects' nutrition during the research period using a food recall questionnaire. In this study, with the guidance and recommendations made at the beginning of the study, the subjects tried to follow a similar dietary pattern to reduce the effects of nutrition on the desired variables. In addition, estimated body fat percentage and muscle mass were estimated using a multifrequency impedance body composition analyzer (Mediana, Seoul, South Korea) and the MSCA in this study was calculated based on formulas proposed by Heymsfield *et al.* and Knapik *et al.* for measuring the amount of hypertrophic changes in the arm (upper body) and thigh (lower body) [26,27].

Conclusions

In general, the results showed that supplementation with Cr-HCl and CrM increases GH, IGF-1 levels, the ratio of follistatin/myostatin, testosterone/cortisol, muscle strength (bench press, leg press), arm and thigh MCSA, SMM and significantly decreases cortisol, ACTH levels and PBF. Hormonal adaptations, strength and body composition in the RT+Cr-HCl group were not significant compared to the RT+CrM-LP and RT+CrM-WLP groups and only changes in the supplementary groups were significant compared to the control group. The results showed the effects of Cr-HCl and CrM on hormonal compatibility, strength and hypertrophy, but Cr-HCl does not seem to have more effects than CrM. 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 affects performance and hormonal indicators, due to its very 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.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors would like to thank the athletes who participated in this study. This study was supported by Scientific Research (A) (20H00574) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Braissant O, Henry H, Beard E, Uldry J. Creatine deficiency syndromes and the importance of creatine synthesis in the brain. Amino Acids 2011;40:1315-1324. https://doi.org/10.1007/s00726-011-0852-z
2. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev 2000;80:1107-1213. https://doi.org/10.1152/physrev.2000.80.3.1107
3. Bagchi D, Nair S, Sen CD. *Nutrition and Enhanced Sports Performance (Second Edition)*. Elsevier, 2019, 818 p.
4. Bassit RA, Curi R, Rosa LC. Creatine supplementation reduces plasma levels of pro-inflammatory cytokines and PGE 2 after a half-ironman competition. Amino Acids 2008;35:425-431. https://doi.org/10.1007/s00726-007-0582-4
5. Guimaraes-Ferreira L, Pinheiro CHJ, Gerlinger-Romero F, Vitzel KF, Nachbar RT, Curi R, Nunes MT. Short-term creatine supplementation decreases reactive oxygen species content with no changes in expression and activity of antioxidant enzymes in skeletal muscle. Eur J Appl Physiol 2012;112:3905-3911. https://doi.org/10.1007/s00421-012-2378-9
6. Kreider RB, Kalman DS, Antonio J, Ziegenfuss TN, Wildman R, Collins R, Candow DG, ET AL. International Society of Sports Nutrition position stand: Safety and efficacy of creatine supplementation in exercise, sport, and medicine. J Int Soc Sports Nutr 2017;14:18. https://doi.org/10.1186/s12970-017-0173-z

7. Becque MD, Lochmann JD, Melrose DR. Effects of oral creatine supplementation on muscular strength and body composition. Med Sci Sports Exerc 2000;32:654-658. https://doi.org/10.1097/00005768-200003000-00016
