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COMBINED EFFECT OF CREATINE MONOHYDRATE OR CREATINE HYDROCHLORIDE AND CAFFEINE SUPPLEMENTATION IN RUNNERS'PERFORMANCE AND BODY COMPOSITION

Jeferson Oliveira Santana[1], Elias de França[1]
Diana Madureira[1], Bruno Rodrigues[2], Erico Chagas Caperuto[1]

ABSTRACT

Background Creatine monohydrate (CrM) and caffeine are two of the main substances used to enhance athletic performance although some studies say that they impair each other and CrM could promote weight gain. Objective evaluate the association of CrM or creatine hydrochloride (CrHCl) with caffeine (Caf) supplementation on runners' performance and body composition. Methods 16 individuals, both genders (20-30 years) were randomly divided in 3 groups 1) CrM+Caf (n=6), 2) CrHCl+Caf (n=5) and 3) Placebo+Caf (n=5), they did four running sessions per week, during four weeks. Supplementation was given on a double blind manner, CrM+Caf (20g.day^{-1} per 7 days + 5 g.day^{-1} per 21 days of CrM), CrHCl+Caf (6g.day^{-1} per 7 days + 1.5 g.day^{-1} per 21 days of CrHCl) and Placebo+Caf (20g.day^{-1} per 7 days + 5 g.day^{-1} per 21 days of resistance starch). Caffeine ingestion was acutely administered for all groups, 6mg/kg^{-1} body weight, only in the last day of the experimental protocol. We collected PRE and POST-treatment, body fat (BF), body weight (BW), 10 km time trial (TT), rating of perceived exertion (RPE), delayed onset muscle soreness (DOMS) and gastrointestinal discomfort perception (GDP). Results No differences were found between groups for RPE, DOMS, BF, BW and GDP. TT decreased significantly for CrM+Caf and CrHCl+Caf group, but no for Placebo+Caf. BF decreased significantly in CrHCl+Caf group and lean body mass increased in CrM+Caf and CrHCl+Caf group. Conclusion These data suggest that either CrM or CrHCLsupplementation works synergistically with acute CAF supplementation improving running performance.

Key words: Caffeine. Creatine Monohydrate. Creatine Hydrochloride. Ergogenic. Running.

1-Metabolism of exercise Research and Study group (GEPAME), São Judas University, São Paulo-SP, Brazil.
2-Physical Education Department, UNICAMP, Campinas-SP, Brazil.

RESUMO

Efeito da combinação da suplementação de creatina monohidrato ou creatina cloridrato e cafeína na performance e na composição corporal de corredores

Creatina monohidratada (CrM) e cafeína são duas substâncias utilizadas para melhorar a performance atlética, embora alguns estudos sugerem que estas substâncias se prejudicam mutuamente, além da CrM promover ganho de peso. avaliar a associação da CrM ou creatina cloridrato (CrHCl) com a cafeína (Caf), na performance e composição corporal de corredores. 16 indivíduos, de ambos os gêneros (20-30 anos) foram divididos em 3 grupos 1) CrM+Caf (n= 6), 2) CrHCl+Caf (n= 5) e 3) Placebo+Caf (n= 5) para realizarem quatro treinos semanais (durante quatro semanas) suplementados com CrM (20g/dia por 7 dias + 5 g/dia por 21 dias) ou CrHCl (6g/dia por 7 dias + 1,5 g/dia por 21 dias) ou amido resistente (Placebo, 20g/dia por 7 dias + 5 g/dia por 21 dias) junto da suplementação aguda de cafeína (6mg/kg de peso corporal), que foi administrada somente no último dia do protocolo. Foram avaliados PRE e PÓS suplementação: gordura corporal (GC), peso corporal (PC), performance 10 km (P10km), percepção subjetiva de esforço (PSE), dor muscular de início tardio (DIT) e percepção de desconforto gastrointestinal (PDG). Não foram encontradas diferenças entre os grupos para PSE, DIT, GC, PC e PDG. O P10km diminuiu significativamente no grupo CrM+Caf e CrHCl+Caf, mas não para o grupo Placebo+Caf. A GC diminuiu significativamente no grupo CrHCl+Caf e a massa corporal magra aumentou no grupo CrM+Caf e CrHCl+Caf. Estes dados sugerem que a suplementação de CrM ou CrHCLfunciona sinergicamente com a suplementação aguda de Cafeína, melhorando a performance na corrida.

