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Acute citrulline-malate ingestion does not enhance performance in judo athletes

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Key words: citrulline-malate, supplementation, *judo*, performance, judo special fitness test.

Abstract

Background. Judo is a combat sport that involves grappling and throwing techniques, and multiple high-intensity efforts. Citrulline Malate (CM) is a nutritional supplement used to enhance physical performance, albeit not all studies have corroborated its ergogenic properties during high-intensity exercise.

Problem and aim. To the best of our knowledge, the effects of CM on judo performance has not been investigated. To fill this gap the aim of the current study was to investigate the effects of acute CM ingestion on performance in judo athletes.

Methods. Using a randomized crossover and double-blinded design, 10 judo athletes ingested 8g of CM (2:1 proportion) or placebo 60 minutes prior to the execution of three bouts of the Special Judo Fitness Test (SJFT). The number of throws, the SJFT index, blood lactate concentration before and after each bout and rating of perceived exertion (RPE) after each bout were assessed.

Results. Acute CM ingestion did not result in an increase of throws during three sets of the SJFT, nor in the SJFT index and RPE. The blood lactate concentration was higher in the CM condition only after the second SJFT bout, with no differences at any other time points.

Conclusions. Acute CM ingestion by judo athletes did not improve performance in the SJFT.

Judo is a Japanese martial art/combat sport created by Jigoro Kano in the late 19th century [Nippon Budokan 2011]. It involves grappling, throwing techniques and multiple high-intensity efforts. Although technical and tactical skills are fundamental for competitive success [Franchini *et al.* 2008; Miarka *et al.* 2012], physical fitness also plays an essential role for judo athletes training and competing [Franchini *et al.*, 2011a, 2014]. Based on this, strategies to improve physical performance are beneficial for judo athletes. In that context, nutritional supplements have been investigated to improve judo performance [Artioli *et al.* 2007; Astley *et al.* 2017; de Andrade Kratz *et al.* 2017].

Citrulline malate [CM] is a nutritional supplement composed of citrulline and malate, usually in a proportion of 2:1 (citrulline:malate). Citrulline is an amino acid that participates in the urea cycle, and malate is an intermediate in the Krebs cycle, involved in the oxidative metabolism. CM has been investigated as an ergogenic aid to improve performance by attenuating fatigue and increasing aerobic ATP production [Perez-Guisado, Jakeman 2010]. It has also been documented that CM supplementation results in increased levels of arginine and ornithine. Arginine is a precursor of nitric oxide, which increases blood flow and thus contributes to oxygen and nutrients delivery to the contracting muscles.

Furthermore, citrulline, due to its urea cycle action, would be involved in the attenuation of ammonia accumulation during exhaustive exercise, thus contributing to fatigue attenuation [Callis *et al*. 1991].

Most investigations on the effects of CM for physical performance improvement involved resistance training exercises. For example, using a randomized double-blinded controlled study, Perez-Guisado and Jakeman [2010] investigated the effects of acute CM ingestion [8g at 2:1 proportion 60 min prior to exercise] on repeated sets of upper body resistance exercise. Authors demonstrated that CM ingestion resulted in a 52% increase at the number of repetitions during 8 sets of bench press exercise done until volitional failure. Similarly, Wax *et al*. [2015] using the same supplementation protocol found that CM ingestion was capable to increase exercise volume during repeated sets of lower limb resistance exercise. These results suggest that acute CM ingestion improves performance, attenuating muscle fatigue during repeated sets of resistance exercise. Despite these promising results, not all studies corroborate these findings, with some reporting no ergogenic effect of CM on resistance exercise performance [Chappell *et al*. 2018; da Silva *et al*. 2017].

To the best of our knowledge, the effects of CM ingestion on judo performance are unknown. Furthermore, the studies evaluating the effects of CM on high-intensity exercise performance have presented conflicting results. To fill this gap in, the purpose of the present study was to investigate the effect of acute CM ingestion [8g] on judo-related performance. Our hypothesis was that pre-exercise CM ingestion would improve judo-related performance.