8. Burke DG, Candow DG, Chilibeck PD, MacNeil LG, Roy BD, Tarnopolsky MA, Ziegenfuss T. Effect of creatine supplementation and resistance-exercise training on muscle insulin-like growth factor in young adults. Int J Sport Nutr 2008;18:389. https://doi.org/10.1123/ijsnem.18.4.389
9. Willoughby DS, Rosene JM. Effects of oral creatine and resistance training on myogenic regulatory factor expression. Med Sci Sports Exerc 2003;35:923-929. https://doi.org/10.1249/01.MSS.0000069746.05241.F0
10. Kreider RB, Jager R, Purpura M. Bioavailability, Efficacy, Safety, and Regulatory Status of Creatine and Related Compounds: A Critical Review. Nutrients 2022;14:1035. https://doi.org/10.3390/nu14051035
11. Fazio, C, Elder CL, Harris MM. Efficacy of alternative forms of creatine supplementation on improving performance and body composition in healthy subjects: A systematic review. J Strength Cond Res 2022;36:2663-2670. https://doi.org/10.1519/JSC.0000000000003873
12. Jager R, Purpura M, Shao A, Inoue T, Kreider RB. Analysis of the efficacy, safety, and regulatory status of novel forms of creatine. Amino Acids 2011;40:1369-1383. https://doi.org/10.1007/s00726-011-0874-6
13. Gufford BT, Sriraghavan K, Miller NJ, Miller DW, Gu XC, Vennerstrom JL, Robinson DH. Physicochemical characterization of creatine N-methylguanidinium salts. J Diet Suppl 2010;7:240-252. https://doi.org/10.3109/19390211.2010.491507
14. Alraddadi EA, Lillico R, Vennerstrom JL, Lakowski TM, Miller DW. Absolute oral bioavailability of creatine monohydrate in rats: Debunking a myth. Pharmaceutics 2018;10:31. https://doi.org/10.3390/pharmaceutics10010031
15. Dash AK, Miller DW, Huai-Yan H, Carnazzo J, Stout JR. Evaluation of creatine transport using Caco-2 monolayers as an in vitro model for intestinal absorption. J Pharm Sci 2001;90:1593-1598. https://doi.org/10.1002/jps.1109
16. Stoppani J. Supplement Breakdown: Creatine HCl; JS Stoppani Blog: Westlake Village, CA, USA, 2021;24.
17. Kreider RB. Species-specific responses to creatine supplementation. Am J Physiol Regul Integr Comp Physiol 2003;285:R725-R726. https://doi.org/10.1152/ajpregu.00375.2003
18. Tayebi M, Arazi H. Is creatine hydrochloride better than creatine monohydrate for the improvement of physical performance and hormonal changes in young trained men? Sci Sports 2020;35:e135-e141. https://doi.org/10.1016/j.scispo.2019.07.013
19. de França E, Avelar B, Yoshioka C, Santana JO, Madureira D, Rocha LY, Zocoler CA, ET AL. Creatine HCl and Creatine Monohydrate Improve Strength but Only Creatine HCl Induced Changes on Body Composition in Recreational Weightlifters. Food Sci Nutr 2015;6:1624. https://doi.org/10.4236/fns.2015.617167
20. Arazi H, Rahmaninia F, Hosseini K, Asadi A. Effects of short term creatine supplementation and resistance exercises on resting hormonal and cardiovascular responses. Sci Sports 2015;30:105-109. https://doi.org/10.1016/j.scispo.2014.03.006
21. Fleck SJ, Kraemer W. *Designing Resistance Training Programs*. Human Kinetics, 2014, 508 p. https://doi.org/10.5040/9781718225107
22. Baechle TR, Earle RW, Wathen D. Resistance training. In: *Essentials of Strength Training and Conditioning, 3rd ed*. Baechle TR, Earle RW (eds). Champaign, IL: Human Kinetics, 2008, pp 401.
23. Negaresh R, Ranjbar R, Habibi A, Mokhtarzade M, Fokin A, Gharibvand M. The effect of resistance training on quadriceps muscle volume and some growth factors in elderly and young men. Adv Gerontol 2017;30:880-887.