Palavras-chave: Cafeína. Creatina Monohidrato. Creatina Cloridrato. Ergogênico. Corredores.

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INTRODUCTION

Parallel with the growth of running around the world, with an increasing number of marathons, half marathons and 10 km runs, there is the growing demand for nutritional ergogenic aids.

Some of these athletes want better health and physical fitness, but most of them challenge themselves to better performance and competitive objectives. Some of the most popular supplements are Caffeine and Creatine (Cr) supplements (Tarnopolsky, 2011).

Although creatine monohydrate (CrM) is more popular among resistance training individuals, it is a consensus that it helps to improve performance in high intensity exercises. CrM is used with caution by athletes that might have problems with weight gain since it is one of the documented side effects of it and one reason why it is not very popular with runners (Lanhers and collaborators, 2015; Tang and collaborators, 2013;).

With this CrM limitation, a new molecule was recently introduced in the market, known as creatine Hydrocloride (CrHCl). Compared to CrM, CrHCl is 40 times more soluble in water (Gufford and collaborators, 2010), it has a greater permeability in the intestinal tract (Gufford and collaborators, 2013), besides having a higher molecular weight (Pubchem, 2017) (this implies higher reverse osmosis than CrM).

Those features suggest that greater solubility and permeability could decrease the amount of Cr needed to fuel the muscle (lower dosages), and could increase reverse osmosis avoiding weight gain due to water retention.

The market offers many supplements that combine caffeine (a ergogenic supplement (Schubert e Astorino, 2013)) with Cr, known as pre-workout. There are few studies evaluating the combination of these two supplements (Caffeine plus Cr). Some studies suggest that the Caffeine and Cr supplementation in combination can bring positive and satisfactory results for high-intensity intermittent exercise (Doherty and collaborators, 2002; Lee and collaborators, 2011).

However, some other papers tried thi simultaneous combination of Cr with Caffeine and showed that the ergogenic benefit of Cr was blunted (Hespel and collaborators, 2002; Vanakoski and collaborators, 1998; Vandenberghe and collaborators, 1996). There are no studies addressing this issue in runners.

Those facts elicited some of the questions raised in this study, does CrM promotes weight gain in endurance athletes that have very different fluid dynamics when compared to strength athletes? Does it make a difference if we use CrHCl, a more soluble molecule? Does CrHCl works for specific running strength and does that mean performance improvement? And most importantly, does CrHCl and caffeine have a synergistic effect when taken together by this population?

This study aims to evaluate the effects of CrM or CrHCl supplementation associated with Caffeine on performance and anthropometric variables of runners.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics Committee of São Judas Tadeu University (CEP/USJT), registered under the number, CAAE: 157140013.0.0000.0089. All individuals were informed about all procedures and signed the Informed Consent Form.

Subjects

We evaluated 16 healthy individuals, from both genders with an average of 20 to 30 years old. We established as inclusion criteria: to be a healthy recreational runner, who usually run 10 km or more for training, to have a training frequency of 3 to 4 days for week and with at least 6 months of experience on running.

Subjects were selected from personal contacts and selection was done using an interview, in which we evaluated all the exclusion criteria. We established as exclusion criteria: to be diagnosed with an injury or disease that would impair the subject participation, not to have a current medical approval for physical activity, to consume supplements that might interfere on the experimental design, and to consume caffeine containing products frequently.

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Experimental Design

All variables were collected in two different moments: Pre (PRE) and Post (POST) experimental protocol.

After the 16 individuals performed an initial 10 km running test (PRE) they were randomly divided in 3 groups, 1) CrM plus Caf (CrM+Caf; n=6), 2) CrHCl plus Caf (CrHCl+Caf; n=5) and 3) Resistant Starch (RS, used as Cr placebo) plus Caf (RS+Caf; n=5).

It should be emphasized that after the distribution of individuals on the group, as verified on Tables 1, 2 and 3, the groups did not present any differences (p> 0.05) on PRE-experimental protocol variables assessed in this study.