Methods

Participants

Ten male judo athletes (aged 19.4 ± 1.8 and 78.7 ± 10.5 kg of body mass) with at least 10 years of practice were recruited. The sample size was chosen based on the effect size of 1, alpha level of 0.05 and a power (1-b) of 0.80, which indicated that a minimum of ten participants were necessary (G*Power, version 3.1.9.4). The institution's Human Research Ethics Committee approved the procedures used in this study and all the athletes gave informed consent to take part, in accordance with the 1964 Declaration of Helsinki.

Procedures

Each participant attended on three occasions with a 7-day interval between visits. At the first visit, the athletes were guided about the protocols used in the study, signed the informed consent form and performed three sets of the Special Judo Fitness Test (SJFT) as familiarization.

In two subsequent visits, participants were submitted to 3 series of the SJFT. Each set consisted of three periods: A) 15 seconds; B) 30 seconds; C) 30 seconds with intervals of 10 seconds between periods of effort and 1-minute rest between sets. The heart rate was obtained through a heart rate monitor immediately and 1 minute after the end of the test. The rating of perceived effort (RPE) was evaluated after each series with the 6-20 Borg scale [Borg 1982]. In addition, finger-stick capillary whole blood was collected before and after each bout of SJFT for blood lactate assessment (Accutrend Plus system, Roche, Switzerland). The total number of throws and the heart rate measured with a heart rate monitor (Polar Electro Oy, Kempele, Finland) were used for the calculation of the SJFT index calculated according to the equation proposed by Sterkowicz [1995]:

INDEX = FINAL HR [BPM] + HR 1 MIN AFTER END OF TEST [BPM] / TOTAL NUMBER OF THROWS

On the experimental testing sessions, participants received 8g of CM (citrulline and malate in 2:1 proportion; Gamma Insumos Farmaceuticos, Sao Paulo, Brazil) diluted in 300 ml of flavored water or the placebo solution (300ml of flavored water) in a randomized and double-blinded fashion. CM and placebo solutions were identical in color and flavor. To evaluate the blinding, after the last experimental session participants were asked: "in which trial do you believe you ingested the supplement?". 20% guessed correctly, 20% guessed incorrectly, and 60% answered "I don't know". The ingestion was performed 60 minutes before the tests, as used in previous studies that demonstrated ergogenic action of citrulline-malate [Perez-Guisado, Jakeman 2010; Wax *et al*. 2015]. The participants were asked to follow the same diet and exercise practices before each trial but to abstain from nutritional supplements and caffeine-containing foods and drinks during all data collection period.

Statistical analysis

Data were analyzed for normality (Gaussian distribution) using the Shapiro-Wilk test. As data were normally distributed, parametric analyzes were used. The number of throws per set, SJFT index, blood lactate concentration and RPE were assessed using a 2-way repeated measures analysis of variance (ANOVA) using the Geisser–Greenhouse epsilon correction for violations of the sphericity assumption. Post hoc analysis using Bonferroni adjustments were performed where any significant interactions and main effects were found. Total number of throws was assessed using paired sample t-tests for each variable. A p-value of 0.05 was used to establish statistical significance.

Cohen's d was calculated as following: $(Mean_{CM} - Mean_{Placebo}) / SD_{Pooled}$. Cohen's d = 0.2 was considered a "small" effect size, 0.5 a "medium" effect size, 0.8 a "large" effect size, and 1.3 a "very large" effect size [Cohen 1988;

Rosenthal, 1996]. Partial eta square (partial η^2) was calculated using the following equation: Sum of squares / (Sum of squares + Residual error). Partial $\eta^2 = 0.01$ was considered a "small" effect size, 0.06 a "medium" effect size, and 0.14 a "large" effect size [Cohen, 1988]. Classifications were indicated after the respective effect sizes on the results section. Data Data are presented as Mean ± SD. The statistical software GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was used for all analysis.

Results

The number of throws during three bouts of the SJFT decreased after each bout, but no differences were observed between CM and placebo conditions (SJFT bout main effect: $F[1.8,16.4] = 29$; $P < 0.0001$; partial $\eta^2 = 0.81$ (large); CM main effect: $F[1,9] = 1.97$; $P = 0.19$; partial $\eta^2 = 0.25$ (large); interaction $F[1.9,17.6] = 1.0$; $P = 0.36$; partial $\eta^2 = 0.1$ (medium); Figure 1A). When the total number of throws was analysed, also no difference was found between conditions ($P = 0.19$; Cohen's $d = 0.51$ (medium); Figure 1B). The JSFT SJFT index augmented during the three bouts of SJFT, with no differences between CM and placebo conditions (SJFT bout main effect: $F[1.6,14.3] = 29.6$; $P < 0.001$; partial $\eta^2 = 0.80$ (large); CM main effect: $F[1,9] = 1.97$; $P = 0.19$; partial $\eta^2 = 0.18$ (large)18; interaction $F[1.9,17.5] = 0.27$; $P = 0.76$; partial $\eta^2 = 0.03$ (small); Figure 1C).

