

Acute Citrulline Malate Supplementation Does Not Improve Anaerobic Capacity in Healthy Young Adults: A Pilot Study

Original Research

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Abstract

Introduction: The aim of this study was to investigate the potential ergogenic properties of citrulline malate (CM) during a 300-yard shuttle run.

Methods: Thirty-two recreationally active subjects (M=24; F=8) participated in a randomized, placebo-controlled, double-blind study. Participants completed baseline testing which consisted of two 300-yard shuttle runs for time. Immediately post-exercise blood lactate was taken via a finger stick. An average of the two trials (time in seconds and blood lactate in mmols/L) were recorded and used for analysis. One week later, participants were randomly assigned to one of four groups (control, placebo, 4 grams CM, or 8 grams of CM) and repeated the same exercise protocol. Data were analyzed using a two-way repeated measures analysis of variance.

Results: No main effect for shuttle-run time ($F=0.149$; $p=0.702$) or shuttle run time by group interaction was observed ($F=0.672$; $p=0.576$). There was a main effect for blood lactate ($F=17.079$; $p<0.001$) with lactate accumulation during the pre-test (11.64 ± 2.83 mmol/L) being significantly greater compared to the post-treatment lactate levels (9.65 ± 1.94 mmol/L). There was no blood lactate by group interaction ($F=0.867$; $p=0.47$).

Conclusions: These results indicated that acute CM supplementation did not improve anaerobic performance in healthy, young adults.

Key Words: Shuttle Run, Blood Lactate, Nitric Oxide

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Introduction

The popularity of pre-workout nutritional supplements continues to grow among athletes and fitness enthusiasts. Citrulline malate (CM), an over-the-counter supplement, has received attention for its potential to improve exercise ¹⁻

⁴ and sports ^{4,5} performance. Studies report reduced muscle fatigue ⁶, improved aerobic energy production ⁷⁻⁹, and augmented force output from CM use ¹⁰⁻¹⁴.

The putative factors leading to improved performance from CM are increases in nitric oxide (NO) production ¹⁵, L-arginine bioavailability ¹⁶, and ATP production ³. These beneficial responses during exercise may be due to the synergistic combination of both L-citrulline and malate. L-citrulline is a nonessential amino acid that is synthesized by the conversion of L-arginine to NO when catalyzed by the enzyme NO synthase ⁵. Compared to L-arginine, the oral supplementation of L-citrulline avoids hepatic metabolism and is transported to the kidneys where roughly 80% can be directly converted to L-arginine ¹. This provides a more effective means of elevating plasma arginine concentrations thereby increasing NO levels. Malate is an intermediate of the Krebs cycle and may augment energy production and increase the rate of adenosine triphosphate (ATP) production during exercise and improve rates of phosphocreatine (PCr) recovery post-exercise ³. Therefore, an increased efficiency of ATP production in combination with an elevation in blood flow to skeletal muscle has the potential to improve exercise performance.

The majority of CM research has focused on strength performance involving upper ^{10-12,17-19} and lower body ^{6,10,13,20} musculature. Three recent reviews demonstrate small but significant improvements in muscle strength, endurance and power following an acute dose of CM ¹⁻³. As a result of these observed benefits, it has been suggested that CM supplementation could translate to enhanced on-field performance for competitive athletes. Due to the challenges associated with assessing on-field performance, one experimental approach is to employ simulated play and field tests. Currently there is little research examining the effects of CM on timed sports-specific drills or field tests like distance runs, jump tests, or sprint performance. Given the positive effects observed in exercise performance under controlled laboratory conditions, it's important that CM also be assessed using practical field tests. One common field test is the 300-yard shuttle run. A 300-yard shuttle run measures an athlete's anaerobic power and it effectively simulates on-field playing conditions ²¹. To our knowledge, no previous study of CM has been conducted using the 300-yard shuttle run.

The aim of this study is to examine the acute effects of CM on anaerobic capacity (run times and blood lactate) during a 300-yard shuttle run in healthy young men and women. The hypothesis for this study is that acute CM supplementation will produce faster shuttle run times (SRT) and reduce blood lactate (BLA) accumulation.

Methods

Participants

A convenience sample of thirty-two recreationally active subjects (M=24; F=8) were recruited from university physical education classes for this randomized, placebo-controlled, and double-blind study (Table 1). Before the investigation, subjects completed a health history questionnaire and signed a statement of informed consent. To qualify for this study (i.e., inclusion criteria), the subjects were classified as low-risk individuals as categorized by the American College of Sports Medicine ²². The exclusion criteria of the study included the following: (a) musculoskeletal problems, (b) metabolic disorders, (c) cardiorespiratory ailments, (d) blood disorders, (e) history of psychological disorders, (f) use of tobacco products, (g) consuming more than 10 alcoholic beverages per week, (h) taking medication, (i) use of over-the-counter supplements in the past 3 months. All experimental procedures were reviewed and approved by the university's Institutional Review Board before the initiation of the study.