The groups received printed instructions about the supplementation protocol and training protocol. Training protocol was standardized for 4 running sessions for week, during 4 weeks. It consisted of 2 sessions where the objective was to improve running speed doing intervals or pace training above the estimated individual threshold and 2 sessions where the objective was endurance training. Training was carried out in treadmills and/or parks or in the streets.

The two types of Cr supplementation and RS supplementation were offered in a double-blind model, after the practitioners were randomly divided in three groups, as seen in Figure 1.

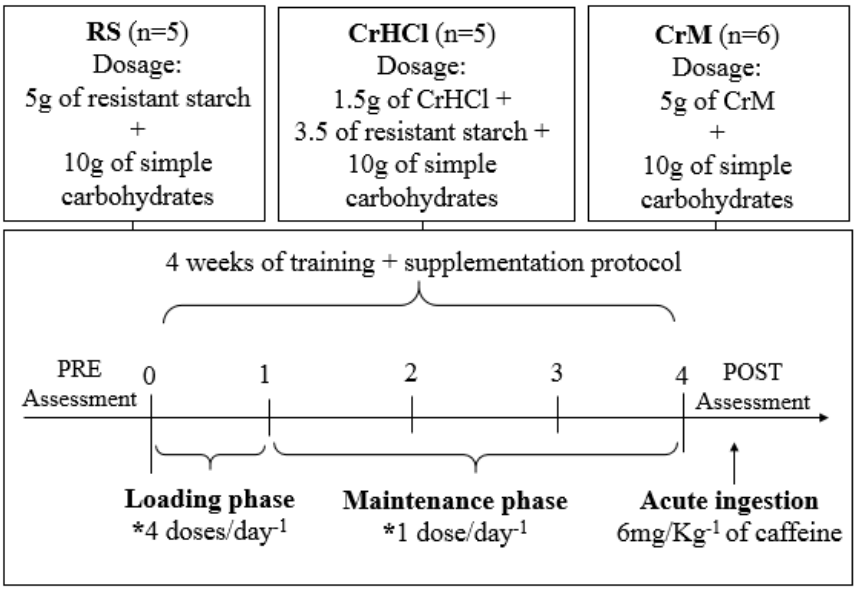

Figure 1 - Experimental design. PRE and POST-assessments: Anthropometric measures, BORG, EVA, GDP, 10 km time trial test; * Refers to dosage conditions of RS, CrHCl and CrM groups.

During the first week (7 days) of experiment, participants supplemented with either 20g/day^{-1} of CrM or 6g/day^{-1} of CrHCl or 20g/day^{-1} of RS on four equal doses (5g CrM, 1.5g CrHCl and 5g RS), every 4 hours (loading phase).

To ensure that all groups had the same supplement ingestion volume, CrHCl+Caf group ingested 1.5g (of HCl) associated with 3.5g of RS in each dose.

Furthermore, all of the groups ingested 10g of simple carbohydrate. Therefore, CrM+Caf group ingested four doses of 5g of CrM and 10g of simple carbohydrate, CrHCl+Caf group ingested four doses of 1.5g of CrHCl plus 3.5g of RS and 10g of simple carbohydrate, and RS+Caf ingested four doses of 5g of Resistant Starch and 10g of simple carbohydrate. In the next 3 weeks (21 days), rather than four doses, the subjects ingested only one dose of the supplement (maintenance phase).

Caffeine supplementation (6mg/Kg^{-1} body weight) was given only once (acutely) for all groups. The ingestion of caffeine occurred 15 minutes before the final test (10 km running) at the last day of the experimental protocol (last day of week 4).

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Variables analyzed

The time trial test (TT) was a 10 km run measured twice in the same circuit, already known for all the subjects. We used a Casio chronograph to TT register.

Gastrointestinal Discomfort Perception (GDP) was evaluated immediately after the 10 km run with an analog visual scale, ranging from 0 to 10. This scale is based on the VAS scale (described by Sousa 2000), that supposed to register the subjective discomfort sensation felt by participant during the test (possibly due to the caffeine ingestion).

