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Original Article

Influence of acute Ctrulline-malate supplementation on maximal strength and anaerobic power in combat athletes

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Abstract:
Citrulline is a precursor of arginine prior to the conversion to nitric oxide. It is believed that L-citrulline intake may increase anaerobic performance in non-athletes. The present study examines the influence of acute citrulline-malate supplementation on maximal isometric handgrip strength, Wingate anaerobic cycling and fractional exhaled nitric oxide in well-trained combat athletes. Twelve athletes consisting of 7 men and 5 women judokas participated in this study (age: 28.2 ± 4.4 years, mass: 71.3 ± 7.4 kg, height: 165.8 ± 5.6 cm, training experience: 8.4 ± 5.1 years and training volume: 7.5 ± 1.9 hour·week^{-1}). In a double-blind, randomised and crossover design, all measurements were conducted an hour after participants had orally ingested citrulline-malate (citrulline-malate; 8 g maltodextrin + 250 mg·kg^{-1} citrulline-malate) and the placebo (8 g maltodextrin) separated by a 10-day washout period. Citrulline-malate increases relative peak power as compared to the placebo (citrulline-malate: 10.6 ± 1.6 vs. placebo: 10.1 ± 1.2 Watts·kg^{-1}; p=0.02). A significant increase can be seen in the average grip strength when citrulline-malate is used as compared to the placebo (citrulline-malate: 34.1 ± 2.2 *vs*. placebo: 32.0 ± 3.1 kg; p<0.01). The resting fractional exhaled nitric oxide is also significantly greater compared to the placebo (citrulline-malate: 44.1 ± 2.2 *vs*. placebo: 32.0 ± 3.1 parts per billion; p=0.02). This result indicates that acute citrulline-malate supplementation can enhance anaerobic performance, likely through the improvement in nitric oxide. It has ergogenic potential for combat athletes.
Key Words: - **Nitric oxide, Exercise performance, Citrulline, Supplementation, Combat, Judo**

Introduction

Combat sports account for around 25 percent of all Olympic medals and cover a wide variety of contact sports in which two opponents with similar physical features engage in physical conflict. Combat sports mimic sport modalities with intermittent dynamics where short bursts of maximal intensity are interrupted with lesser intensities (López-González et al., 2018). Combat strategy demands significant involvement from both oxidative energy metabolism (Campos et al., 2012) and non-oxidative metabolism (glycolysis and the greater phosphagen system) (Nordin et al., 2021) following bouts of intense physical activity (Bridge et al., 2014).

L-citrulline combined with malate is typically marketed as a sports supplement, a compound known to regulate cellular energy production (Gonzalez & Trexler, 2020). L-citrulline supplementation is favoured by athletes since it is not on the list of prohibited substances. The alleged advantage of L-citrulline supplementation is its effective change into L-arginine, the substrate for endothelial nitric oxide synthase (eNOS) (Linoby et al., 2020; Stanelle et al., 2020). Recent evidence has shown a relationship between higher NO bioavailability and enhanced high-intensity exercise performance (Bailey et al., 2016; Jumat et al., 2021; Stanelle et al., 2020) as well as maximum exercise performance (Bailey et al., 2015; Linoby, Md Yusof, et al., 2020a). Citrulline malate (CIT_m) was shown to lessen fatigue and boost muscular force output in animal experiments (Stear et al., 2010).

Anaerobic capacity is the work produced by skeletal muscles during maximal or supramaximal exercise using anaerobic energy transfer systems (Tatlibal & Zencir, 2022). Anaerobic power is described as the ability to function without oxygen when insufficient oxygen enters the body, yet the body can continue working during exercise (Işildak, 2021). Anaerobic power is one of the two elements that comprise anaerobic performance. The term power refers to the maximum amount of power obtained per unit of time for explosive charges. The ATP-PC system provides anaerobic power for ATP regeneration under extreme loads (Bencke et al., 2002).

Power is essential, especially in sports that require speed and explosive power. However, maximum acceleration and speed are equivalent to force. Combat sports have psychophysiological requirements (Alesi et al., 2014; Padulo et al., 2014) and demand a well-developed upper and lower limb muscle strength (Tabben et al., 2013). These features highlight the significance of anaerobic fitness development *via* anaerobic power and

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anaerobic capacity to obtain a high level of fitness. The muscle strength of the lower limbs is the most important fitness feature since it is essential for kicking.

