Acute Citrulline Malate Supplementation Does Not Improve 1-km Time-Trial Performance in Trained Female Kayak Paddlers

Original Research

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Abstract

Introduction: Citrulline malate (CM) has been reported to enhance performance during resistance training and high-intensity exercise; however, its ergogenic effects during kayak exercise have not been investigated. This study examined the effects of acute CM ingestion on 1-km kayak ergometer time-trial (TT) performance and ratings of perceived exertion (RPE) in trained female youth kayakers.

Methods: Fifteen trained female youth kayakers (age: 16.3 ± 1.1 y; body mass: 55.5 ± 6.7 kg; height: 164.3 ± 3.7 cm) completed three conditions in a randomized, double-blind, placebo-controlled crossover design: CM (8 g), placebo (PL; 6 g citric acid), and a control condition (CON; no supplement). Participants ingested CM and PL 60 min before a 1-km kayak ergometer TT. RPE (6–20) was recorded immediately upon TT completion.

Results: The TT completion time differed across conditions ($p = 0.001$; $\eta p^2 = 0.44$). Compared with CON (270.8 ± 5.31 s), TT was faster following CM (262.6 ± 4.69 s; $p = 0.016$) and PL (261.8 ± 4.55 s; $p < 0.001$), with no difference between CM and PL ($p = 1.000$). The RPE also differed across conditions ($p = 0.025$; $\eta p^2 = 0.23$); however, Bonferroni-adjusted pairwise comparisons did not reach statistical significance (CON vs CM: $p = 0.080$; CON vs PL: $p = 0.107$; CM vs PL: $p = 1.000$).

Conclusion: Acute CM supplementation did not improve 1-km kayak ergometer TT performance compared with placebo in trained female youth kayakers. The faster times observed in both CM and placebo compared with control may reflect non-specific effects (e.g., placebo/taste or pre-trial routines) and warrant further investigation.

Key Words: exercise performance, nitric oxide, ergogenic aids

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Introduction

L-citrulline is a non-essential amino acid found abundantly in watermelon[1] and sold as citrulline malate (CM) in the sports nutrition market with a 1:1 and 2:1 ratio.[2,3] In the last decade, sports nutrition scientists have conducted several

studies on CM that collectively suggest improvements in exercise performance.[3-5] The possible ergogenic effects of CM are mediated by its capacity to increase nitric oxide (NO) level.[3]

L-citrulline is an endogenous precursor of arginine through conversion by lyase and argininosuccinate synthase, providing substrate for NO synthesis via nitric oxide synthase (NOS).[6] L-citrulline is a more efficient substrate for elevating L-arginine concentrations and NO bioactivity than its supplementation.[7] At higher NO levels, L-citrulline could increase muscle blood flow, oxidative substrates, and gas exchange, and enhance muscle contractility.[8] Moreover, L-citrulline could facilitate ammonia buffering capabilities in the liver as a urea cycle component, improve pyruvate's oxidative utilization, and decrease lactate production.[9] All these physiological mechanisms related to CM supplementation may lead to delayed muscle fatigue,[10] improve oxidative ATP production,[11] and attenuate ratings of perceived exertion (RPE) when used to measure effort, exertion, breathlessness, and fatigue during strenuous exercise.[9]

While several studies have investigated acute CM supplementation during high-intensity efforts[12-14] and during resistance training with equivocal results,[2,3,12,15] few studies have examined cardiorespiratory endurance exercise modalities.[5,16] Cardiorespiratory endurance includes all sports modalities in which ATP is mainly resynthesized via oxidative phosphorylation.[17] Maximal oxygen consumption (VO_2max) reflects the maximum capability of the pulmonary, cardiovascular, and muscle systems to take up, transport, and utilize oxygen, predominantly in contracting muscle mitochondria.[18] However, the minimum intensity corresponding to VO_2max is maintained at approximately 5 minutes,[17] the maximum effort with a duration longer than 1 minute mainly depends on oxidative metabolism, known as endurance-intensive efforts.[19] Various studies have assessed the effects of CM on cardiorespiratory endurance.[20-24] In this regard, a study using Bruce's graded treadmill test reported that neither pre-exercise watermelon juice nor L-citrulline supplementation (6g) increased time-to-exhaustion (TTE) performance.[25] Gliss et al[20] utilized a submaximal intensity test to assess the acute effect of CM supplementation (8g) during a cycling TTE protocol at 90% of peak oxygen consumption (VO_2peak) on recreationally active males. However, the study did not demonstrate any significant ergogenic effects of acute CM supplementation under these conditions. Bailey et al[21] also did not report any ergogenic effect of L-citrulline-rich watermelon juice (~3.4 g/d for 16 days) in recreational athletes during a cycle ergometer at 70% VO_2peak. Stanelle et al[22] found a reduction of more than 5% in time to cover during the 40-km time trial (TT) in trained cyclists, whereas another study failed to find significant differences in half-marathon performance after acute ingestion of watermelon juice enriched with 3.45 g of L-citrulline compared with a PL in recreational runners.[26] Regarding endurance-intensive efforts, Hickner et al[4] reported that 24 h of supplementation with L-citrulline (9 g) and an acute supplement intake 3 hours before graded treadmill running improved TTE performance in 17 young active males and females. In another study, 7 days of citrulline supplementation at 2.4 g/day improved 4-km cycling TT performance by 1.5%.[23]

