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DOES CITRULLINE MALATE ENHANCE PHYSICAL PERFORMANCE

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14. ABSTRACT.
The purpose of this study was to investigate the ability of citrulline malate (CM) to enhance physical performance. Our hypothesis was that CM ingestion prior to exercise would result in increased maximal or peak oxygen consumption and time-to-exhaustion. Twelve subjects, 7 men and 5 women, after signing their consent and completing medical screening, participated in this randomized double blind, repeated measures design. Each subject completed three VO_2max testing sessions. Initial testing was accomplished to provide a baseline for their secondary and tertiary tests. Following this baseline test, subjects ingested 6 g/d of either CM or a placebo for 14 days. Subjects then returned for their second VO_2 max test. After the second test, subjects ingested 6 g/d of the other condition's capsules for 14 days, and then returned for their third and final VO_2 max test. VO_2max, lactate threshold, maximum watts reached, ratings of perceived exertion and pre- and post-test blood urea nitrogen levels were compared between conditions. There were no significant differences between groups for any of the performance variables measured nor was there a difference between groups in RPE. Although previous studies have claimed to observe an ergogenic effect of CM ingestion, its effects do not appear to include enhancing maximal oxygen uptake or exercise time-to-exhaustion.

15. SUBJECT TERMS
Citrulline malate, ergogenic aid, nutritional supplement, exercise

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Executive Summary

Purpose:

This study was investigated the ability of citrulline malate (CM) to enhance physical performance. We hypothesized that CM ingestion prior to exercise would result in increased maximal or peak oxygen consumption and time-to-exhaustion.

Methods:

Twelve subjects; seven men and five women, after signing their informed consent document and completing medical screening, participated in this randomized double-blind, repeated measures design. Each subject completed three VO_2max testing sessions. Initial testing was accomplished to provide a baseline or control for their secondary and tertiary tests. Following the baseline test, subjects ingested 6 g/d of either CM or a placebo for 14 days. Subjects then returned for their second VO_2 max test. After the second test, subjects ingested 6 g/d of the other condition's capsules for 14 days and then returned for their third and final VO_2max test. VO_2max, lactate threshold, maximum watts reached (time-to-exhaustion), ratings of perceived exertion (RPE) and pre- and post-test blood urea nitrogen levels were compared between conditions.

Results and Conclusions:

There were no significant differences between groups for any of the performance variables measured, nor was there a difference between groups in RPE. Although previous studies have claimed to observe an ergogenic effect of CM ingestion, its effects do not appear to include enhancing maximal oxygen uptake or increasing exercise time-to-exhaustion. Different dosing regimens and/or physical applications may be necessary to observe an ergogenic effect.

INTRODUCTION

Objective

The purpose of this proposed study was to investigate the ability of citrulline malate (CM) to enhance physical performance. We hypothesized that CM ingestion prior to exercise would result in an increased maximal oxygen uptake (VO_2max) and exercise time-to-exhaustion.

Background

The physical and mental demands placed on Air Force special operations personnel and other Battlefield Airmen are among the most significant in the U.S. military. As those demands continue to increase with the execution of the Global War on Terror, providing every advantage to our special operators is critical. One potential method to provide such an advantage is the use of nutritional ergogenic aids.

Citrulline malate (CM), a combination of the amino acid citrulline and the acid salt malate, may possess ergogenic properties. In the 1980s, CM was demonstrated to be an effective treatment for clinical asthenia (Callis et al., 1989). More recently, Bendahan et al. (2002) found that 14 days of CM ingestion prior to exhaustive finger flexion resulted in a significant reduction in the sensation of fatigue, a 34% increase in the rate of oxidative ATP production during exercise, and a 20% increase in the rate of phosphocreatine recovery after exercise, indicating a larger contribution of oxidative ATP synthesis to energy production. Conspicuously, they did not measure or report work performance. Wu et al. (2007) found that rats fed L-malate for 30 days increased their swimming time by 27%. Perez-Guisado and Jakeman (2010) observed that CM ingestion dramatically improved bench press performance in pre-fatigued subjects.

The primary purpose of this study was to test the ability of CM ingestion to improve physical performance, specifically maximal oxygen uptake (VO_2max) and exercising time-to-exhaustion. A secondary purpose was to examine the ability of CM ingestion to influence resting and post-exercise blood urea nitrogen (BUN) levels. Such an effect would indicate a potential mechanism of CM's action on performance.