A study revealed that L-citrulline supplementation increases maximal grip strength and peak power (during a maximal anaerobic test) as compared to the placebo ($p<0.05$) (Glenn et al., 2016). In a subsequent trial conducted by the same set of researchers, the L-citrulline group exhibited substantial improvement in strength capacity compared to the placebo group ($p<0.05$) (Glenn et al., 2015). High-intensity exercise results in the development of ammonia in the blood and the activation of phosphofructokinase, which significantly improves glycolysis (Takeda et al., 2011). Anaerobic glycolysis will lead to blood lactate development and greater fatigue as the rate of glycolysis increases during high intensity exercise (Linoby, Kusrin, et al., 2020). According to a clinical study, L-citrulline boosts plasma L-arginine levels more efficiently than L-arginine supplementation in healthy human volunteers, depending on the dosage (Suzuki et al., 2017). Citrulline can be considered as an effective source of arginine and NO, potentially enhancing athletic performance.

The previous report shows that L-citrulline can be a beneficial dietary supplement for improving intense anaerobic performance with some rest intervals (Gonzalez & Trexler, 2020). However, it is currently unclear if oral CIT_m supplementation can reproduce these benefits in highly trained combat athletes. The purpose of this study is to determine the effect of acute citrulline-malate supplementation on maximal isometric handgrip strength, anaerobic cycling performance and fractional exhaled nitric oxide (FeNO) in well-trained combat athletes. We hypothesise that acute supplementation of CIT_m will increase combat athletes' anaerobic power and enhance their endogenous nitric oxide bioavailability.

Material & methods

Participants

Twelve trained judokas participated in the experimental trials (age: 23.6 ± 4.1 yrs, mass: 69.2 ± 8.1 kg, height: 167.9 ± 7.7 cm, training experience: 10.1 ± 6.4 yrs, training volume: 8.7 ± 1.7 $hrs \cdot wk^{-1}$). Prior to the lab visit, participants were asked to continue their normal physical activity and refrain from consuming any nutritional supplements, including dietary caffeine. Participants were also asked to consume low-dietary fats (Asraff et al., 2022) and restrict eating within two hours prior to the lab visit (Linoby, Jumat, et al., 2020). The eligibility requirements of the current study include: (1) healthy adults between 18 to 30-year-olds, (2) at least 5 years of endurance training experience, (3) a minimum of 6 combat-specific training per week, (3) no significant changes in body weight (≥5%) 3 months prior to the commencement of the study, (4) do not use dietary supplements (excluding macronutrients) or illegal drugs; and (5) engaged with any investigational research agent within the previous 3 months. Participants were asked to submit a written informed consent after receiving a thorough explanation of the study's methodology as well as the potential risks and benefits of participation. The study conformed to the Declaration of Helsinki and was authorised by the Institutional Human Research Ethics Committee.

Procedure

During the familiarisation session, randomly assigned individuals were asked to consume CIT_m (8 g maltodextrin + 250 $mg \cdot kg^{-1}$ citrulline-malate) or a placebo PLA (maltodextrin, 8 g) 60 minutes prior to the first assessment (i.e., FeNO assessment), separated by a 10-day washout interval. The CIT_m and maltodextrin powder were obtained from Myprotein, Malaysia (with Good Manufacturing Practice (GMP) and ISO17025 quality certification). Upon arriving at the laboratory, participants were asked to breathe into a portable breath nitric oxide chemiluminescence analyser (NIOX MINO™, Aerocrine, Sweden). The valve connected to the analyser was set at a flow rate of 50 $mL \cdot sec^{-1}$, according to the American Thoracic Society regulations (ATS/ERS, 2005). Maximal voluntary isometric contractions were subsequently measured using a JAMAR Hydraulic Hand Dynamometer (Sammons Preston Rolyan, Bolingbrook, Chicago, USA). The test was conducted using the dominant hand, as detailed by Linoby et al. (2020). The measurements for maximal voluntary isometric contractions were computed as follows: (a) the average grip strength (mean of 3 trials) and (b) the maximum grip strength (highest value of attempts). Anaerobic capacity and relative peak power were measured using the Wingate cycling test data, as described by Glenn et al. (2016).