We analyzed the Ratio of Perceived Exertion (RPE) using the Borg scale in order to verify if the test was done at their maximal effort (Borg e Noble, 1974). The Borg scale range 0 to 10 scale (the adapted version), where 0 is a very light effort and 10 is exhaustive effort. It is a validated instrument used extensively in exercise research. The RPE was evaluated immediately after the 10 km running test.

Delayed Onset Muscle Soreness (DOMS) was evaluated by asking the subjects for their perception of pain 48 hours after the last test. They supposed to rank the perception using the visual analog scale (VAS), that also ranged from 0 to 10, with 0 meaning no pain at all and 10 meaning worst pain imaginable.

Body weight was measured in a digital scale, with the subjects with a running t-shirt and shorts (the same ones on PRE and POST-protocol), with no shoes. The POST weight was measured one day after the final test, so there was no interference of the test on the subject weight.

Body composition was measured using a Lange skinfold caliper, using the 7 skinfold protocol (Jackson e Pollock, 1978), percentage of body fat (%BF), fat mass and lean body mass (LBM) were calculated from the values obtained using the Physical test 7.0 physical evaluation software.

All variables (and that is more relevant to the 10 km test, which was conducted in an outside environment) were measured in the same time of the day and ambient temperature and humidity were equivalents, the study was conducted during the same season and climate conditions had little and not relevant changes (as observed in the weather forecast local services).

PRE test variables were measured before the supplementation and training protocol in two different days. One day where 10 km TT test and, RPE and GDP. The second day was body composition assessment and DOMS levels were collected 48 hours after the 10 km running test. POST test variables were measured after 4 weeks of training and supplementation protocol, also in two different days.

Statistical Analysis

Results were expressed as mean and standard deviation. After Shapiro Wilk normality test, the variables were analyzed through a repeated measures ANOVA test to compare the groups (with Tukey's post hoc) and to observe the interaction (supplement x time). When comparing PRE *vs.* POST-treatment, paired t-tests were used.

The effect size (ES; Cohen's *d*) of mean delta change and 95% confidence interval (95% CI) for both 1) PRE- vs POS-treatment and 2) between groups were calculated. Significance was set as $p<0.05$ and the Statistical Package for the Social Sciences (SPSS) 20.0 software for windows was used.

RESULTS

Performance results

Table 1 presents 10 km performance before and after the experimental protocol. There was no group x time interaction ($p = 0.385$) or difference between groups in the PRE or POST-treatments. However, both supplemented groups decreased their running time significantly, which did not occur for the RS+Caf group, when we compare PRE with POST-treatments.

Effort (RPE), discomfort (GDP) and pain (DOMS) variables

Table 2 shows no significant decrease on RPE between all groups, with no group x time interaction ($p= 0.91$) or significant time effect ($p= 0.12$).

There is a moderate ES on RPE decrease for RS+Caf and CrM+Caf, in CrHCl+Caf group the ES was negative, but low. There was no difference on RPE between

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all groups when we compared PRE and POST-treatments (p> 0.05).

At the POST moment, the ES was weak when we compared RS+Caf and CrM+Caf groups (ES= - 0.092), however, there was a moderate negative ES for CrHCl+Caf when compared with the two others groups (ES= - 0.628 and -0.592, CrM+Caf and RS+Caf, respectively).

Despite there was a reduction on DOMS (PRE vs. POST treatments) in all groups (time effect, p= 0.07), only in the CrM+Caf group it was significant (Table 2).

There was no difference between groups on DOMS (or group x time interaction, *p*= 0.95), however, the effect size shows that individuals of the CrM+Caf group presented lower values compared to others groups (CrM+Caf vs. RS+Caf: ES=0.970; CrM+Caf vs. CrHCl+Caf: ES=0.972); CrHCl+Caf group presented a weak and negative value of ES when compared to RS+Caf group (ES= -0.239).

GDP did not increase significantly (time effect, *p*= 0.22), however, the large ES value indicates that CrM+Caf and CrHCl+Caf, presented a relevant increase on this variable (Table 2).

Although there was no difference between groups (or group x time interaction, *p*= 0.39), the large ES values show that more than 50%[10] of the subjects that supplemented with both Cr increased the GDP when compared to RS+Caf (CrM+Caf *vs.* RS+Caf: ES= -0.934; CrHCl+Caf *vs.* RS+Caf: ES= -0.801). CrHCl presented a weak and negative ES value when compared with CrM+Caf group (ES= -0.348).