However, to our knowledge, no studies have yet been conducted on kayak TT performance following CM supplementation. Olympic flat-water kayak is a popular sport in 500-m and 1000-m races, with about 86% of energy derived from oxidative metabolism during a 1000-m kayak match.[24,27] To enhance kayak exercise performance, a few studies have examined ergogenic substances such as creatine monohydrate[28], β-alanine[29], and beetroot juice (BRJ) during kayak TT performance.[30,31] As a NO-boosting ergogenic aid, BRJ effects show discrepancies. In this regard, Peeling et al[30] found an increased performance during a 500-m TT in international female kayakers and a trend to significant effect (with enhanced performance in 5 of 6 participants) in work done during a 4-min kayak ergometer test after 70 mL BRJ supplementation (4.8 mmol nitrate) in international male kayakers, whereas Muggeridge et al[31] found no ergogenic effects of BRJ on TT performance during a 1-km kayak ergometer test after 70 mL BRJ in trained male kayakers. Given the limited studies investigating the ergogenic effects of CM during kayak exercise, this study aimed to determine whether acute ingestion of 8 g CM improves 1-km kayak ergometer TT performance in trained female kayakers. The primary hypothesis was that CM would reduce 1-km TT completion time compared with placebo (PL) and control (CON). The secondary hypothesis was that CM would reduce post-trial RPE compared with PL and CON. An acute 8 g CM dose was selected because 6–8 g is one of the most commonly used acute bolus doses in the adult CM performance literature and reviews[2,3], facilitating methodological alignment and comparison across studies. Based on the athletes' mean body mass (~55.5 kg), this dose corresponds to ~0.14 $g \cdot kg^{-1}$. Oral L-citrulline is generally well tolerated in humans, with few adverse events reported in exercise studies, and gastrointestinal discomfort being the most commonly reported minor complaint when it occurs;[25] tolerability has also been reported for single oral doses up to 15 g in healthy volunteers.[32] Given limited supplementation data in youth athletes, CM was administered as a single, supervised acute dose following health screening and exclusion of recent ergogenic aid use.

Methods

Participants

Fifteen female youth national-team kayakers with a minimum of four years of structured training experience (age: 16.3 ± 1.10 years; body mass: 55.5 ± 6.7 kg; height: 164.3 ± 3.7 cm) were recruited for this study. Participants were healthy and free of injury, as determined by a health screening questionnaire, and had not used ergogenic aids (e.g., creatine, β-alanine, or NO–boosting supplements) for at least 3 months prior to participation. All participants were informed of the study procedures, potential risks, and benefits before providing written informed consent. For participants under 18 years of age, written informed consent from a parent or guardian and participant assent were obtained. The Islamic Azad University of Borujerd Ethics Committee approved the study (IR.IAU.B.REC.1401.04.02). Training age was not quantified beyond the eligibility criterion (≥4 years), and biological maturation status (e.g., maturity offset/peak height velocity) was not assessed; these factors may contribute to inter-individual variability in supplement responsiveness in youth athletes.

An a priori power analysis for this crossover design was not performed because no published data were available on 1-km kayak ergometer time-trial responses to CM in trained female youth athletes, limiting the defensibility of assumptions about the expected effect size and within-subject correlation. Therefore, a pragmatic recruitment strategy was used, and all eligible athletes from the national youth program who met the inclusion criteria were enrolled (n=15). Because each participant completed all experimental conditions, the crossover design reduces between-subject variability and improves statistical efficiency compared with a parallel-group design. Effect sizes and 95% confidence intervals are reported alongside p-values to aid interpretation.