Measurements

By using the standard Wingate cycling test protocol (WAnT), the relative mean power (defined as the mean output of power produced throughout the test divided by the participant's body mass) and relative peak power (defined as 5 seconds of power output produced during the test divided by the participant's body mass) were determined. The anaerobic capacity was measured using a cycle-ergometer (Monark 894 Peak Bike, Monark AB, Varberg, Sweden). The warm-up session consisted of 3- to 4-second maximum sprints accomplished 4 times per minute. Under guidance, static stretches of the quadriceps, hamstring and abductors were then conducted. As previously mentioned (Hernández-Belmonte et al., 2020), the 30-s WAnT procedure requires a standard resistance of 7.5% of body mass. The participants cycled at 60 revolutions per minute (rpm) and began following a verbal cue by the tester. When the cycling speed reached 70 rpm, the cycling electromagnetic brakes automatically activated with the sampling rate of the ergometer set at 50 hertz. Participants were always seated with toe clips used during the test. All participants received the same verbal support throughout the test. Using the built-in Anaerobic Test Software, performance outputs were derived (in watts). The following criteria were determined as the outcome of the test: (1) WAnT-PP (equal to the maximum value observed in the first 10 s) and (2) WAnT-MP (defined as the average performance during the test).

The measurement of maximal voluntary isometric contractions was performed using a JAMAR Hydraulic Hand dynamometer (Sammons Preston Rolyan, United Kingdom). Participants sat in an upright position with their feet flat on the floor. They held the dynamometer in a standardised hand position for each trial, with their forearms, wrists and shoulders at a neutral position and their elbows flexed at 90 degrees. Prior to each test, participants underwent a warm-up session consisting of 10, 6 and 10 submaximal voluntary isometric contractions at 25%, 50% and 25% of 1RM, respectively. The maximal voluntary isometric contractions test was conducted for the handgrip method. It consisted of 3 maximal contractions sustained for 5 seconds, separated by a rest interval of 60 seconds (Linoby et al., 2020). The maximum grip strength value measured throughout the test was recorded as the test outcome.

After the exercise testing familiarisation session, each participant was asked to use the portable breath nitric oxide chemiluminescence analyser before the actual test to get accustomed to the fractional exhaled nitric oxide (FeNO) device (NIOX MINO™, Aerocrine, Sweden). The analysis was conducted by qualified individuals in accordance with the American Thoracic Society's approved procedures (Linoby et al., 2020). Participants were directly told to inhale and exhale air into the analyser's probe-connected valve (flow rate of 50 $mL \cdot s^{-1}$). A consecutive evaluation of FeNO (repeated 3 times) yielded a coefficient of variation (CV) of 3.8%.

Statistical Analysis

By using a two-tailed, paired-sample t-test, the present study assessed the difference in maximal isometric handgrip strength, Wingate anaerobic cycling parameters and FeNO indices between the supplementations. GraphPad Prism was used for data analysis. A $p<0.05$ was considered statistically significant (Version 7.0b, GraphPad Software Inc., La Jolla, California, US). Data was presented as mean ± SD unless stated otherwise.

Results

Anaerobic Capacity

The results revealed that CIT_m increases the relative peak power (CIT_m: 10.64 ± 1.6 $W \cdot kg^{-1}$ *vs*. PLA: 10.13 ± 1.2 $W \cdot kg^{-1}$; p=0.02; Figure 1A) but not anaerobic capacity (CIT_m: 6.23 ± 0.9 $W \cdot kg^{-1}$ *vs*. PLA: 5.94 ± 1.2 $W \cdot kg^{-1}$; p=0.092) during the Wingate test when compared to PLA.