Blood lactate concentration increased during the execution of the three bouts of the SJFT (SJFT bout main effect: $F[2.5,22.2] = 52$; $P < 0.0001$; partial $\eta^2 = 0.90$ (large); CM main effect: $F[1,9] = 2.09$; $P = 0.18$; partial $\eta^2 = 0.07$ (medium); interaction $F[2.8,25.3] = 4.7$; $P = 0.0102$; partial $\eta^2 = 0.34$ (large); Figure 2A). When the CM and placebo conditions were compared, a significant difference was observed only at Post-II ($P < 0.05$). RPE also increased during the test protocol, but no differences were observed between conditions (SJFT bout main effect: $F[1.5,13.5] = 7.3$; $P = 0.0104$; partial $\eta^2 = 0.63$ (large); CM main effect: $F[1,9] = 0.39$; $P = 0.55$; partial $\eta^2 = 0.17$ (large); interaction $F[1.6,14.6] = 0.53$; $P = 0.56$; partial $\eta^2 = 0.06$ (medium); Figure 2B).

Discussion

This study examined whether acute CM ingestion enhances performance of judo athletes assessed by a specific judo physical test, the SJFT. The number of throws during 3 sets of the SJFT, the SJFT index, as well as RPE and blood lactate levels were not different in CM compared to placebo condition. Based on these results, acute

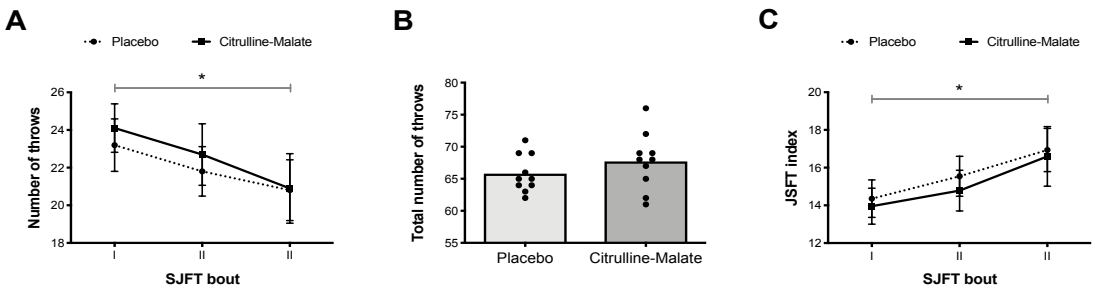

Figure 1: Effects of citrulline-malate ingestion on Judo Special Judo Fitness Test performance. A) number of throws in each set. B) total number of throws. C) JSFT SJFT index after each set. *P < 0.05 ANOVA Main Effect for SJFT bout (time factor).

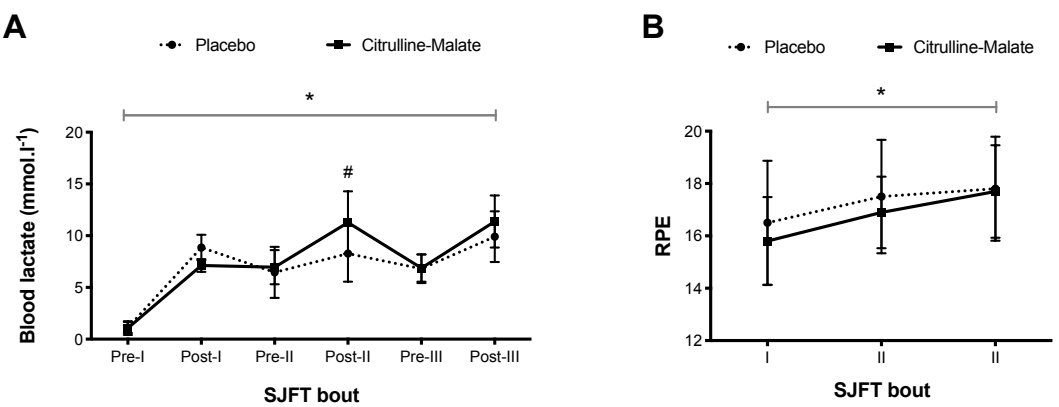

Figure 2: A) Blood lactate concentration before and after each SJFT set. B) RPE after each set of the SJFT. *P < 0.05 ANOVA Main Effect for SJFT bout (time factor). #P < 0.05 CM vs Placebo on Post-II time point.

