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Acute citrulline-malate supplementation is ineffective during aerobic cycling and subsequent anaerobic performance in recreationally active males

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Abstract

Citrulline-malate (CM) purportedly increases exercise performance through increased nitric oxide production. The effects of CM on muscular strength performance are well-documented; however, the benefits of CM on aerobic and anaerobic biking performance are not well researched. Therefore, the present investigation examined the acute CM supplementation effects on aerobic and anaerobic cycling performance in recreationally active males. *Methods*: 28 recreationally active males (20.9 ± 2.8 years) completed randomized, double-blind, crossover trials consuming CM (12g dextrose + 8g CM) or a placebo (12g dextrose). Participants performed an aerobic cycling protocol (time-to-exhaustion [TTE]), followed by a subsequent 30-second Wingate cycling test, 60-minutes after supplement consumption. *Results*: Dependent t-tests showed no significant differences ($p > 0.05$) for TTE (PLA: 315.4 s ± 137.7 s; CM: 314.1 s ± 107.1 s) and Total Work Completed (TWC) (PLA: 74.7 ± 34.1 kilojoules (kJ); CM: 74.1 ± 26.4 kJ) during the aerobic cycling protocol. Dependent t-tests also showed no significant differences ($p > 0.05$) for mean watts (PLA: 586.1 ± 87.7 Watts (W); CM: 588.0 ± 93.0 W), peak watts (PLA: 773.0 ± 136.7 W; CM: 786.7 ± 133.0 W), and fatigue index (PLA: 12.9 ± 6.4 FI; CM: 14.3 ± 7.2 FI) during the Wingate protocol. Repeated-measures ANOVA results indicated a significant effect between each 5 s interval ($p < 0.001$), but no differences were observed between trials ($p > 0.05$). *Conclusion***:** Acute CM supplementation in recreationally active males provides no ergogenic benefit in aerobic cycling performance followed by an anaerobic cycling test.

Keywords: Citrulline-Malate; Ergogenic Aids; Cycling; Nitric Oxide

Introduction

Athletes are consistently looking for an edge to achieve peak performance during competition. Although important, daily dietary habits may not be adequate when competing at high levels of athletic performance. As a result, many individuals employ the use of ergogenic aids, substances, or devices to improve exercise performance (Bucci, 1993).

One particular category of ergogenic aids that has recently increased in popularity involves those purported to increase nitric oxide (NO) production. NO is an important modulator of blood flow and mitochondrial respiration during physical exercise (Shen et al., 1994). It is also suggested that the increase in blood flow derived from NO synthesis may improve recovery processes of the activated tissue. However, it is challenging to supplement with NO itself because it is a gas with a short half-life (Bloomer, 2010). Thus, athletes and researchers have used intermediates to the NO pathway as potential supplements. One such precursor is L-arginine; however, when dietary L-arginine is consumed, it is metabolized in the liver reducing its bioavailability. Thus, alternatives for increasing NO bioavailability are being sought. L-citrulline is another NO intermediate that is directly converted to L-arginine, avoiding hepatic metabolism, theoretically making this substance more effective at increasing vasodilation resulting in enhanced athletic performance. L-citrulline – an organic compound and non-essential amino acid naturally found in watermelon – has been promoted as an effective and legal means of increasing athletic performance (Rimando & Perkins-Veazie, 2005).

Due to the perceived ergogenic benefits of L-citrulline, the volume of research involving supplementation has increased substantially in recent years (Suzuki et al., 2016; van Wijck et al.,

2014; Bailey et al., 2015). Acute ingestion of oral L-citrulline has elicited positive increases in plasma citrulline/arginine levels (van Wijck et al., 2014), plasma nitrite (Bailey et al., 2015), and resulted in blood pressure improvements, VO_2 kinetics, and enhanced time to exhaustion while increasing the total work completed during high-intensity exercise compared to placebo (PLA) (Bailey et al., 2015). Additionally, previous research has determined long-term L-citrulline supplementation reduced time to complete a cycle ergometer exercise trial (Suzuki et al., 2016). However, acute supplementation has produced equivocal results on overall exercise performance (Cutrufello et al., 2014; Hicker et al, 2006;) indicating the need for further investigations into the efficacy of short-term use.