Table 1 - Time to perform a 10 km run from CrM+Caf, CrHCl+Caf, and RS+Caf groups PRE and POST-supplementation protocol (time in minutes).

	RS+Caf (n=5)		CrM+Caf (n=6)		CrHCl+Caf (n=5)	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
TT (minutes)	56.4 ± 7.2	52.2 ± 6.6	59.7 ± 12.2	52.7 ± 7.3*	56.4 ± 5.9	51.2 ± 7.4*
95% IC, ES	-10.18 to 1.7, 0.599		-12.30 to -1.69, 0.697		-8.16 to - 2.23, 0.778	

Legends: * p <0.05 when compared to PRE. 95% IC, 95% Interval Confidence; ES, Effect Size.

Table 2 - Ratio of Perceived Exertion (RPE), Delayed Onset Muscle Soreness (DOMS) and gastrointestinal discomfort (GDP) after a 10km run.

	RS+Caf (n=5)		CrM+Caf (n=6)		CrHCl+Caf (n=5)	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
RPE	5.8 ± 1.9	5.2 ± 0.7	6.0 ± 1.0	5.2 ± 1.6	6.0 ± 2.2	6.6 ± 0.9
95% IC, ES	-3.83 to 2.63, 0.419		-3.90 to 2.23, 0.599		-1.28 to 2.48, -0.369	
DOMS	4.8 ± 2.4	4.0 ± 0.8	5.5 ± 1.4	3.3 ± 1.8*	4.8 ± 1.6	4.4 ± 1.7
95% IC, ES	-2.41 to 0.81, 0.533		-3.97 to -0.35, 0.228		-3.25 to 2.45, 0.242	
GDP	2.6 ± 1.3	3.0 ± 0.8	0.5 ± 0.2	2.8 ± 1.0#	0.8 ± 0.6	2.4 ± 1.3#
95% IC, ES	-1.85 to 2.65, -0.153		-0.61 to 5.27, -1.380		-0.28 to 3.48, -0.889	

Legends: * p <0.05 when compared to PRE; 95% IC, 95% Interval Confidence; ES, Effect Size.

Body composition

Table 3 describes the mean and standard deviation (PRE and POST-experimental protocol) of total body weight, % body fat, lean body mass and fat mass.

There was no group x time interaction (*p*= 0.82) in body weight. It was found a moderate and positive ES for both Cr groups when compared to RS+Caf group (CrHCl+Caf vs. RS+Caf: ES= 0.541; CrM+Caf vs. RS+Caf: ES= 0.283).

In addition, there was no significant difference between CrM+Caf and CrHCl+Caf groups, moreover the ES (d= 0.033) shows that the weight changes on both groups were similar. Albeit it was found a significant time effect (p<0.01), there was no significant changes on the average weight (kg) in all groups.

There was no group x time interaction (p= 0.34) in BF%. There was a decrease in %BF in all groups comparing PRE vs. POST-treatment (time effect, p=0.01), however, the

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only CrHCl+Caf group significantly decrease (see Table 3).

Despite there was no difference on the BF% between groups, ES was strong and positive for CrHCl+Caf (ES=0.936) and weak for CrM+Caf (ES=0.187) when compared to RS+Caf group.

The CrHCl+Caf group showed a more consistent %BF decrease compared to CrM+Caf group (ES= 0.598). However, there was no significant differences between groups (CrM+Caf vs. RS+Caf: p= 0.98, ES= -0.124; CrHCl+Caf vs. RS+Caf: p= 0.73, ES= 0.936; CrM+Caf vs. CrHCl+Caf: p= 0.60, ES= 0.598).

When we compared LBM (PRE vs. POST-treatment), we found a significant increase only in CrM+Caf, however, without group x time interaction or difference between groups (see table 3).

We observed a significant decrease in fat mass in PRE vs. POST-treatment only in CrHCl treatment. However, no significant difference was found between groups (CrHCl+Caf vs. RS+Caf: p= 0.30 ES= -0.801; CrHCl+Caf vs. CrM+Caf: p= 0.63, ES= -0.332; CrM+Caf vs. RS+Caf: p= 0.20, ES= 0.904).

