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Acute citrulline-malate supplementation improves maximal strength and anaerobic power in female, masters athletes tennis players

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Abstract
Citrulline-malate (CM) is a precursor to nitric-oxide (NO) in the NO synthase (NOS) pathway and is suggested to increase exercise performance in younger individuals. With age, NO production decreases and augmented NO production may provide beneficial effects on sports performance among masters athletes (MAs). Purpose: To examine the effects of acute CM supplementation on grip strength, vertical power, and anaerobic cycling performance in female, MA tennis players. Methods: Seventeen female MA (51 ± 9 years) completed two double-blind, randomized trials consuming CM (12 g dextrose + 8 g CM) and placebo (PLA) (12 g dextrose). One hour after consumption, subjects completed grip strength, vertical power, and Wingate anaerobic cycling assessments in respective order. Maximal and average grip strength, peak and average vertical power, anaerobic capacity, peak power, explosive power, and ability to sustain anaerobic power were calculated from the tests. Results: When consuming CM, participants exhibited greater maximal (p = .042) and average (p = .045) grip strength compared to PLA. No differences existed between trials for peak (p = .51) or average (p = .51) vertical power. For the Wingate, peak power (p < .001) and explosive power (p < .001) were significantly greater when consuming CM compared to PLA. For the ability to sustain power, a significant effect (p < .001) was observed for time within trials, but no significant differences were observed between trials regarding supplement consumed. Conclusions: These data suggest that consuming CM before competition has the potential to improve tennis match-play performance in masters tennis athletes. However, this study utilized a controlled laboratory environment and research evaluating direct application to on-court performance is warranted.

Keywords: Ergogenic aid; nitric-oxide; Wingate; amino-acid; sport performance; aging

Introduction
The National Senior Games began in 1987, attracting 2500 athletes; the number of master’s competitors has increased exponentially (almost 500%) since the inauguration with 12,000 competitors participating at the 2015 games (National Senior Games Association, 2013). As participation in masters athletic events continues to increase, there is a natural increase in competition (Jokl, Sethi, & Cooper, 2004). In order to maintain a physical advantage, masters athlete (MA) are continually searching for methods to enhance sport performance.

Supplemental ergogenic aid use is reported among MA because of the ability to increase sport performance (Beshgetoor & Nichols, 2003). One subset of these ergogenic aids includes substances eliciting increases in nitric-oxide (NO) production (Bescos, Sureda, Tur, & Pons, 2012). Augments in NO production cause increased vasodilation of the blood vessels and ultimately lead to greater blood flow in the working musculature. Based on this mechanism, citrulline-malate (CM) has recently experienced an increased interest for its effectiveness during exercise performance. l-citrulline is a non-essential amino acid naturally found in watermelon (Curis et al.,...
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2005) and when combined with malate (intermediate in the tricarboxylic acid cycle) increases exercise capacity during repeated bouts of sub-maximal resistance exercise to exhaustion in males (Perez-Guisado & Jakeman, 2010; Wax et al., 2014) and females (Glenn, Gray, Wethington et al., in press). Additionally, L-citrulline alone may have ergogenic properties when supplemented independently from malate (Bailey et al., 2015; van Wijck et al., 2014). In healthy men, acute ingestion of oral L-citrulline has reported positive increases in plasma citrulline/arginine levels (van Wijck et al., 2014) and plasma nitrite (Bailey et al., 2015) during exercise compared to placebo (PLA). Although one previous study (van Wijck et al., 2014) involving L-citrulline, independent from malate, did not evaluate performance factors associated with exercise (subjects cycled for 60 min at 70% VO2max but performance measures were not evaluated), another investigation (Bailey et al., 2015) demonstrated improvements in blood pressure, VO2 kinetics, and enhanced time to exhaustion while increasing the total work completed during severe-intensity exercise. Taken together, the aforementioned studies suggest that L-citrulline supplementation may have beneficial influences on the NO synthase (NOS) pathway (with or without the inclusion of malate) which may result in performance enhancement during repeated bouts of high-intensity exercise and/or continual exercise that has a start–stop nature.