Unlike L-citrulline, supplementation of citrulline-malate (CM) has demonstrated positive ergogenic effects on acute exercise performance (Glenn et al., 2017; Perez-Guisado, 2010; Wax, Kavazis, Weldon, & Sperlak, 2015). The inclusion of malate, an intermediate of the Krebs cycle, appears to increase aerobic energy availability due to an increase in ATP production rates, blood flow, and mitochondrial respiration during exercise (Bendahan et al., 2012; Shen et al., 1994). L-citrulline and malate combined could be the underlying reason for physiological changes leading to improved physical performance. Recently, researchers found an acute dose (8 g) of CM increased resistance exercise performance to exhaustion for upper- and lower-body musculature when lifting at 80% and 60% of one-repetition maximum in resistance trained males and females, respectively (Glenn et al., 2017; Wax, Kavazis, Weldon, & Sperlak, 2015). An increase in total number of sets, repetitions completed, as well as a 40% reduction in muscle soreness at 24 hours and 48 hours post-training in resistance trained individuals was also observed during acute CM supplementation (Perez-Guisado, 2010). Conversely, Cunniffe and colleagues (2016) found that 12 g of CM failed to improve multiple bouts of anaerobic cycling

performance and subsequent aerobic cycling capacity in well-trained men. This outcome could be due to the rigorous protocol, 10 x 15-seconds (s) maximal cycle sprints with 30 s rest followed by a time-to-exhaustion test at 100% of individual peak power, in well-trained men. Overall, CM demonstrated ergogenic potential for athletes engaged in high-intensity anaerobic exercises with short rest periods (weightlifting, Wingate, sprints, etc.), as well for any athlete seeking to reduce muscle soreness and improve recovery rates (Perez-Guisado, 2010).

There is evidence that acute CM ingestion may improve anaerobic exercise and long-term supplementation with L-citrulline improves aerobic performance, but no performance data exist on the acute effects of CM on subsequent anaerobic performance after a maximally exhaustive bout of aerobic exercise. This concept is applicable to athletes that sprint to the finish of races after an extensive stretch of aerobic exercise. Therefore, the purpose of this study was to investigate the effects of CM on short, high intensity exercise performance after a preliminary bout of exhaustive endurance exercise in young, healthy males. Based on purported CM mechanisms, we hypothesized CM supplementation would augment total work completed (TWC) total time to complete the Time-To-Exhaustion (TTE) test. Additionally, we hypothesized that CM would increase mean and peak power while decreasing fatigue during the Wingate cycling test.

Methods

Subjects

A total of 28 young (20.9 ± 2.8 years) males participated in this study. Inclusion criteria consisted of the following: (1) 18-29 years of age, (2) classified low-risk for exercise testing as categorized by the American College of Sports Medicine (ACSM, 2013, p.26), and (3)

participation in endurance-based exercise (running, cycling, swimming, etc.) a minimum of two times per week for ≥1 year determined via the rapid assessment of physical activity form (RAPA) (Topolski et al., 2006). Low-risk for exercise testing can be described by absence of known or signs/symptoms of cardiovascular, pulmonary and metabolic diseases. In addition, the exclusion of two or more cardiovascular disease risk (age, family history, cigarette smoking, sedentary lifestyle, obesity, hypertension, dyslipidemia, or prediabetes. Subjects reporting certain lifestyle factors (i.e. smoking) or diseases (i.e. diabetes, asthma) that decrease NO production were excluded from participation. Subjects consuming any supplements that may counteract with CM within the previous 6 months including branched-chain amino acids, creatine monohydrate, protein, L-arginine, and/or CM were excluded from participation. To ensure subjects met inclusion criteria, all individuals completed a health history questionnaire before testing began. All subjects were required to sign a statement of informed consent approved by Louisiana Tech University's Institutional Review Board.

Procedure

This study utilized a randomized, double-blind, crossover design where subjects served as their own controls. All trials were scheduled at the same time of day (± 1 hour) to ensure chronobiological control; trials were also separated by at least one week to allow for sufficient recovery/washout between visits (Glenn et al., 2016). Subjects completed 4 total visits to the laboratory. The first visit included signing of the informed consent, completion of a health history questionnaire, demographic measurements, and establishment of peak aerobic capacity (VO_{2peak}). On the second visit, subjects were familiarized to the exercise tests and testing order performed during subsequent trials (described below).

The final visits (visits 3 and 4) involved consuming the following supplement doses in randomized order: 12 g dextrose (PLA) or 8 g citrulline-malate (CM) + 12 g dextrose. During visits 2, 3 and 4, subjects completed a time to exhaustion protocol on a cycle ergometer, followed by a 5-minute recovery and finally, a 30 s all out Wingate cycling test. All measurements and methods for each variable being assessed in this investigation are described in detail below.