Table 3 - Weight (kg), percentage of body fat (%), lean body mass (kg) and fat mass (kg) of experimental groups from PRE and POST-protocol.

	RS+Caf (n=5)		CrM+Caf (n=6)		CrHCl+Caf (n=5)	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Weight (kg)	70.8 ± 13.4	70.4 ± 13.4	71.0 ± 15.0	71.7 ± 15.0	69.6 ± 8.9	68.8 ± 7.8
95% IC, ES	-1.91 to 1.31, 0.022		- 4.96 to 2.86, -0.086		-2.71 to 0.79, 0.110	
% Body fat	16.6 ± 7.7	16.6 ± 7.9	15.9 ± 8.2	15.0 ± 7.2	20.3 ± 3.9	18.7 ± 3.9*
95% IC, ES	-2.25 to 0.63, 0.106		-2.47 to 0.55, 0.164		-2.30 to - 0.85, 0.410	
Lean body mass (kg)	58.9 ± 11.6	59.4 ± 12.2	59.5 ± 13.0	60.8 ± 12.9*	55.5 ± 9.4	55.9 ± 7.9
95% IC, ES	-0.78 to 1.69, 0.038		0.29 to 2.15, -0.100		- 0.80 to 1.70, -0.046	
Fat mass (kg)	11.9 ± 5.6	11.0 ± 5.3	11.5 ± 6.8	10.9 ± 5.9	14.1 ± 4.6	12.8 ± 3.9*
95% IC, ES	-2.03 to 0.29, 0.158		-1.76 to 0.65, 0.087		-2.30 to -0.85, 0.304	

Legends: * p < 0.05 compared to PRE; 95% IC, 95% Interval Confidence; ES, Effect Size.

DISCUSSION

This study aimed to analyze the effects of the combination of CrM plus Caffeine or CrHCl plus Caffeine supplementation in running performance, anthropometric variables and subjective perception of DOMS, GDP and RPE.

Creatine and Caffeine combination

The main findings of this study are that both forms of Cr associated with Caffeine improved running performance and the ergogenic effect of CrM or CrHCl is not decreased with acute Caffeine intake.

Although there were no differences between the groups, probably due to the small number of participants and the heterogonous distribution of the groups (a limitation of this study) the significant decrease (between PRE and POST) found in the TT in both Cr supplemented groups is probably due to the synergistic effects of caffeine plus Cr.

These results corroborate with Lee and collaborators (2011) and Doherty and collaborators (2002) studies, who found similar results in a high intensity intermittent exercise model although our study is probably the first one that have seen these results in runners.

Other studies (Hespel and collaborators, 2002; Vanakoski and collaborators, 1998; Vandenberghe and collaborators, 1996) have reported that when caffeine is consumed simultaneously with Cr supplementation it blunts its ergogenic effect. We believe that this negative interference between Caffeine and Cr supplementation might be linked to the supplementation protocol proposed in these studies. For example, when we examine the supplementation protocol of the first study that raised this issue (Vandenberghe and collaborators, 1996) and the other study that corroborates it the experimental design was composed of Cr supplementation for seven days with concomitant Caf supplementation (5 mg·kg body wt-1·day-1) on days 5, 6 and 7

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and 20 hours before the exercise test (Hespel and collaborators 2002).

In these studies, the Cr group had a better performance than the Cr+Caf group and this, in turn, had similar performance to the placebo group. We believe that the caffeine supplementation protocol used in these studies (Hespel and collaborators, 2002; Vandenberghe and collaborators, 1996) does not give the right time to the caffeine reach its peak plasma concentration (Teekachunhatean and collaborators, 2013).

Moreover, Hespel and collaborators (2002) states that three consecutive days of Caf supplementation, per se, might be deleterious to athletic performance.

Vanakoski and collaborators (1998) study, was designed with Cr (3 x 100 mg· kg-1·d-1 for three days) and Caffeine (7 mg ·kg-1) supplementation alone or in combination.

There were no effects on performance improvement. It is currently established that 0.3 g·kg-1·d-1 at least for 5 days (of Cr supplementation) are necessary to promote ergogenic benefits (Hall e Trojian, 2013).