Based on the intensity and start–stop nature of the sport, one particular sport that may experience benefits from CM supplementation is tennis. One of the most popular sports worldwide, tennis is a multifaceted sport requiring a multitude of different physical characteristics (i.e. grip strength, power, anaerobic capacity) to achieve success (Kovacs, 2006). Grip strength significantly relates to reactive power, jump power, and speed (Girard & Millet, 2009), while ensuring proper contact between the racket head and ball (Kovacs, 2006). Additionally, explosive power significantly relates to performance status of elite players (Roetert, Brown, Piorkowski, & Woods, 1996) and becomes essential as serve efficiency in elite players is dependent on muscle preload and the use of stored energy in the lower body (Girard, Micallef, & Millet, 2005). High anaerobic capacity is also important for tennis success due to the explosive nature of groundstrokes and rapid changes in direction (Kovacs, 2007), and finally, the ability to sustain power throughout long rallies’ is critical as points can last upwards of 15 s (Kovacs, 2006). When combined, these factors provide athletes the ability to achieve tennis match-play success (Kovacs, 2006, 2007).

While beneficial during repeated bouts of sub-maximal resistance exercise to exhaustion (Glenn, Gray, Wethington et al., in press; Perez-Guisado & Jakeman, 2010; Wax et al., 2014), other investigations assessing the acute effects of L-citrulline/CM supplementation on exercise performance in younger individuals are equivocal (Cortefello, Gadomski, & Zavorsky, 2015; Hickner et al., 2006). To date, the effects of CM have only been observed in younger individuals, yet NO production decreases with advancing age (Soucy et al., 2006). One of the reasons for this decrease in NO may be attributed to increased arginase activity (Santhanam, Christianson, Nyhan, & Berkowitz, 2008). L-citrulline inhibits the arginase enzyme (Shearer, Richards, Mills, & Caldwell, 1997) and supplementation may increase L-arginine availability. Therefore, supplementation with L-citrulline would be expected to increase L-arginine availability and, subsequently, the potential from NO synthesis from NOS. Based on this aspect, it cannot be assumed CM supplementation in aging individuals will elicit similar effects as younger individuals with regard to exercise performance; thus, research is necessary in masters populations. Therefore, the purpose of this investigation was to examine the effects of acute CM supplementation on grip strength, vertical power, and anaerobic cycling performance in female MA tennis athletes.

Based on previous literature, we hypothesized an acute 8 g CM dose would increase performance during grip strength, vertical power, and anaerobic cycling evaluations.

Methods

Participants

Based on previous CM investigations in females (Glenn, Gray, Wethington et al., in press), for significant differences to be determined between two dependent means with a power of 0.8 and an alpha level of 0.05, a minimum a priori sample of 15 participants was required (G*Power version 3.1.9.2, Kiel, Germany). As a result, this study included 17 masters-aged female tennis players from the Southern region of the USA (Table I). Inclusion criteria consisted of the following: categorized as low-risk by the American College of Sports Medicine, playing tennis at least two times per week over a two-year period (minimum), participation in sanctioned, competitive tennis events for at least two years (United States Tennis Association), and no previous CM or alternative NO-producing-related supplementation within the last year. Additionally, the amino acid creatine (synthesized from L-arginine) is suggested to increase creatine delivery to skeletal
muscle (Paddon-Jones, Borsheim, & Wolfe, 2004); therefore, based on CM’s role as a precursor to L-arginine, subjects consuming supplemental creatine were excluded from participation. Depending on the competition and/or organization, age cut-offs for masters competition can vary from 30 (World Masters Games, 2015) to 50 years (International Tennis Federation, 2015) and for the purposes of this investigation, the average (40 years) was used as minimum the age cut-off. Each participant completed a health history questionnaire and signed a statement of informed consent. Measures and procedures were approved by the University’s Institutional Review Board prior to testing.