If CM possesses the ability to safely improve performance with short-term (two-week) dosing regimens, it could be used by Battlefield Airmen immediately prior to deployment or in anticipation of upcoming physical challenges and may aid in the execution of difficult tasks.

METHODS

Volunteers

Twelve moderately trained subjects; seven men and five women, participated in this three-trial, repeated measures design. Each subject had to meet the following five criteria to be included:

1. Age 18-44
2. Met the American College of Sports Medicine (ACSM) standard for low risk of coronary artery disease (ACSM, 2000) as having no more than one of the following risk factors:
    a. Family History
    b. Hypertension
    c. Cigarette Smoking
    d. Obesity (BMI > 30)
    e. Hyperlipidemia
    f. Diabetes Mellitus or Impaired Fasting Glucose
3. Participated in aerobic exercise for ≥ 120 min/wk
4. Did not use any form of nutritional/herbal supplements for 30 days prior to study
5. Was not pregnant

All subjects visited the lab three times during a seven-week period. Each visit lasted approximately one hour. Subject demographics are presented in Table 1.

Table 1. Subject Demographics

	Height (m)	**Mass (kg)**	**Age (yr)**
Males (7)	1.75 ± 0.06	77.5 ± 2.8	33.7 ± 8.6
Females (5)	1.67 ± 0.05	68.8 ± 18.9	29.2 ± 7.8
Total (12)	1.71 ± 0.08	73.9 ± 11.9	31.8 ± 6.9

Experimental Design and Assessment Overview

Informed Consent and Facilities

The AFRL Wright Site Institutional Review Board approved the use of human subjects in this study. Prior to participation, subjects were informed of the risks and discomforts associated with this study and their written consent was obtained. After consent was given, a medical screening/clearance was performed by the medical monitor. All testing was completed at the Brook-City Base Human Performance Laboratory.

Assessment Procedures

Each subject completed three VO_2max tests. Prior to each test, the subject was medically cleared. Once cleared, a 3.0 ml blood sample was drawn and later assayed for BUN levels. Each subject then completed an 8-12 minute, incremental VO_2 peak test on a Racermate Velotron Dynafit Pro Cycle Ergometer (Racermate, Seattle WA) (See Figure 1).

Figure 1. Racermate Velotron Dynafit Pro.

During the VO_2max test, each subject pedaled at a cadence of 80-90 rpm while the resistance was gradually increased at a rate of 20-35 watts per minute. The rate of resistance increase was based on each subject's size, fitness level, and exercise history in order to have them reach volitional fatigue between 8 and 12 minutes. A plastic facemask with a 6 foot expired gas tube was worn during the entire test to collect expired gases that were analyzed using the Parvo Medics' TrueOne® 2400 Metabolic Cart (ParvoMedics, Sandy, UT) (See Figure 2).

Figure 2. ParvoMedics' metabolic cart.

Blood lactate levels were obtained prior to the start of the test, each minute during the test, and one minute post-test using a Lactate Pro portable blood lactate analyzer (FaCT Canada Consulting Ltd, Quesnel, BC Canada) (See Figure 3) in order to determine the subjects' lactate thresholds for each condition. Approximately 5 µL of blood obtained by finger stick was used for each blood lactate collection.

Figure 1. Lactate Pro Portable Blood Lactate Analyzer.

Treatments

The first VO_2max test, or baseline, was administered without any treatment. At the end of this test, subjects visited our "code-keeper." This code-keeper randomly assigned a treatment order to each subject and provided them with a 14-d supply of color-coded capsules corresponding to CM (6 g/d of citrulline malate) or PL (6 g/d of a placebo of white flour). The code-keeper did not know which color corresponded to each treatment nor did he relay subject color-code information to any of the test proctors.

Subjects returned after two weeks of taking either CM or PL for their second VO_2max test. After the second test, subjects received a 14-d (6 g/d) supply of the other color capsules. They took these capsules for 14 days and then returned for their third and final VO_2max test. During visits two and three, subjects completed a questionnaire detailing what side effects from the capsules, if any, they had experienced during the previous two weeks.

<u>Data Analysis</u>

VO_2max, lactate threshold, time-to-exhaustion (TTE), maximum watt level attained, ratings of perceived exertion (RPE) and pre- and post-test BUN levels between conditions in a two-factor repeated measures (time and condition) ANOVA were compared. When a significant condition or condition by time interaction was found, Student's t-tests were performed to identify specific differences between conditions.