The caffeine dose used in that study (Vanakoski and collaborators, 1998) might increase performance in TT tests (Ganio and collaborators, 2009), time to exhaustion (Donghia and collaborators, 2016) and maximal voluntary contraction (Warren and collaborators, 2010), but there are other studies where torque (the variable used by Vanakoski and collaborators, 1998) still show some controversial results with caffeine supplementation (Glaister and collaborators, 2014).

Using the valuable contribution that those studies offered and other protocols suggested by the literature as effectives (Lanhers and collaborators, 2015), as was also done by Doherty and collaborators (2002) and Lee and collaborators (2011) we built the protocol used in this study and we could observe performance improvements with the Cr+caf combination.

Endurance Performance

Classically the ergogenic effects of Cr supplementation are credited to their role in ATP resynthesis from ADP in cytosolic cellular environment.

Another not less important role of Cr in the ATP resynthesis is its participation in the biochemical process responsible for the ATP output and ADP input in the electron transport chain ETC (Tewari and collaborators, 2012; Vendelin and collaborators, 2004).

This process might be helpful to endurance events (through Cr supplementation), since this process might be responsible for 80% of the energy provided by the ETC to the muscle cell cytosol (Aliev and collaborators, 2011).

Another possible benefit of Cr supplementation to endurance is its impact on mitochondria energy production with consequent muscle glycogen sparing. There are several studies with Cr supplementation that observed, for the same workload, decreased plasma Lactate in the Cr supplemented group (Cooper and collaborators, 2012; Santos and collaborators, 2004b), along with these findings, greater muscle glycogen sparing and even less protein breakdown were noted (Tang and collaborators, 2013).

These biochemical changes observed with Cr supplementation are related to a better ETC performance, i.e., to better use muscle glycogen (in the oxidative phosphorylation pathway). These improvements in the ATP resynthesis promoted by Cr might prevent a decrease in performance in regular and long endurance events (Gejl and collaborators, 2013).

This contribution of Cr to oxidative phosphorylation, in theory, might be responsible for the improving in the 10 km TT performance in both Cr groups.

Gastrointestinal Discomfort Perception

When the participants in both groups ingested caffeine (6 mg·kg-1 prescribed exactly for each individual) in the final test, 8 of 16 participants reported gastrointestinal discomfort, although the caffeine dose could be considered low for regular users.

This dose has been proven effective for performance improvement without affecting other parameters, such as the gastrointestinal state (Spriet, 2014).

This probably happened because the participants added the caffeine effects and their own nervousness once they were eager to run the final 10 km test. That might have made them more sensitive to the supplementation.

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Other studies reported the same (Lee and collaborators, 2011; Astorino and collaborators, 2012), where participants reported mild tremor symptoms, nausea, gastrointestinal discomfort and anxiety after caffeine intake. Surely, a control group for the caffeine supplementation (a limitation of our study) would allow us to draw better conclusions about these results.

Rate of Perceived Exertion

When we compared the RPE group's data in PRE and POST-supplementation, there were no significant differences. Lee and collaborators (2011) also showed that acute ingestion of caffeine after 5 days of Cr supplementation increased performance in high-intensity interval exercise, with no significant differences in RPE, data corroborated by another study (Schneiker and collaborators, 2006).

As evidenced by the ES values in our results, RS+Caf and CrM+Caf groups showed a decrease in RPE despite the high intensity of the training (evidenced by the reduction in TT to complete the 10 km test), these results might be linked to effect of caffeine supplementation on RPE (Doherty and collaborators, 2002).

On the other hand, the CrHCl+Caf group had a negative ES value, suggesting a stronger perception of the intensity experienced by this group (De Morree e Marcora, 2015), given that this group had a larger improvement in the 10k TT (ES value) compared to CrM+Caf and RS+Caf groups.

Delayed Onset of Muscular Soreness

Despite the high intensity training, participants experienced a reduction of the DOMS perception (ES values). However, only the CrM+Caf group showed significant reduction. That suggests that both, caffeine and CrM, can have a synergistic action in reducing the DOMS perception.

Santos and collaborators (2004a) demonstrated a reduction in inflammatory markers associated with DOMS, after long distance running in athletes supplemented with CrM. These results were attributed to the mechanical protection promoted by the intra cellular water increase in the muscle cell.