CM provided for this investigation was 3rd party lab tested for supplement purity and authenticity (Powder City Inc., Philadelphia, PA). An outside researcher mixed supplements in a sealed shaker combined with a commercially available, cherry flavoring. To ensure preservation of the double-blind design, subjects consumed each supplement while wearing a nose clip in order to further mask any taste and/or smell discrepancies. After consuming the supplement, participants underwent a 1-hour seated rest period.

Food logs were distributed to all participants to record food and fluid intake for the 24 h prior to each trial. Participants were given copies of their initial dietary report and asked to replicate the first trial's dietary intake for the subsequent trial. Upon each subjects return to the laboratory, they were asked to verbally confirm their dietary adherence prior to testing. To further account for dietary intake affecting outcome measures on testing days, participants fasted 3 hours prior to each trial. All participants were instructed to refrain from vigorous exercise, alcohol for 24 hours prior to testing, and caffeine 12 hours prior to each trial. Participants replicated the same attire for all trials and wore clothes/shoes in which they normally exercise.

Performance measures

Demographic measurements. Demographic measurements included body mass, height, and body fat percentage. Height was assessed with a stadiometer (Detecto, Webb City, MO).

Body mass and body fat was measured with InBody 720 (InBody CO., Cerritos, CA) multi-frequency bioelectrical impedance analyzer. Physical activity levels were also recorded using the RAPA.

Peak aerobic capacity (VO_{2peak}). VO_{2peak} was assessed via a graded exercise test (GXT) on a Velotron Dynafit Pro cycle (Racer Mate, Seattle, WA) electronically braked cycle ergometer. Prior to testing, seat and handlebar preferences were established for each individual and recorded for use in all future testing sessions. Subjects warmed-up at 50 W for 5 minutes at a self-selected cadence. Upon completion of the warm-up, the resistance increased 25 W every 2 minutes until the participant could no longer maintain 60 revolutions per minute (RPM). VO_{2peak} was measured using breath-by-breath analysis and analyzed via open-circuit spirometry (COSMED, Rome, Italy). The highest 15 s VO_{2peak} value recorded was used as the peak measurement provided it met at least two of the following criteria: a) a plateau in heart rate or heart rate is within 10% of the age predicted maximum, b) a plateau in VO_2 (an increase of no more than 150 $ml \cdot min^{-1}$), and/or c) a respiratory exchange ratio value > 1.15 (Smith et al. 2009). Test retest reliability (ICC = 0.98) and coefficient of variation (5.18 %) for this protocol have been previously demonstrated (Smith et al. 2009).

Time-to-exhaustion. Prior to TTE testing, the predetermined seat height and handlebar settings were adjusted for each participant. After completing the same warm-up as used for the GXT, subjects completed a TTE at 90% of their previously recorded VO_{2peak} (Simmonds et al. 2010; Weber and Schneider 2001). TTE was defined as the amount of time participants could maintain intensity above 40 RPM pedaling cadence (Astorino et al. 2000; Vivodtzev et al. 2011). Subject's total time to complete the TTE test was recorded (in seconds) along with the total work completed (TWC [kJ] =TTE time*exercise intensity (W)/1000) during the protocol. Reliability

and coefficient of variation for TTE protocol have been reported as ICC = 0.71 and 3.8 %, respectively (Smith et al. 2009).

Wingate cycling test. The Wingate cycling test was completed immediately after TTE completion. For the Wingate protocol, subjects performed a 5-minute cool down, which also served as the warm-up, after the TTE test against 50 W at a self-selected pedaling cadence. Following the warm-up period, a 5 s countdown was provided after which the participants were instructed to pedal as quickly as possible against a predetermined resistance (7.5% body mass) (Glenn, Smith, Moyen, Binns, & Gray, 2015). Based on previous recommendations, major points of emphasis to participants before testing was that the Wingate: a) starts at a specified pedaling cadence, b) is a maximal sprint, c) requires participants to remain seated during the trial, and d) participants should not stop until instructed (Bar-Or, 1987). Mean watts, peak watts, and fatigue index were measured throughout the test.