CM ingestion does not exert an ergogenic effect in judo athletes assessed through the SJFT.

Both the anaerobic and aerobic capacities seem to be important for judo athletes (Franchini *et al*. 2011b; Julio *et al.* 2017). During combats, the athlete relies on fast actions to unbalance and apply throwing techniques on the opponent. On the other hand, during judo tournaments multiple matches occur on the same day, and the aerobic system is required for recovery between matches, as well as in the interval between high-intensity actions in a match. The SJFT was developed by Sterkowicz to assess both anaerobic and aerobic fitness in judo athletes using a movement pattern specific from the modality (*ippon seoi nague* technique). Franchini *et al.* [2011b] demonstrated that during the SJFT the alactic anaerobic energy system represent the major contribution (42.3%), albeit both lactic and aerobic energy systems contributing significantly (29.5 and 28.2%, respectively). A study published by Julio *et al.* [2017] demonstrated that during simulated judo matches there is a predominance of the oxidative system (70%), with a contribution of the lactic anaerobic and alactic anaerobic systems contribute of 8% and 21%, respectively. Despite different profile of the energy-system contributions, the SJFT has been widely used by researches and coaches to assess physical fitness in judo athletes, being a valid test for that population. Also, previous studies have used the SJFT protocol to investigate the effects of nutritional supplements on judo performance, for example with caffeine [Astley *et al*. 2017], sodium bicarbonate [Artioli *et al*. 2007] and beta-alanine [de Andrade Kratz *et al*. 2017] supplementation. Furthermore, it was previously demonstrated that the SJFT is a valid tool to discriminate athletes with different performance levels [Franchini *et al*. 2005b].

Investigations with CM and physical performance have provided controversial results. Perez-Guisado and Jakerman [2010] demonstrated that acute CM ingestion improved anaerobic performance in repeated sets of resistance exercise [8 sets of barbell bench press at 80% 1RM]. Similarly, Wax *et al* reported an increased performance in response to CM ingestion on lower limb [Wax *et al*. 2015] and upper limb [Wax *et al*. 2016] resistance exercise trained individuals, as well as Glenn *et al* using a higher load corresponding of 80% 1RM in moderately trained females. However, two investigations have failed in demonstrate positive effects of CM ingestion on resistance exercise performance, with untrained young adults using 90% of 10RM load [da Silva *et al*. 2017] and moderately trained males and females during a specific resistance training protocol, the German Volume Training [Chappell *et al*. 2018].

As cited before, a significant number of investigations focused on resistance exercise performance. Two other studies have used different protocols, but also presenting conflicting results. Glenn *et al* [2016] investigated the effects of 8g of CM on muscle strength and power in female tennis players. Maximal and average grip strength were improved in CM condition compared to placebo, as well as Wingate anaerobic cycling performance. However, no differences between conditions were observed in vertical jump performance. On the other hand, Cunniffe *et al.* [2016], investigating the effects of 12g of CM prior to repeated high-intensity cycling sprints in well-trained males did not observe any improvement on cycling performance after CM ingestion. A recent meta-analysis investigated the effect of citrulline (not necessarily in combination with malate) on high-intensity strength and power performance [Trexler *et al*. 2019]. After the analysis of 13 independent studies, the small effect size in favor of citrulline (0.20) indicates that citrulline ingestion prior to high-intensity exercise may exert a small benefit for performance. It is important to note that from the 13 studies included, 10 used citrulline in combination with malate, as CM. When only the studies using CM were analyzed, the effect size was only slightly higher (0.22). Therefore, based on those data, if CM can promote performance improvements, it seems to be with small magnitude.