Protocol

In a randomized, double-blind, placebo-controlled study design, participants, who were experienced in the use of a kayak ergometer as a performance measurement tool, attended the laboratory four times (Figure 1). Visits comprised one familiarization session and three experimental sessions, including CM, PL, and CON, with a one-week washout between visits. During the familiarization session, participants were informed of the test procedure and performed the exercise test protocol once.

Participants, outcome assessors, and the investigator supervising testing were blinded to the supplement condition during the CM and PL sessions. Participants ingested CM and PL in identical volumes (200 mL) 60 min before the 1-km time trial, and the PL was selected to match the taste/acidity profiles. The CON session involved no supplementation; therefore, blinding was not applicable.

The trials were administered to each participant on the same day and at the same time to minimize circadian rhythm effects. Before the familiarization session, the participants were instructed to record their dietary intake for 24 hours. During the experimental trials, they had to replicate their dietary intake as recorded in the food diaries. They were asked to avoid caffeine-rich beverages and coffee 24 h before the test and strenuous exercise 48 h before each session. The Hooper index questionnaire was used in each session to monitor fatigue.[33,34]

Experimental Session

Participants arrived at the laboratory one hour before starting the test. They ingested 8 g of CM (BULK SUPPLEMENTS, Henderson, NV, USA) at a 2:1 L-citrulline-to-malic acid ratio, or 6 g of citric acid as PL dissolved in 200 mL of water.[15] Citric acid was chosen to reproduce the taste of CM as used in previous studies.[15] Each participant completed a 1-km fixed-distance TT on a kayak ergometer (KayakPro SUPErgo, Miami, FL, USA) 1 hour after ingestion. The ergometer drag setting was adjusted for youth kayakers via a flywheel damper set to 1.5 to provide appropriate resistance for female youth lightweight kayakers. Each test was preceded by enough time for general and specific warm-ups,[29] and accompanied by strong verbal encouragement. The RPE (6–20) was measured at the end of the test. Participants were queried for adverse events (e.g., gastrointestinal discomfort, headache, dizziness) during each visit and were instructed to report any delayed symptoms within 24 h of ingestion.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Normality of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test. Differences among conditions (CM, PL, CON) were analyzed using one-way repeated-measures ANOVA. When a significant main effect was observed, pairwise comparisons were performed with Bonferroni adjustment, and exact adjusted p-values are reported. Effect sizes for

main effects were reported as partial eta squared (ηp^2). All analyses were conducted in SPSS (Version 21; SPSS Inc., Chicago, IL, USA).

Results

The 1-km TT completion time showed a significant effect of condition ($F = 11.0$, $p = 0.001$; $\eta p^2 = 0.44$; Figure 1). Post hoc comparisons (Bonferroni-adjusted) indicated faster completion times in both the CM condition (262.6 ± 4.69 s; $p = 0.016$) and the PL condition (261.8 ± 4.55 s; $p < 0.001$) compared with CON (270.8 ± 5.31 s). There was no difference between CM and PL ($p = 1.000$).

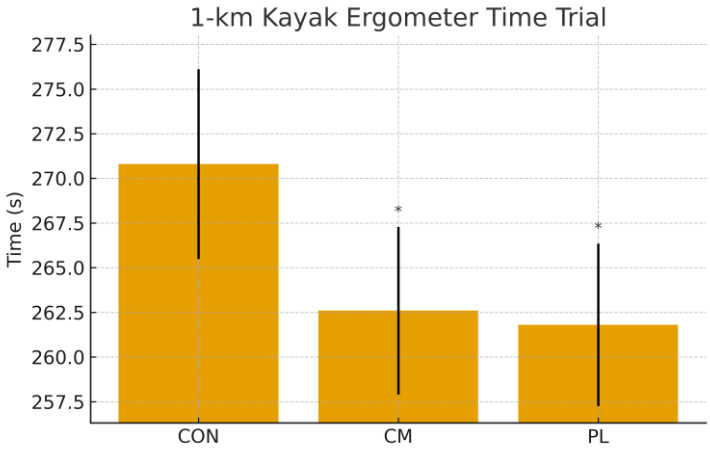

Figure 1. 1-km kayak ergometer time-trial completion time (s) across conditions in trained female youth kayak paddlers (n=15). Bars represent mean ± SD. Conditions: CM = citrulline malate (8 g, 60 min pre-trial), PL = placebo (6 g citric acid, 60 min pre-trial), CON = control (no supplement). One-way repeated-measures ANOVA: $p = 0.001$ ($F = 11.0$). Bonferroni-adjusted pairwise comparisons: CM vs CON $p = 0.016$; PL vs CON $p < 0.001$; CM vs PL $p = 1.000$. * indicates $p < 0.05$ vs CON (Bonferroni-adjusted). Asterisks are displayed above the CM and PL bars.