Heart rate and rating of perceived exertion. Heart rate via Polar heart rate monitors (Polar heart rate monitor, Lake Success, NY) and rating of perceived exertion (RPE) via the Omni scale was measured throughout the 1-hour rest period (0, 15, 30, 45, and 60 minute) intervals to ensure the subject was in a rested state. Both measures were also taken during the TTE protocols. During the TTE, measures were taken at 60 s intervals throughout the cycling protocol and immediately after reaching volitional exhaustion. For the Wingate, heart rate, and RPE measurements were recorded immediately before and after completion of the Wingate trial.

Blinding efficacy and side-effects. Upon completion of the supplement intervention, participants were asked which supplement they believed they consumed. Participants were also asked if they experienced any side-effects throughout the course of each trial related to the supplement ingested.

Statistical analyses. Statistical Package for the Social Sciences (SPSS, version 22) (Armonk, NY) 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.

Paired *t*-tests determined the differences between trials for TTE and Wingate variables. TTE variables included: TTE time and TWC. Wingate variables included: mean watts, peak watts, and fatigue index. Statistical significance was set at $\alpha < 0.05$ for *t*-test analyses. Additionally, muscular power was calculated during each 5 s interval throughout the Wingate cycling test and a 2 (trial) x 6 (time) repeated-measures analysis of variance was utilized to evaluate sustained relative power. For significant *F*-scores, the Bonferroni correction was applied to determine the appropriate α level ($p > 0.05$). All values are reported as mean ± *SD*.

Results

All subjects were deemed recreationally active through the RAPA scale with the average subject engaging in moderate exercise 5 or more times a week for at least 30 minutes. According to American College of Sports Medicine, our subjects' average VO_{2peak} of 56.3 ± 8.4 ml•kg^{-1}•min^{-1} fell within the ~65th percentile which displayed that they engaged in endurance exercise (Table 1). Of 32 subjects recruited and consented, 28 completed all trials and were included in final analyses. No side effects were reported during or after supplement trials.

TIME-TO-EXHAUSTION

TTE total time and TWC were compared between trials. There were no significant differences observed for TTE total time ($p = 0.94$; PLA: 315.4 ± 137.7; CM: 314.1 ± 107.1) nor TWC ($p = 0.86$; PLA: 74.7 ± 34.1; CM: 74.1 ± 26.4) between supplement trials (Figure 1 & 2).

WINGATE CYCLING TEST

Mean watts, peak watts, and fatigue index were evaluated throughout the Wingate cycling test for each trial. No significant differences were observed between trials for mean or peak watts (Table 2). There were also no differences between CM and PLA for fatigue index (Table 2). For sustained relative power, a significant effect was observed for time within the trials ($p < 0.001$); however, no significant differences ($p > 0.05$) were observed between trials with regard to supplement consumed.

Heart Rate (HR) and Ratings of Perceived Exertion (RPE)

There were no significant differences ($p > 0.05$) in HR or RPE between trials. However, after the 5-minute recovery post-Wingate, CM trials showed a trend for a lower recovery perceived exertion compared to PLA trials (CM = 3.0, PLA = 3.7; $p = 0.08$).

Discussion

The purpose of this study was to investigate the effects of CM on short, high intensity exercise performance after a preliminary bout of exhaustive endurance exercise in young, healthy males. Our *a priori* hypothesis was that CM supplementation would augment TWC and total time to complete the TTE test. Additionally, we hypothesized that CM would increase mean and peak power while decreasing fatigue during the Wingate cycling test. Our hypotheses were not supported as subjects showed no significant difference in TWC, total time, and fatigue variables after consuming CM compared to the placebo.

Throughout both aerobic and anaerobic components of the cycling protocol, CM failed to significantly increase performance. Cunniffe and colleagues (2016) found similar results while examining an acute dose of CM on cycling performance in well-trained men. Their study however investigated the anaerobic Wingate test before the aerobic TTE test. Nevertheless, they examined 12 g of CM on performance variables while we used 8 g of CM. 6-8 g of CM has