Caffeine reduced pain in the leg muscles during high-intensity exercise or in the following days to exercise (Gliottoni e Motl, 2008; Kim e Lee, 2014).

On the other hand, Astorino and collaborators (2012) found no differences for muscle pain in the legs in physically active women in an 8.2 km pedaling an all-out test, suggesting that the effect of caffeine may be ineffective in some cases. That effect is apparently dependent on the ambient temperature (Spriet, 2014).

All participants in our study did the tests at the same time, so the ambient temperature was not the responsible for the differences. De França and collaborators (2015) has been suggested that CrHCl has a different effect on water dynamics than CrM (confirmed by recent unpublished data from our laboratory), with less water being absorbed by the cells, that might possibly be one explanation, for the significant reduction being only noticed in the CrM+Caf group.

Body composition

One of our hypotheses was that CrHCl would promote less weight gain or would help athletes to lose more weight when combined with training.

However, as seen in our results, there was no differences in the total body weight for both types of Cr or when compared to placebo supplementation. Our premise was that the fact that an increased need for water to dilute the CrM in relation to CrHCl (Gufford and collaborators, 2010), would represent an increase in water retention in individuals who would supplement with CrM (Francaux e Poortmans, 1999; Powers and collaborators, 2003), and would therefore increase their body weight (Branch, 2003).

The lack of change in body weight can be related to our training protocol intensity, or even to a dehydrated state promoted by our training protocol (intense aerobic exercise). One of CrM side effects is weight gain, as described by Murphy and collaborators (2005) who found an increase in the total weight in sedentary individuals, with a protocol of 20 g/day-1 in the first 7 days, and over 10 g/day-1 for the remaining 21 days.

Tang and collaborators (2013), also observed weight gain in athletes during a supplementation protocol of 12 g/day-1 for 15

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days with moderate exercise. Both types of Cr used in this study did not influence body weight, going against several studies in the literature (Branch, 2003; Francaux e Poortmans, 1999; Murphy and collaborators 2005; Powers and collaborators, 2003; Tang and collaborators, 2013).

The only clear difference from this to the other studies is that we used intense endurance exercise as the experimental intervention. Future studies should focus on body weight changes with CrM or CrHCl supplementation and endurance exercise or circuit training to understand if the weight gain side effect is exclusively seen on resistant training.

We observed a small increase in LBM only for CrM+Caf group, with the ES value for the CrM+Caf group showing a greater gain in LBM. Although there are studies that suggest that the weight gain associated with CrM supplementation can result mostly from LBM (Kreider, 2003), there are others that link it to water retention (which increase LBM) and not muscle mass (Mendes and collaborators, 2012), this might explain the greater gains (although not significant) for the CrM+Caf group when compared to the CrHCl+Caf and RS+Caf groups.

A study evaluating the effects of 28 days of CrM supplementation on metabolism and cycling performance during a simulated road race found (by hydrostatic weighing) a significant increase in the total weight from pre to post supplementation in the CrM group, accompanied by a non-significant decrease in the fat percentage (Hickner and collaborators, 2010).

Mendes and collaborators (2012) observed (by DXA and BIA) a significant increase in total body weight and lean body weight in 18 swimmers after CrM supplementation. Furthermore, it seems that CrM supplementation prevents the loss of protein during endurance practice (Tang and collaborators, 2013).

These studies support the data found in our study which identified an increase in LBM in the CrM group from PRE to POST-treatment. There was no significant increase for total body weight or fat mass for both Cr supplementation, our data are consistent with previous studies (Branch, 2003; Lee and collaborators, 2011).

CONCLUSION

The association of CrM or CrCrHCl supplementation with caffeine worked synergistically to promote improvements in 10 km TT.

This study showed that CrM and CrHCl supplementation associated with endurance training did not significantly increase total body weight, but promoted an increase in LBM.

Despite the high-intensity training, RPE decreased for CrM+Caf, but not for CrHCl+Caf. The association between CrM+Caf decreased the perception of DOMS.

Higher gastrointestinal discomfort was shown for both supplementation protocols, but due to the lack of a Caffeine control group we cannot explore further this result.

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