Procedures

This study utilized a PLA-controlled, randomized, double-blind, crossover design. Participants reported to the laboratory for three visits. The initial visit included signing of an informed consent, completion of health history questionnaire, demographic/body composition measurements, and familiarization to the exercise protocols (described in detail below). The utilization of a familiarization trial ensured that subjects would be comfortable with procedures and allow for the immediate transition between exercise tests (grip strength, vertical power, and anaerobic cycling) with minimal rest in-between. A balance beam and stadiometer (Detecto, Webb City, MO, USA) were used to assess mass and height, respectively. Body fat and lean mass were measured by dual-energy X-ray absorptiometry (DXA; General Electric, Fairfield, CT, USA).

Table I. Subject demographic data

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Body mass (kg)</th>
<th>Body fat (%)</th>
<th>Years training</th>
<th>Times playing per week</th>
<th>Hours playing per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>51</td>
<td>167.6</td>
<td>66.6</td>
<td>32.8</td>
<td>10.0</td>
<td>3.4</td>
</tr>
<tr>
<td>(SD)</td>
<td>(9)</td>
<td>(6.4)</td>
<td>(9.5)</td>
<td>(7.7)</td>
<td>(8.2)</td>
<td>(0.9)</td>
</tr>
</tbody>
</table>

Notes: Subject age range was 40–68 years. Data are expressed as mean ± SD (n = 17).

Supplementation. For the intervention trials (visits 2 and 3), subjects consumed the following in randomized order: PLA (12 g dextrose, NowFoods, Bloomington, IL, USA), CM (12 g dextrose + 8 g CM; Powder City, Philadelphia, PA, USA) 1 h (Glenn, Gray, Wethington et al., in press; Perez-Guisado & Jakeman, 2010; Wax et al., 2014; Wax, Kavazis, & Luckett, 2015) before performing the exercise tests (hand-grip, vertical jump, and anaerobic cycling performance). The CM used for this investigation was third-party lab tested for purity prior to use. All supplementation visits were at the same time (±1 h) within trials, ensuring chronobiological control. Supplements were mixed in a sealed shaker by an outside researcher isolated from subject interaction. A cherry flavouring (Mio™, Northfield, IL, USA) was added to mask for taste, ensuring the double blind was maintained. To additionally blind taste, dextrose was added to both conditions; the same amount of dextrose was added to both trials to ensure that any additional effects could be isolated to CM consumption (Glenn, Gray, Wethington et al., in press). Finally, subjects wore a nose plug during consumption to confirm that no taste differences were discernable between trials.

Grip strength

Hand-grip testing measured isometric grip strength (Bohannon & Schaubert, 2005). All tests were assessed by a trained technician and measured in kg via a handheld dynamometer (Creative Health Products, Ann Arbor, MI, USA). All grip strength measurements were performed on the preferred hand with the subject standing, arm down at the side, wrist in neutral position, and the middle joint of the middle finger maintained at 90°. For the purposes of this study, preferred hand was determined as the hand used to hold the racket when hitting forehand groundstrokes. Participants maximally squeezed the dynamometer for 5 s as standard encouragement was provided. The test was repeated three times on the preferred hand with 60 s rest between attempts. Final isometric strength measurements were calculated as (a) average grip strength (the average measurements among the three attempts) and (b) maximal grip strength (greatest of the three attempts). High test–retest reliability
Vertical power

To assess vertical power, subjects first determined their vertical jump height via the Vertec™ Jump Trainer (Sports Imports, Columbus, OH). Subjects stood facing the apparatus, directly beneath and parallel to the plastic vanes. Without slouching and feet flat on the floor, subjects were instructed to raise their dominant hand, pushing through the furthest vane within reach to establish initial reach height. When jumping, subjects performed a counter-movement jump (no stepping or running starts were permitted). When ready, the subject jumped as high as possible, in line with the longitudinal axis of the apparatus, pushing their hand through the highest achievable vane. Subjects continued until they recorded two consecutive failed attempts (60 s rest was provided between jumps). A failed attempt occurred when the subject was unable to touch the next highest vane. Maximal jump height was calculated as the difference between final jump height and initial reach (Glenn, Vincenzo, Gray, & Binns, 2014). Maximal vertical jump performance was used to calculate average and peak vertical power based on the following, previously established equations (Johnson & Bahamonde, 1996):