shown to have impact on performance (Glenn et al., 2017; Perez-Guisado, 2010; Wax, Kavazis, Weldon, & Sperlak, 2015); moreover, higher dosages of CM have been positively correlated with GI distress (Perez-Guisado, 2010). Although our subjects were not well-trained athletes, they were recreationally active males who exercised on average 4.8 times per week. Therefore, taken together with the results from Cunniffe et al. (2016), CM may have limited ergogenic effects in subjects that are moderate to well-trained during cycling exercises. This may be attributed to the fact that NO is increased in response to acute bouts of exercise (Bode-Böger et al., 1994; Clarkson et al., 1999; Hickner et al., 1997; Rognmo et al., 2008) and increased as an adaptation to regular exercise training (Edwards et al., 2004; Poveda et al., 1997; Tordi et al., 2006). Well-trained individuals also have higher resting circulating NO than their sedentary counterparts (Bloomer, 2010). With NO circulating levels being high, trained individuals may receive no ergogenic benefit taking CM. In particular, our subjects had an average VO_2 peak of 56.3 ± 8.4 ml•kg^{-1}•min^{-1} which falls within the ~65th percentile indicating our subjects were trained (ACSM, 2013, p.84).

In this current study, HR and RPE values were similar between trials. However, others have shown that CM has had significant effects on increasing HR (Cunniffe et al., 2016). This could be due to the methodology of previous research using multiple Wingate tests before the TTE test increasing HR levels throughout the study (Cunniffe et al., 2016). However, both supplement and placebo trials followed similar protocols, so this would not explain why CM trials had a higher average HR than placebo trials. This may be an aspect that needs further investigation. Furthermore, Glenn and affiliates (2017) used resistance trained females completing a weightlifting protocol which may have prompted lower RPE during CM trials due

to the nature of the protocol isolating certain muscle groups and may have different cardiovascular and metabolic demands to our chosen experimental approach.

Considerations and Limitations

To our current knowledge, this is the first study examining CM's effect on aerobic exercise with subsequent anaerobic exercise. Neither blood flow nor NO levels were directly evaluated during the supplement and placebo trials; thus, discussion about proposed mechanisms cannot be confirmed. Future research is essential to advance mechanistic backing for aforementioned experimental increases in performance. The precise mechanisms with which exogenous CM affects performance have yet been established; however, several theories have been suggested. A well-supported hypothesis includes L-citrulline's role in the NO pathway (Bescos et al., 2012). L-citrulline bypasses hepatic metabolism and therefore a higher possible effective approach of increasing levels of extracellular L-arginine, than L-arginine supplementation itself (Hartman, Torre, & Prior, 1994), leading to increase NO production. When combined with malate, L-citrulline appears to increase the rate of ATP production (Bendahan et al., 2012), blood flow, and mitochondrial respiration during exercise (Shen et al., 1994). Combined, it may be these physiological changes leading to greater physical capability. More research is required to determine how CM enhances resistance exercise performance, reduces muscle soreness, and decreases feelings of fatigue at a physiological level.

Other limitations included the inability to directly control dietary intake and exercise activity. Although participants reported no strenuous activity and indicated consistent dietary intake 24 hours before trials, this is contingent on subject honesty and accurate recordings.

Conclusion

In conclusion, the results of this investigation demonstrate that young recreationally trained males ingesting an acute amount of CM may not experience significant advantages when maximally biking for an extended period of time followed by an all-out sprint. Athletes who engage in competition with intermediate to long distances followed by an all-out sprint may not benefit ingesting CM and should investigate alternate forms of performance enhancement methods. Further research is needed to determine whether additional populations (women, master athletes, etc.) may benefit from an acute intake of CM under a similar protocol.

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Table 11

Demographic data for all study subjects

Variable	Mean	SD
Age	20.9	2.8
Height (cm)	175.3	6.9
Weight (kg)	75.9	9.8
% Body Fat	14.5	5.4
VO_2 Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	56.3	8.4

Note. SD: Standard Deviation

Table 2

Wingate Variables for all study subjects

Variable	Supplement	Mean	SD	*p*-value
Mean watts	CM	588.0	93.0	.805
	PLA	586.1	87.7	
Peak watts	CM	786.7	133.0	.244
	PLA	773.0	136.7	
Fatigue Index	CM	14.3	7.2	.077
	PLA	12.9	6.4	

Note. CM: Citrulline-Malate, PLA: Placebo, SD: Standard deviation, **p*-value = .05