The RPE showed a significant effect of condition ($F = 4.21$, $p = 0.025$; $\eta p^2 = 0.23$; Figure 2). Although mean RPE was lowest in CON (17.9 ± 0.30), post hoc comparisons (Bonferroni-adjusted) did not reach statistical significance versus CM (18.6 ± 0.31; $p = 0.080$) or PL (18.6 ± 0.30; $p = 0.107$). No difference was observed between CM and PL ($p = 1.000$). Accordingly, no significance symbols are shown in Figure 2.

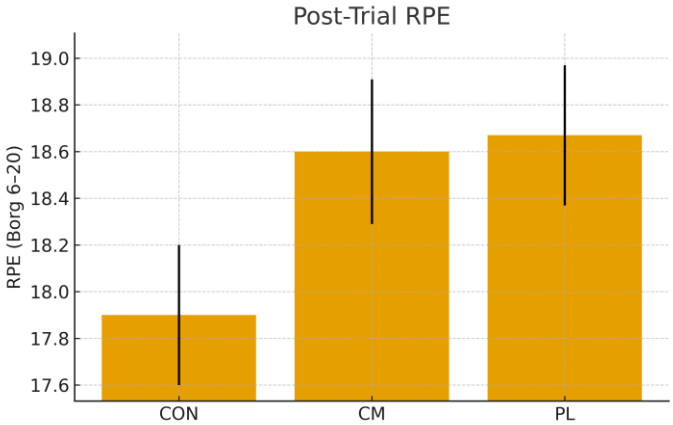

Figure 2. Ratings of perceived exertion (RPE; Borg 6–20) recorded immediately after the 1-km kayak ergometer time trial across conditions in trained female youth kayak paddlers (n=15). Bars represent mean ± SD. Conditions: CM = citrulline malate (8 g, 60 min pre-trial), PL = placebo (6 g citric acid, 60 min pre-trial), CON = control (no supplement). One-way repeated-measures ANOVA: $p = 0.025$ ($F = 4.21$). Bonferroni-adjusted pairwise comparisons: CON vs CM

p = 0.080; CON vs PL p = 0.107; CM vs PL p = 1.000. No pairwise comparisons were significant; therefore, no significance symbols are shown.

Discussion

The main findings of this investigation were that CM supplementation significantly improved 1-km kayak ergometer performance compared with CON, but not with PL treatments. Despite a small positive effect, it did not have a significant impact on RPE. However, similar results were observed following PL supplementation, with significantly improved performance and greater RPE compared with CON.

To examine the effects of NO precursor supplements on kayak performance, Peeling,[30] in contrast to our findings, reported a significant reduction in the time to cover during 500-m TT kayak performance in female-trained athletes after BRJ supplementation. Conversely, Muggeridge et al[31] reported no ergogenic effect of BRJ supplementation on 1-km TT performance in trained kayakers. These contrasting BRJ findings underscore that kayak-specific ergogenic outcomes may depend on race distance (e.g., 500 m vs 1-km), testing modality (on-water vs ergometer), and participant characteristics (e.g., sex and training status), which should be considered when interpreting and comparing supplementation studies in kayakers.[30,31] While this study did not observe an ergogenic benefit of acute CM supplementation compared to PL in trained female youth kayakers, other studies have assessed the effect of CM supplementation during endurance-intensive efforts. Bailey et al[35] reported that CM supplementation significantly improved performance following a 60-second all-out sprint in recreationally active men. Other studies also reported that CM supplementation improved TTE performance during a gradual running test[4] and a 4-km cycling TT.[23]