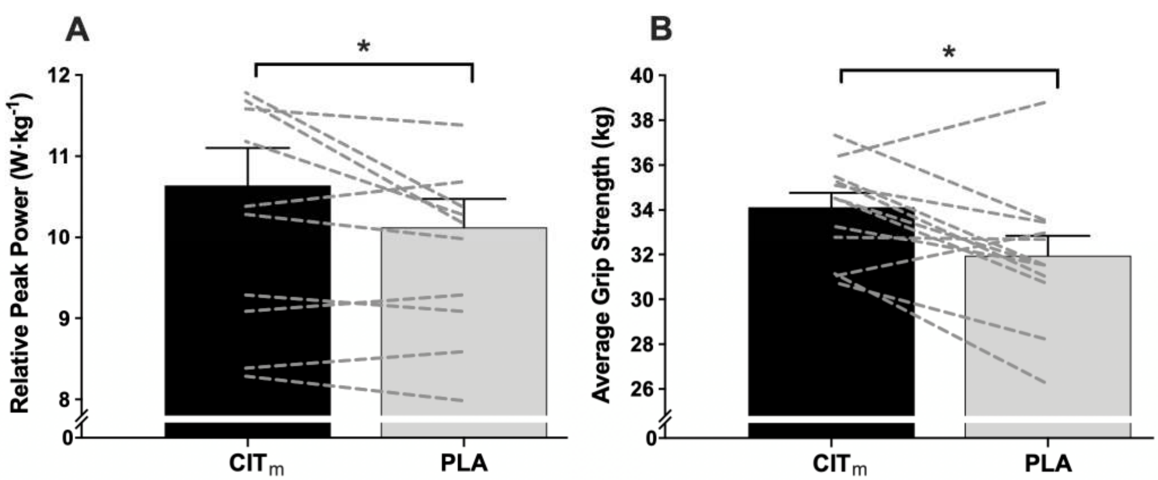

Fig. 1. After CIT_m and PLA conditions, the Wingate test reaches its maximum power (panel A). Analysis of the average grip strength was accomplished during maximal voluntary isometric contractions under CIT_m and PLA conditions (panel B). Individual subject responses are represented by dashed lines. The solid line represents the group mean ± SEM. *Significantly different (p<0.05)

Maximal Voluntary Isometric Contractions

A substantial improvement was seen in the average grip strength for the CIT_m group (CIT_m: 34.1 ± 2.2 kg) versus the PLA group (32.0 ± 3.1 kg; p=0.01; Figure 1A). However, the increase in maximal grip strength was insignificant (CIT_m: 35.7 ± 2.2 kg versus PLA: 33.7 ± 4.4 kg; p=0.07).

Fractional Exhaled Nitric Oxide (FeNO)

Resting FeNO was higher in the CIT_m condition than in the PLA condition (CIT_m: 44.1 ± 2.2 ppb *vs.* PLA: 32.0 ± 3.1 ppb; p=0.018).

Discussion

The success of combat sports is dependent on multiple physiological variables. Upper body strength and strong anaerobic performance are significant variables (Artioli et al., 2019). A large body of evidence support the hypothesis that L-citrulline plays a significant role in nitric oxide-generating pathway (Linoby, Md Yusof, et al., 2020b). Citrulline appears to boost the rate of adenosine triphosphate production when mixed with malate (Goron et al., 2019) and the rate of muscle oxygenation during exercise (Rashid et al., 2020). This eventually results in greater physical ability. In the current study, oral CIT_m supplementation enhanced resting FeNO by ~38%. Acute L-citrulline intake has been shown to increase FeNO levels (Holguin et al., 2019). Although studies have found potential physiological responses to L-citrulline supplementation associated with an increase in nitric oxide production (Schwedhelm et al., 2008; Sureda et al., 2009, 2010), only a few existing research had examined the ergogenic influence of L-citrulline supplementation, particularly in the trained population (Dewhurst-Trigg et al., 2018).

Several researchers who used greater doses of CIT_m (8 $g \cdot day^{-1}$) demonstrated a significant increase in strength (Pérez-Guisado & Jakeman, 2010). However, these findings could not be replicated in a study using a relatively similar method (Caballero-García et al., 2021). Mechanistically, CIT_m consumption simultaneously improves skeletal and muscular strength with higher energy turnover, decreased pH-to-power ratio and decreased ATP expenditure (Airin et al., 2014; Farney et al., 2019). The present study revealed that CIT_m administration may enhance skeletal muscle contractile power and efficiency, which is expected to lead to an optimal response during anaerobic performance (Bailey et al., 2015). The relationship between isometric handgrip strength and total strength is highly relevant in sports that involve significant strength generation (Rogers et al., 2017). In hand-to-hand combat, grabbing requires a substantial degree of handgrip strength to acquire a physical edge over an opponent. The current research proves that the average grip strength measured by maximal voluntary isometric contractions is ~6.8% greater in CIT_m than in PLA. The capacity of judokas to rapidly enhance grip strength may provide an athletic advantage during competition. Although improved grip strength due to CIT_m treatment has been previously reported in racquet-based sports (Glenn et al., 2016), the present study provides new data which demonstrates its ergogenic potential for combat athletes.

Conclusions

Intense activities, such as combat sports, may enhance from supplements that boost anaerobic performance. The ingestion of CIT increased the relative peak power during anaerobic cycling by 5%. Citrulline combined with malate compound has shown to increase the power output generation during the anaerobic capacity test. This indicates that CIT_m may have profound effects on maximal anaerobic performance. In this context, an acute dose of 250 $mg \cdot kg^{-1}$ CIT_m can potentially increase the peak power and hand grip strength, which will enhance performance in combat sports. No adverse reactions were detected which, compared to previous research (Glenn et al., 2015), indicates that athletes can safely consume CIT_m with no side effects.

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Conflicts of interest: There is no conflict of interest.

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