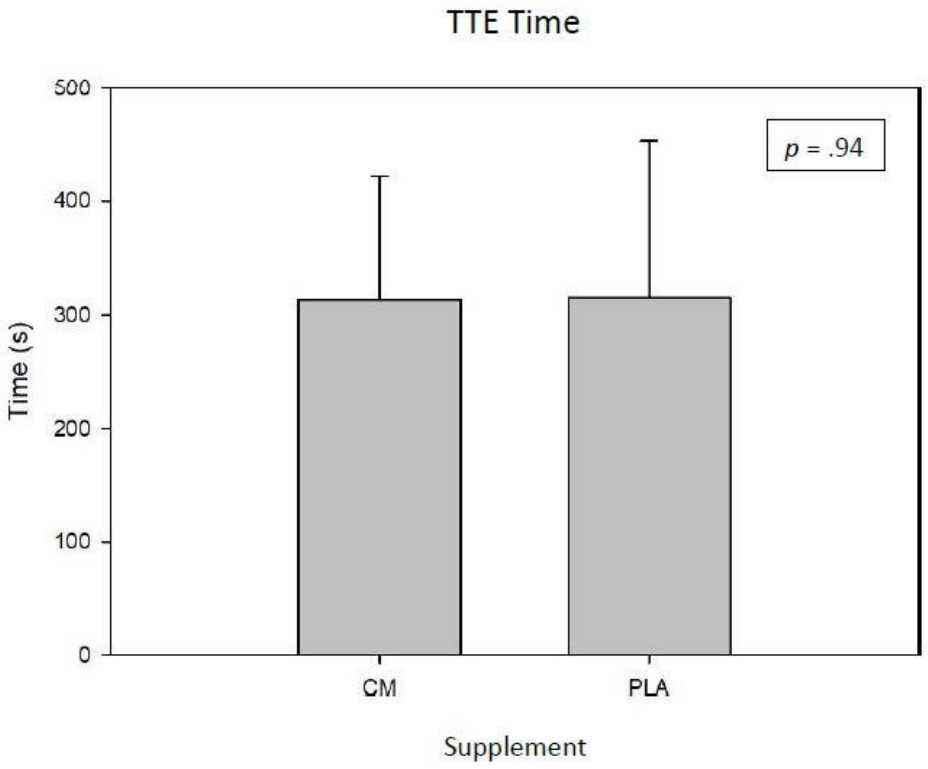

Figure 1. Average time to exhaustion (TTE) time for supplement trials. CM = Citrulline Malate, PLA = Placebo

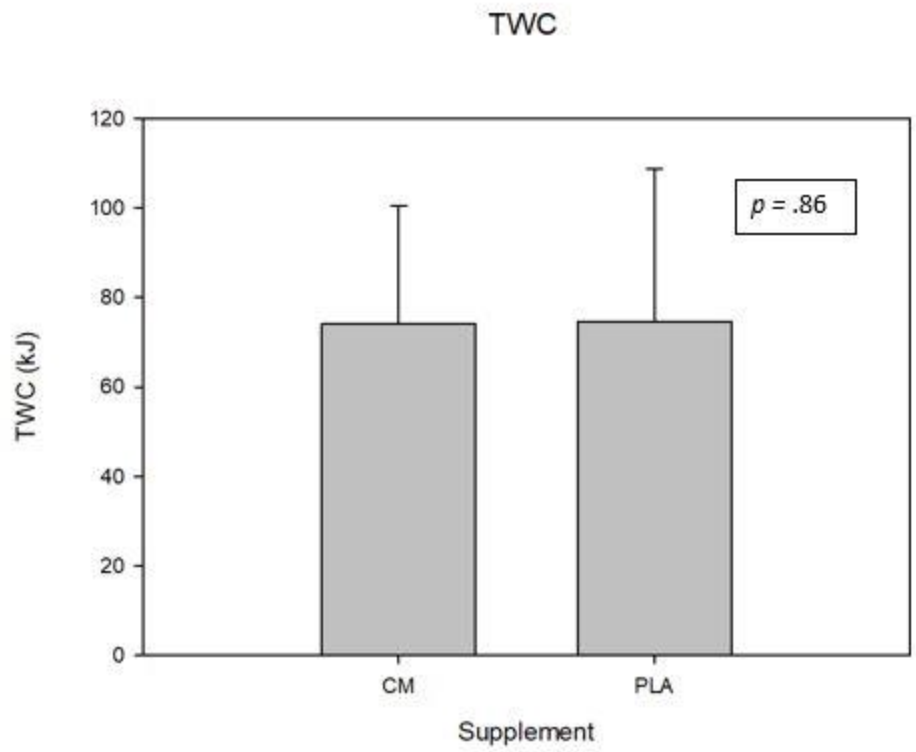

Figure 2. Average total work completed (TWC) for supplement trials. Citrulline-malate (CM), placebo (PLA).