Perez-Guisado and Jakerman [2010] stated that a single dose of CM may improve high-intensity anaerobic exercise that rely on anaerobic metabolism and result in increased lactate. However, our results, similarly to others [Chappell *et al*. 2018; Cunniffe *et al*. 2016; da Silva *et al*. 2017], do not corroborate that claim. It was previously demonstrated that the blood lactate concentration after the SJFT was correlated with blood lactate after a judo combat [Franchini *et al*., 2005a]. Despite increased blood lactate concentration during SJFT sets, we have found that acute CM ingestion did not promote increased performance when compared to placebo condition.

The mechanism of action of CM is still unclear, but some have been proposed. Firstly, citrulline is a precursor of nitric oxide, which promotes vasodilation and, consequently, nutrient delivery and metabolites clearance, regulates muscle contractility and may be involved in muscle satellite cells activation. Malate is an intermediate of Krebs cycle and CM supplementation may increase ATP resynthesis [Bendahan *et al*., 2002]. No differences were observed in blood lactate concentration, suggesting the CM ingestion did not result in an increased glycolytic contribution during the SJFT.

Furthermore, no differences on RPE were observed between conditions, suggesting that CM ingestion did not influence perceptual response to high-intensity physical exercise. This observation is in accordance with the study of Gonzalez *et al*. [2018] with resistance-trained men, but contrary to the results of Glenn *et al*. [2017] with resistance-trained women, which presented a lower RPE after the ingestion of 8g of CM prior to six sets of upper and lower body resistance exercise. Due to a limited number of studies, further investigation is necessary to better understand the effects of CM

on perceptual responses to exercise. A limitation of the current study is that, albeit widely used, the SJFT presents differences on the energy systems contributions, compared to simulated judo matches [Franchini *et al.* 2011b; Julio *et al.* 2017]. The investigation of CM ingestion on judo match performance can shed light on the effects of this nutritional supplement in a competitive setting. Moreover, the participants of this study were not top-level competitors.

Conclusion

Acute CM ingestion (8g, 60 minutes prior to exercise) did not improve performance in a judo-related anaerobic test. Neither physical performance nor perceived exertion were different between CM and placebo conditions. Despite a higher blood lactate concentration after the second bout of SJFT in CM condition, the blood lactate concentration during the three sets was similar between conditions. Based on these results, CM supplementation does not seem to be an effective ergogenic aid to improve judo performance.

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Znaczne spożycie suplementu jabłczanu cytryuliny nie poprawia wyników u zawodników judo

Słowa kluczowe: jabłczan cytryuliny, suplementacja, judo, wydajność, specjalny test sprawności fizycznej judo

Streszczenie:

Tło. Judo jest sportem walki, w którym wykorzystuje się techniki chwytania, rzucania i wiele innych intensywnych działań. Jabłczan cytruliny (*Citrulline Malate*) jest suplementem diety stosowanym w celu zwiększenia wydolności fizycznej, choć nie wszystkie badania potwierdziły jego właściwości ergogeniczne podczas ćwiczeń o dużej intensywności.

Problem i cel. Zgodnie z wiedzą autorów, nie zbadano wpływu jabłczanu cytruliny na wydolność w judo. W celu wypełnienia tej luki, celem niniejszego badania było zbadanie wpływu znacznego spożycia suplementu na wydajność zawodników judo.

Metody. Zastosowano losową próbę krzyżową i próbę podwójnie ślepą. 10 zawodników judo spożyło 8g jabłczanu cytruliny (proporcja 2:1) lub placebo 60 minut przed wykonaniem trzech serii Specjalnego Testu Sprawności Fizycznej Judo (SJFT). Oceniano liczbę rzutów, wskaźnik SJFT, stężenie mleczanów we krwi przed i po każdej walce oraz ocenę postrzeganego wysiłku (RPE) po każdej walce.

Wyniki. Znaczne spożycie jabłczanu cytruliny nie spowodowało zwiększenia ilości rzutów podczas trzech testów SJFT, ani indeksu SJFT i RPE. Stężenie mleczanów we krwi, po spożyciu suplementu było wyższe dopiero po drugiej serii SJFT, bez różnic w pozostałych punktach czasowych.

Wnioski. Znaczne spożycie jabłczanu cytruliny przez zawodników judo nie poprawia wyników w SJFT.