1. Average vertical power (W) = 43.8·jump height (cm) + 32.7·body mass (kg) 16.8·participant height (cm) + 721
2. Peak vertical power (W) = 78.6·jump height (cm) + 60.3·body mass (kg) 15.3·participant height (cm) – 1245

After calculation of average and peak vertical power, the final products (measured in W) were divided by participant body mass (expressed as W/kg) and the same calculation was applied to peak power output in order to establish relative values. The 5 s power intervals were compared within and between trials to determine if subjects could better sustain power output throughout the 30 s test when consuming CM; this measure provides a more detailed perspective on performance than peak and/or average power independently. Anaerobic capacity includes the mean power output divided by participant body mass (expressed as W/kg). An additional measure, previously described as explosive power (peak power/time to reach peak power [reported in W/s]), was also calculated from Wingate data (Popadic, Barak, & Grujic, 2009). Explosive power is defined as, “the ability of the neuromuscular system to momentarily activate and reach initially maximal contractions until peak power is reached” (Popadic et al., 2009).

Blinding efficacy and side effects

After completion of each intervention trial, subjects were asked as which supplement they believed they had consumed. Subjects were also asked if they developed any side effects they believed were related to the ingested supplement.

Statistical analyses

Statistical Package for the Social Sciences (SPSS, version 22) was used to conduct all analyses. Normal distribution of data was assessed using histograms and boxplots. Descriptive statistics (means and standard deviations) were calculated for all data. To ensure that there were no differences in dietary consumption, paired t-tests compared individual macronutrients (carbohydrates, fats, and proteins) and total kilocalorie intake between trials.

For maximal and average grip strength, relative peak and average vertical power, anaerobic capacity, relative peak power, and explosive power, paired t-tests determined differences between trials. Statistical significance was set at \(\alpha = 0.05\) for t-test analyses. Power output was calculated for each 5-s interval throughout the cycling test and a 2 (trial) × 6 (time) repeated-measures analysis of variance was utilized to evaluate sustained relative power. For significant F-scores, post-hoc tests with the applied Bonferroni
correction \( (0.05/15 = 0.003) \) determined the appropriate \( \alpha \) level.

Finally, a \( 2 \times 2 \) Pearson chi-squared analysis indicated the ability of each participant to accurately determine which supplement they had consumed during the intervention trials \( (\alpha = 0.05) \). All values are reported as mean ± SD.

**Results**

No significant differences \( (p > .05) \) were observed for total kilocalorie or individual macronutrient (carbohydrates, fats, proteins) intake between trials, indicating that dietary intake had no effect on overall results. Of the 21 subjects recruited and consented, 17 completed all trials and as none of the subjects was considered outlier, all subjects were included in the final analyses.

When consuming CM, participants generated significantly greater maximal (Figure 1(a)) and average (Figure 1(b)) grip strength compared to PLA \( (p = .042 \text{ and } p = .045, \text{ respectively}) \). Relative peak and average vertical power were assessed via established calculations from vertical jump height and normalized based on participant body mass. No significant differences existed between CM and PLA trials for relative peak vertical power \( (p = .51; 69.9 \pm 5.3 \text{ and } 69.3 \pm 5.2 \text{ W/kg, respectively}) \) and these non-significant results were mirrored when evaluating relative average power \( (p = .51; 21.7 \pm 2.8 \text{ and } 21.4 \pm 2.6 \text{ W/kg, respectively}) \).

Anaerobic capacity, relative peak power, explosive power, and sustained power were evaluated via the Wingate cycling test. No significant differences \( (p \)
= .64) were observed between CM and PLA trials for anaerobic capacity (5.9 ± 0.9 and 5.8 ± 1.0 W/kg, respectively). In contrast, relative peak power (Figure 2(a)) and explosive power (Figure 2(b)) were significantly greater when consuming CM compared to PLA (p < .001 for both variables). For sustained relative power, a significant effect was observed for time within the trials (p < .001); however, no significant differences (p > .05) were observed between trials with regard to supplement consumed (Figure 3).