24. Saremi A, Gharakhanloo R, Sharghi S, Gharaati MR, Larijani B, Omidfar K. Effects of oral creatine and resistance training on serum myostatin and GASP-1. Mol Cell Endocrinol 2010;317:25-30. https://doi.org/10.1016/j.mce.2009.12.019
25. Enes A, Alves RC, Schoenfeld BJ, Oneda G, Perin SC, Trindade TB, ET AL. Rest-pause and drop-set training elicit similar strength and hypertrophy adaptations compared with traditional sets in resistance-trained males. Appl Physiol Nutr Metab 2021;46:1417-1424. https://doi.org/10.1139/apnm-2021-0278
26. Heymsfield SB, McManus C, Smith J, Stevens V, Nixon DW. Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. Am J Clin Nutr 1982;36:680-690. https://doi.org/10.1093/ajcn/36.4.680

27. Knapik JJ, Staab JS, Harman EA. Validity of an anthropometric estimate of thigh muscle cross-sectional area. Med Sci Sports Exerc 1996;28:1523-1530. https://doi.org/10.1097/00005768-199612000-00013
28. Tibana RA, Prestes J, Nascimento Dda C, Martins OV, De Santana FS, Balsamo S. Higher muscle performance in adolescents compared with adults after a resistance training session with different rest intervals. J Strength Cond Res 2012;26:1027-1032. https://doi.org/10.1519/JSC.0b013e31822dfefb
29. Brown LE, Weir J. ASEP procedures recommendation I: Accurate assessment of muscular strength and power. J Exerc Physiol Online 2001;4:1-21.
30. Hakkinen K, Pakarinen A, Kraemer WJ, Hakkinen A, Valkeinen H, Alen M. Selective muscle hypertrophy, changes in EMG, and force, and serum hormones during strength training in older women. J Appl Physiol 2001;91:569-580. https://doi.org/10.1152/jappl.2001.91.2.569
31. Kreider RB, Leutholtz BC, Katch FI, Katch VL. *Exercise and Sport Nutrition*. Santa Barbara, CA: Fitness Technologies Press, 2009.
32. Greenwood MB, Cooke M, Ziegenfuss TS, Kalman D, Antonio J. *Nutritional Supplements in Sports and Exercise (Second Edition)*. Springer International Publishing Switzerland, 2015, 385 p. https://doi.org/10.1007/978-3-319-18230-8
33. Sword DO, Carolina S. Exercise as a Management Strategy for the Overweight and Obese: Where Does Resistance Exercise Fit In? Strength Cond J 2013;35:89-89.
34. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol 2013;4:863. https://doi.org/10.3389/fpsyg.2013.00863
35. Mielgo-Ayuso J, Zourdos M, Urdampilleta A, Calleja-Gonzalez J, Seco J, Cordova A. Relationship of long-term macronutrients intake on anabolic-catabolic hormones in female elite volleyball players. Nutr Hosp 2017;34:1155-1162. https://doi.org/10.20960/nh.763
36. Tayebi MM, Yousefpour M, Ghahari L. Effects of creatine hydrochloride supplementation on physical performance and hormonal changes in soldiers. Phys Act Rev 2021;9:93-99. https://doi.org/10.16926/par.2021.09.11
37. Safdar A, Yardley NJ, Snow R, Melov S, Tarnopolsky MA. Global and targeted gene expression and protein content in skeletal muscle of young men following short-term creatine monohydrate supplementation. Physiol Genomics 2008;32:219-228. https://doi.org/10.1152/physiolgenomics.00157.2007
38. Mobley CB, Fox CD, Ferguson BS, Amin RH, Dalbo VJ, Baier S, Rathmacher JA, ET AL. L-leucine, beta-hydroxy-beta methylbutyric acid (HMB) and creatine monohydrate prevent myostatin-induced Akirin-1/Mighty mRNA down-regulation and myotube atrophy. J Int Soc Sports Nutr 2014;11:38. https://doi.org/10.1186/1550-2783-11-38
39. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. FEBS J 2013;17:4294-4314. https://doi.org/10.1111/febs.12253