Cardiorespiratory endurance performance is mediated by VO_2max economy and the ability to sustain a high fraction of VO_2max during long periods before blood lactate concentration increases.[36] Previously, studies that assessed the effect of CM supplementation during gradual exercise tests failed to modify VO_2max.[4,25] At the same time, a meta-analysis reported a non-significant effect of CM supplementation on running economy, with no ergogenic effects of CM on VO_2 kinetics in endurance efforts.[37] The NO concentrations, however, were not measured in this investigation. The dosage and timing used in the present study were within a range reported to increase NO levels in previously published studies.[38] Therefore, it could be speculated that an increase in NO levels provokes vasodilation and an increase in muscle blood flow, promoting the transfer of oxygen and oxidative substrates (i.e., glucose) to the active muscle, with possible reduced VO_2 and efficiency at submaximal intensities in cardiorespiratory sport modalities.[17] Nevertheless, the absence of ergogenic effects in the current and other studies related to CM supplementation in endurance-intensive efforts[20,21] may be that due to the training status of the athletes, they already possess the metabolic adaptations to pathways that CM supplementation mimics (e.g., higher NO levels, reduced VO_2 during exercise, PCr sparing).[39] Indeed, similar null effects have been noted following BRJ supplementation and passive heat exposure in elite athletes.[40,41] Although CM and BRJ are both often framed as NO-related ergogenic strategies, they act via distinct upstream pathways. L-citrulline and L-arginine availability can be elevated by CM to support NOS-dependent NO synthesis.[7] In contrast, BRJ provides dietary nitrate, which can be reduced to nitrite and then to NO via the nitrate–nitrite-NO pathway.[39] These mechanistic differences may contribute to divergent outcomes across protocols; additionally, BRJ effects in trained athletes may be more sensitive to dosing strategy (including multi-day loading) to induce physiologically small but competitively meaningful performance changes.[42,43]

Youth training age and maturation status may have confounded responses to CM in the present study. Although all participants were trained, adolescence is characterized by substantial inter-individual variability in biological maturation even within a narrow chronological age range, which can influence neuromuscular capacity and physical performance.[44-46] These maturation-related differences may also influence fatigue tolerance and internal load responses (including perceived exertion).[47-49] Maturation-related differences may additionally impact physiological pathways relevant to CM (e.g., NO bioavailability and endothelial/vascular responses), potentially contributing to heterogeneous responses across individuals.[50,51] Future studies in youth athletes should quantify training age and training volume and incorporate an indicator of biological maturity (e.g., maturity offset/years from peak height velocity) to enable stratified analyses or covariate adjustment.

No significant differences were found between CM and PL for either 1-km TT or RPE, suggesting expectancy and placebo effects, likely mediated by supplement taste, similar to previous work on sprint and power performance.[52] Efforts were made to taste-match CM and PL supplementation, which may have contributed to the ergogenic effects observed in both conditions compared to CON.[53] Citric acid, used as the taste-matched PL, can potentially increase intracellular citrate levels in animal models,[54] affecting downstream metabolism related to energy availability. However,

the supplemented dose was unlikely to be either ergogenic or inhibitory.[54,55] Taste responses can also be highly individual, potentially increasing variance within a dataset but allowing for individualization of supplementation strategies in practice.[53]

Participants were deemed appropriately familiar with the 1-km protocol, as it formed part of their regular training and testing cycles, so learning effects due to insufficient familiarization can be discounted. This is supported by mean RPE values that lie within 1 AU across all conditions, indicating that participants exerted near-maximal effort in each trial (Figure 2). Compared to PL and CM conditions, the lower RPE value in CON suggests a placebo effect contributing to the total intervention effect.[56] We also acknowledge that short-duration, high-intensity aerobic activity of similar durations has required more trials to demonstrate familiarization.[57] However, this was impractical in the current study due to the additional time it would have taken away from athletes' training. Finally, because maturation status and detailed training history were not quantified, we could not determine whether these factors influenced individual responses to CM versus placebo; this should be addressed in future adolescent supplementation studies.

Conclusion

In trained female youth kayakers, acute CM ingestion (8 g, 60 min pre-exercise) did not improve 1-km kayak ergometer time-trial performance or post-trial RPE compared with the taste-matched placebo. Although both CM and placebo were faster than the no-supplement control, this pattern may reflect non-specific effects (e.g., expectancy/taste and/or differences in pre-trial routines) rather than a CM-specific ergogenic benefit.

Future research should (a) include larger samples and quantify training age and biological maturation, (b) test alternative dosing strategies (e.g., multi-day loading) and measure mechanistic markers (e.g., NO-related biomarkers), and (c) examine kayak-relevant outcomes across different distances and modalities (on-water vs ergometer) to determine whether any CM effects are context-dependent.

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Conflicts of Interest. The authors declare no conflicts of interest.

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