Pearson chi-squared analysis indicated that the participants were unable to accurately assess which supplement they had consumed based on a 2 (supplement guess) × 2 (accuracy) analysis (p = .49). Accurate guesses for the trials were recorded at 44%. None of the participants reported any side effects they believed were related to the supplement they had consumed.

**Discussion**

The purpose of this investigation was to examine the effects of acute CM supplementation on grip strength, vertical power, and anaerobic cycling performance in female MA tennis athletes. While CM had no beneficial effects on peak or average vertical power, CM may have increased performance with regard to maximal and average grip strength along with anaerobic cycling performance (explosive power and relative peak power). These are the first data evaluating CM supplementation in MAs and as females are underrepresented in sports and exercise medicine research (Costello, Bieuzen, & Bleakley, 2014), current results combine these populations in an effort to provide expanded insight to the use of ergogenic aids for increasing physical ability.

**Grip strength**

Tennis is a unique sport based on the multidimensional array of physiological factors required for success. One of these factors, grip strength, was improved from both maximal and average perspectives in female MA tennis players after acute consumption of 8 g CM. Although muscular strength was previously evaluated in younger individuals during sub-maximal exercise to failure, these are the first data demonstrating effects on maximal strength. Grip strength significantly relates to overall strength (Martin et al., 2006), along with reactive power, jump power, and speed (Girard & Millet, 2009). Additionally, grip strength is necessary to ensure proper contact between the racket head and ball (Kovacs, 2006) and when combined, these factors are beneficial for tennis performance. Grip strength is significantly greater in trained female athletes compared to their untrained counterparts, but this is still only 25% as strong as age-matched males (Leyk et al., 2007). The ability to acutely increase grip strength in female tennis players may provide an athletic advantage during competition, especially for those individuals competing in mixed-sex events (i.e. mixed-doubles) where physical discrepancies are most evident.

**Explosive and peak power**

Tennis involves a high degree of variability in ground stroke activity (i.e. speed, spin type, spin rate). In order to properly respond to this degree of variability, tennis players must have an explosive first step to properly return each stroke (Kovacs, 2006). During the anaerobic cycling test, significant increases were observed for explosive power when consuming CM compared to the PLA. This indicates acute supplementation of CM may minimize the time required to reach maximal power, suggesting that athletes may respond more quickly to the inconsistency of return shots. Relative peak power during the anaerobic cycling test was also significantly greater when consuming CM. The efficiency of the tennis serve in elite players is dependent on the use of stored energy in lower body musculature (Girard et al., 2005) and in tennis (similar to other ground sports), force production involves the transfer of ground reaction forces from the lower- to upper-body and eventually into the tennis racket (Chandler,
Our results are similar to a previous investigation (Bailey et al., 2015), where increases in power output were observed during a maximal sprint test (60 s) after supplementation with L-citruline, indicating L-citruline and CM may have similar effects on maximal sprint performance. Taken together, these data indicate acute consumption of 8 g CM may augment peak power generation while simultaneously decreasing the time required to reach peak power, potentially increasing performance in female tennis MA.

**Sustained power**

Interestingly, no differences were observed between trials for sustained anaerobic power. Previous research involving the effects on exercise performance indicates that CM is most effective during the latter parts of high-intensity exercise after the onset of fatigue (Glenn, Gray, Wethington et al., in press; Wax et al., 2014). The discrepancy in results may be due to the fact that (a) the cycling exercise was only 30 s in duration and may not have been long enough to elicit differences between trials or (b) the previous investigations used sub-maximal bouts to exhaustion with intermittent rest periods (the cycling test utilized in this investigation was one continuous bout). As a result, it appears for CM to be effective in mitigating performance decrements, intermittent, repeated exercise bouts are required when utilizing high-intensity exercise. Based on these results and taken together with previous literature, it appears that CM may have greater benefits for aerobic vs. anaerobic exercise; however, further research must be conducted.