Table 1. Participant Characteristics

Variable	(n = 32)
Age (y)	22.18 ± 3.53
Height (cm)	173.25 ± 12.0
Weight (kg)	73.87 ± 12.27
Body Fat%	17.48 ± 7.14

Data are Means ± SD

Protocol

Subjects reported to the laboratory on two separate occasions at the same time of day with testing being separated by seven days. Session 1 was used to obtain anthropometric data and to do baseline testing which consisted of two 300-yard shuttle runs for time each followed by a blood sample to assess lactate concentrations. Participants were asked to fast three hours prior to each trial and refrain from vigorous exercise for 24 hours before testing. For dietary control, participants were instructed to abstain from caffeine 12 hours prior and to replicate the first trial's dietary intake for the subsequent trial. A standardized warm up included dynamic movements to properly warm up the body before all

testing. Sub-maximal jumps, active and dynamic stretching, and dynamic motions emphasizing quadriceps and hamstrings as agonistic muscle groups were included in the warmup. All participants experienced an identical warmup protocol prior each experimental trial. During session 2, participants were randomly assigned to one of four treatment groups (control=CON, placebo=PLA, 4 grams CM, or 8 grams of CM) and repeated the same exercise protocol.

Testing Procedures

The 300-yard shuttle run measures the functional ability of athletes, specifically anaerobic capacity 21. The test was performed on a gymnasium floor. Two parallel lines were marked with tape on the floor 25 yards apart. The participants began in pairs at the starting line on a whistle of the timekeeper and ran 12 lengths at maximum speed ensuring that foot contact was made on the start line and the 25-yard line when changing directions. A timekeeper recorded run times by hand using a stopwatch. Immediately after each trial, BLa was sampled via a single-use lancet device. The first flow of blood was wiped away, and then approximately 5 ul (2 mm) of blood was loaded on a lactate strip and immediately analyzed using the Lactate Pro Analyzer (ARKRAY, Inc, Shiga, Japan). A rest interval of 5 minutes was given between trials. After both trials were complete, SRT and BLa for each subject were averaged and reported to the nearest 10th of a second and millimoles per liter, respectively.

Supplementation

Subjects were randomly assigned to one of four treatments: 4g of CM (PrimaForce, Burlington, NC, USA), 8g of CM (PrimaForce, Burlington, NC, USA), PLA, or CON. The CM treatment contained 4g or 8g of CM mixed into 20 oz of sugar-free flavored water (Great Value; Wal-Mart Stores, Inc., Bentonville, AR, USA). The PLA treatment contained 20 oz of sugar-free flavored water. The CON treatment contained no administration of a drink. Subjects consumed all treatments 45 minutes prior to the 300-yard shuttle run.

Statistical Analysis

Separate two-way repeated measures analyses of variance (ANOVA) were performed to analyze group (CON, PLA, CM 4g, CM 8g) by time (pre, post) interactions for 300-yard SRT and BLa. Statistical software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) was used to perform all statistical analyses. When appropriate, follow-up paired t-tests were utilized for post hoc comparisons. An alpha level of $p < 0.05$ was considered statistically significant for all comparisons. All data are reported as mean \pm standard deviation (SD).

Results

Results of the statistical analysis revealed no main effect for SRT ($F=0.149$; $p=0.702$), and no interaction between time and group ($F=0.672$; $p=0.576$). The effect sizes for pre and post-treatment SRT were determined to be small for all four groups. Table 1 shows the means, SDs, confidence intervals, and effect sizes by the group assignments for SRT. For BLa a main effect was observed ($F=17.079$; $p<0.001$) with the pre (baseline) BLa level (11.64 ± 2.83 mmol/L) being significantly greater compared to the post-treatment BLa level (9.65 ± 1.94 mmol/L). There was no interaction between BLa and group ($F=0.867$; $p=0.47$). Calculation of Pearson product-moment correlation coefficients determined the pre and post-treatment for lactate levels had a medium effect size for the CM 8g group, while the effect sizes for CON, PLA, and CM 4g groups were small. Table 2 shows the means, SDs, confidence intervals, and effect sizes by the group assignments for BLa.

Table 1. Means, standard deviations, confidence intervals, and effect sizes for SRT.