40. Vingren JL, Kraemer WJ, Ratamess NA, Anderson JM, Volek JS, Maresh CM. Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. Sports Med 2010;40:1037-1053. https://doi.org/10.2165/11536910-000000000-00000
41. Persky AM, Brazeau GA, Hochhaus G. Pharmacokinetics of the dietary supplement creatine. Clin Pharmacokinet 2003;42:557-574. https://doi.org/10.2165/00003088-200342060-00005
42. Deldicque L, Decombaz J, Zbinden Foncea H, Vuichoud J, Poortmans JR, Francaux M. Kinetics of creatine ingested as a food ingredient. Eur J Appl Physiol 2008;102:133-143. https://doi.org/10.1007/s00421-007-0558-9
43. Tuckfield C. First use of creatine hydrochloride in premanifest huntington disease. Med J Aust 2015;202:378-381. https://doi.org/10.5694/mja14.01070
44. Lanhers C, Pereira B, Naughton G, Trousselard M, Lesage FX, Dutheil F. Creatine supplementation and lower limb strength performance: a systematic review and meta-analyses. Sports Med 2015;45:1285-1294. https://doi.org/10.1007/s40279-015-0337-4
45. Lanhers C, Pereira B, Naughton G, Trousselard M, Lesage FX, Dutheil F. Creatine supplementation and upper limb strength performance: A systematic review and meta-analysis. Sports Med 2017;47:163-173. https://doi.org/10.1007/s40279-016-0571-4

46. Wu S-H, Chen K-L, Hsu C, Chen H-C, Chen J-Y, Yu S-Y, Shiu Y-J. Creatine Supplementation for Muscle Growth: A Scoping Review of Randomized Clinical Trials from 2012 to 2021. Nutrients 2022;4:1255. https://doi.org/10.3390/nu14061255
47. Mills S, Candow DG, Forbes SC, Neary JP, Ormsbee MJ, Antonio J. Effects of creatine supplementation during resistance training sessions in physically active young adults. Nutrients 2020;12:1880. https://doi.org/10.3390/nu12061880
48. Chilibeck PD, Kaviani M, Candow DG, Zello GA. Effect of creatine supplementation during resistance training on lean tissue mass and muscular strength in older adults: A meta-analysis. Open Access J Sports Med 2017;8:213-226. https://doi.org/10.2147/OAJSM.S123529
49. Bazzucchi I, Felici F, Sacchetti M. Effect of short-term creatine supplementation on neuromuscular function. Med Sci Sports Exerc 2009;41:1934-1941. https://doi.org/10.1249/MSS.0b013e3181a2c05c
50. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF-1-akt/pKB pathway: insights from genetic models. Skeletal Muscle 2011;1:1-14. https://doi.org/10.1186/2044-5040-1-4
51. Ribeiro AS, Avelar A, Kassiano W, Nunes JP, Schoenfeld BJ, Aguiar AF, Trindade MCC, ET AL. Creatine supplementation does not influence the ratio between intracellular water and skeletal muscle mass in resistance-trained men. Int J Sport Nutr Exerc Metab 2020;30:405-411. https://doi.org/10.1123/ijsnem.2020-0080
52. Forbes SC, Candow DG, Krentz JR, Roberts MD, Young KC. Changes in fat mass following creatine supplementation and resistance training in adults≥ 50 years of age: a meta-analysis. J Funct Morphol Kinesiol 2019;4:62. https://doi.org/10.3390/jfmk4030062
53. Lee N, Kim I, Park S, Han D, Ha S, Kwon M, Kim J, ET AL. Creatine inhibits adipogenesis by downregulating insulin-induced activation of the phosphatidylinositol 3-kinase signaling pathway. Stem Cells Dev 2015;24:983-994. https://doi.org/10.1089/scd.2014.0130
54. Petridou A, Siopi A, Mougios V. Exercise in the management of obesity. Metabolism 2019;92:163-169. https://doi.org/10.1016/j.metabol.2018.10.009
55. Bray GA, Heisel WE, Afshin A, Jensen MD, Dietz WH, Long M, Kushner RF, ET AL. The Science of Obesity Management: An Endocrine Society Scientific Statement. Endocr Rev 2018;39:79-132. https://doi.org/10.1210/er.2017-00253