**Side effects**

No side effects were reported by our subjects in reference to the supplement ingested. Although data are available suggesting that CM can elicit feelings of gastrointestinal discomfort (Perez-Guisado & Jakeman, 2010), there is a growing body of evidence to support the contrary (Glenn, Gray, Wethington et al., in press; Wax et al., 2014). More recent investigations utilizing CM supplementation indicate exogenous intake of up to 8 g is free from side effects in younger males (Wax et al., 2014) and females (Glenn, Gray, Wethington et al., in press) and our current data support this concept in female MA. Nevertheless, these data only support acute supplementation and it cannot be assumed that CM is free from harmful or uncomfortable side effects if consumed regularly. Longitudinal designs are necessary to determine if continual CM consumption exhibits the same outcomes.

**Physiological mechanisms and implications**

**In vivo**, L-citruline is available via exogenous (naturally found in watermelon) and endogenous (produced via conversion of L-arginine to NO) avenues (Curis et al., 2005). When compared to L-arginine (downstream counterpart in the NOS pathway), L-citruline is considered more beneficial for increasing NO production (Bescos et al., 2012; Curis et al., 2005). L-citruline is essentially a masked version of L-citrulline-arginine, avoiding hepatic metabolism as the liver is unable to uptake L-citrulline from portal circulation (Windmueller & Spaeth, 1981). The resultant increase in NO can increase vasodilation of the blood vessels allowing for an increased blood flow to the active musculature, enhancing the delivery of nutrients, augmenting contractile properties, and improving the repair of muscle fibres (Petrovic et al., 2008). With or without the combination with malate, L-citruline appears to provide significant benefit when utilized acutely as an ergogenic aid during exercise (Bailey et al., 2015; Glenn, Gray, Wethington et al., in press; Perez-Guisado & Jakeman, 2010; Wax et al., 2014).

The absence of plasma L-citrulline and L-arginine are a limitation of the current investigation, but previous data demonstrate the efficacy of exogenous L-citruline to increase plasma citrulline and arginine levels (van Wijck et al., 2014) as well as plasma nitrite levels (Bailey et al., 2015). Taken together with the results from the current investigation, it is plausible that the beneficial effects we observed in performance are attributed to these mechanisms for augmenting NO production. In order to elucidate these hypotheses, future investigations should measure levels of NO metabolites in conjunction with the exercise tests in an effort to further evaluate the mechanisms of action behind CM supplementation. Additionally, L-arginine has the potential to be converted to creatine in vivo (Paddon-Jones et al., 2004). This may also be a potential mechanism for the observed increases in performance and warrants further investigation.

A final note associated with this investigation includes the menstrual status of the subjects. The MA included in this investigation were at various stages within the menopausal continuum (pre- and/or peri-menopausal, n = 13; menopausal, n = 4) and future research should investigate the effects of L-citruline (with or without malate) in pre-, peri-, and menopausal females to determine if the magnitude in effectiveness differs among groups.
Conclusion

The results of this study indicate that acute supplementation of 8 g CM may be effective for increasing maximal grip strength and anaerobic cycling power in female MA. As acute CM supplementation may have increased maximal strength, peak power, and explosive power (presumably through increases in plasma L-citrulline and L-arginine concentrations), this indicates consuming 8 g CM before competition may provide strategic advantages for female, masters tennis players. Athletes should take into account prematch timing with regard to supplementation as the current data suggest that these results were observed after a 1h rest interval. Additionally, no side effects were observed which, when taken together with previous literature (Glenn, Gray, Wethington et al., in press; Wax et al., 2014), means that athletes can safely consume CM with no side effects. Important to note is this study did not determine length of effectiveness after supplementation. As such, future research should evaluate how long these beneficial effects are present after an acute dose to develop inmatch recommendations for ergogenic use. Finally, it is important to note this study utilized a controlled laboratory environment and data evaluating direct laboratory environment and data evaluating direct application to on-court performance is warranted.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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