Group	Treatment	$\bar{x} \pm SD$ (sec)	95% CI	r
Control	Pre	71.44 \pm 5.78	66.76 – 76.12	-0.03
	Post	71.75 \pm 5.98	66.86 – 76.64	
Placebo	Pre	70.00 \pm 6.42	65.32 – 74.68	0.03
	Post	69.56 \pm 6.85	64.68 – 74.45	
CM 4g	Pre	68.14 \pm 7.10	63.14 – 73.14	-0.03
	Post	68.61 \pm 8.52	63.39 – 73.84	
CM 8g	Pre	69.56 \pm 6.55	65.15 – 73.97	0.08
	Post	68.56 \pm 5.70	63.95 – 73.16	

Table 2. Means, standard deviations, confidence intervals, and effect sizes for BLA.

Group	Treatment	$\bar{x} \pm SD$ (mmol/L)	95% CI	r
Control	Pre	10.80 \pm 3.26	8.70 – 12.90	0.34
	Post	8.80 \pm 2.15	7.41 – 10.19	
Placebo	Pre	11.45 \pm 3.02	9.35 – 13.55	0.20
	Post	10.45 \pm 1.64	9.06 – 11.84	
CM 4g	Pre	11.81 \pm 2.11	9.57 – 14.06	0.42
	Post	10.06 \pm 1.71	8.57 – 11.55	
CM 8g	Pre	12.42 \pm 2.98	10.44 – 14.41	0.51
	Post	9.37 \pm 2.09	8.05 – 10.68	

Discussion

The purpose of this research was to determine if CM supplementation prior to a 300-yard shuttle run would augment anaerobic exercise performance. Our results demonstrate CM provided no beneficial effects on SRT or BLA levels in healthy young adults. Previous research investigating CM use in other field tests for power (vertical jump), muscle strength (grip strength) and muscular endurance (chin-ups, push-ups) have yielded mixed results. One hour after female tennis players ingested 8g CM, no benefits were found in vertical jump performance (average or peak power) when compared to PLA⁴. However, in the same study, maximal grip strength was significantly greater in the CM group. This contrasts with Edwards et al. who found 7-days of citrulline supplementation (6g/day) had no beneficial effect on maximal grip strength in a group of 16 older physically active women²³. These authors also found no improvements in several other functional performance measures like sit-to-stand and a 6-min walk test. Wax et al¹³ had 14 resistance-trained males perform three sets with repetitions to fatigue for chin-ups, reverse chin-ups, and push-ups. Supplementation included 8g CM, 1-hour before exercise. Total number of repetitions for each exercise was significantly greater when supplementing with CM.

Our data suggests no improvement in anaerobic performance following CM ingestion. This is in agreement with Bezugulov et al. who had 18 elite adult soccer players consume a single dose of either 3g or 6g CM 1-hour prior to performing a field-based soccer test²⁴. No differences were found in physical performance between groups or when compared to PLA. It appears that supplementing with CM before strength-based tests may provide favorable outcomes compared to sustained anaerobic power that's required in the 300-yard shuttle run or other sports-specific tests. CM supplements are also marketed to improve muscle performance through a reduction in BLA concentration, however, most research does not support this assertion^{6,13,20,25}. In our study, we did not detect differences in BLA between the 4g and 8g CM trials or when compared to CON or PLA. A recent systematic review and meta-analysis of the effects of citrulline on BLA by Rhim and colleagues support our findings²⁵.

Several limitations of this study do exist. For this study, participants were physical education students, not athletes as indicated by their above average body fat percentage and SRTs. Fitness of our subjects may have influenced the results. Individuals typically have better results during subsequent repeated trials of a physical test due to a learning effect. However, a 300-yard shuttle run is an all-out effort and participants know it must be repeated with a short rest period. Unfortunately, this may cause participants to pace themselves, knowing there was another intense effort on the way during the second trial of the shuttle run. This might explain why we found significantly lower post-treatment BLA levels across all groups compared to baseline. It is unknown if pacing played a role in our results but should be a consideration for future research incorporating the 300-yard shuttle run.

Also, the pilot study contained a small number of subjects within each treatment group which might have impacted the study results. Finally, as mentioned previously, subjects recruited in this study were healthy but not competitive athletes. Therefore, the anaerobic capacity of our subjects may have limited the benefit of CM supplementation. The 300-yard shuttle run severely taxes the glycolytic system so healthy participant may simply lack the metabolic specificity when compared with anaerobic athletes. For example, Farney et al.⁶ recruited 12 participants who were involved in structured exercise for six months (recreationally trained). In their study, supplementing with 8g of CM was ineffective in reducing fatigue or BLA during high intensity resistance exercise.

Conclusions

The 300-yard shuttle run is an easily administered useful measure of anaerobic capacity. However, the results of this study indicate that supplementation of either 4g or 8g CM 45 minutes prior to exercise did not yield faster run times or reduced BLA concentrations. Based on our findings, we cannot support the acute use of CM for improved exercise or sports performance in a healthy, active population.

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