RESULTS

There were no significant differences between groups for any of the performance variables (Figures 4-7) nor was there a difference between groups in RPE (Figure 8). In all three treatment conditions post-exercise BUN levels were significantly lower than pre-exercise levels. However, there were no differences between groups for pre-exercise or post-exercise BUN levels, nor were there any significant condition by time interactions (Figure 9).

There were no significant differences in the mean number or severity of side effects reported between the PL and CM treatments. In fact, only two subjects reported having any side effects (one incident of gastrointestinal distress and one report of fatigue). These side effects were mild, transitory, and not directly attributable to CM or PL ingestion.

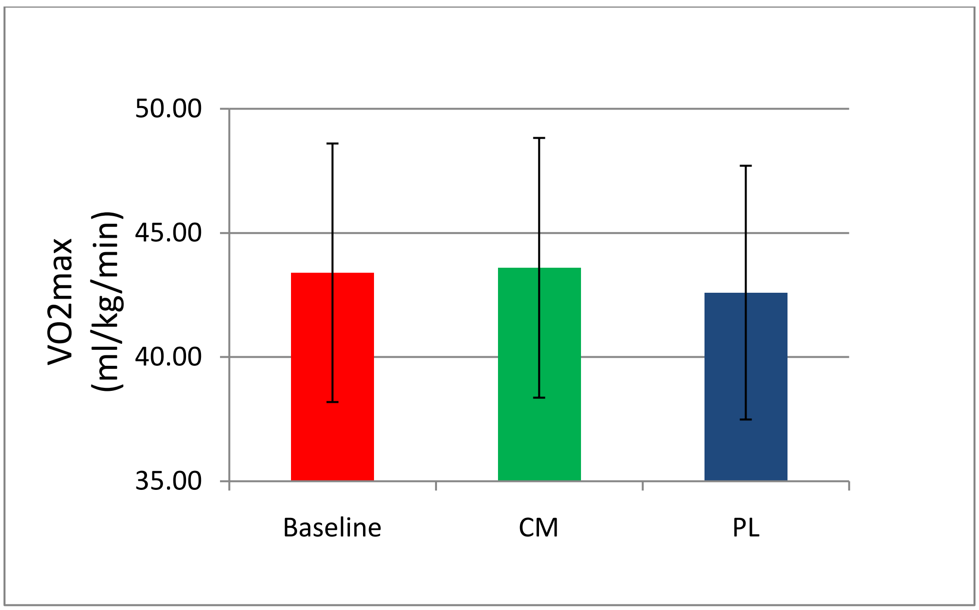

Figure 2. VO2max results for all three conditions.

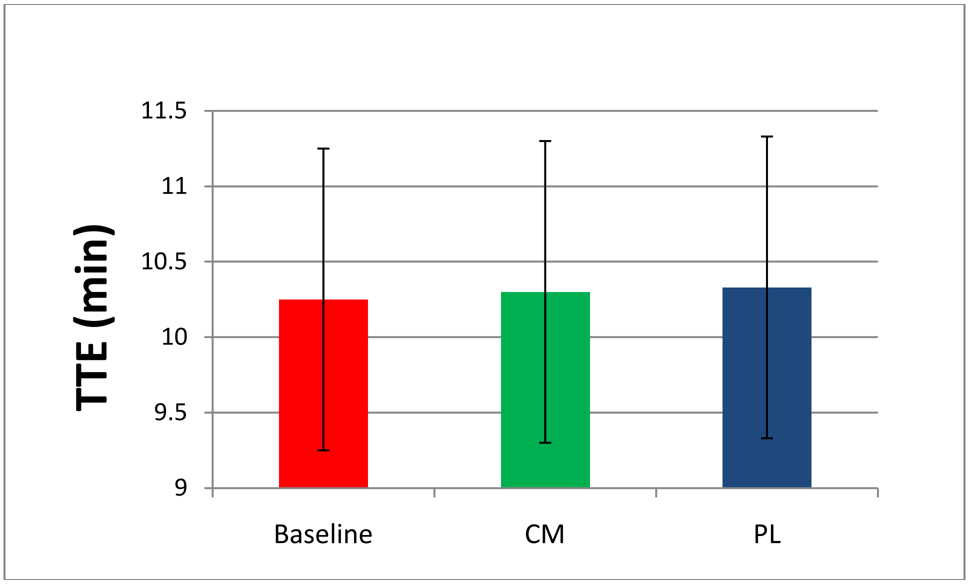

Figure 3. Time to exhaustion for all three conditions.

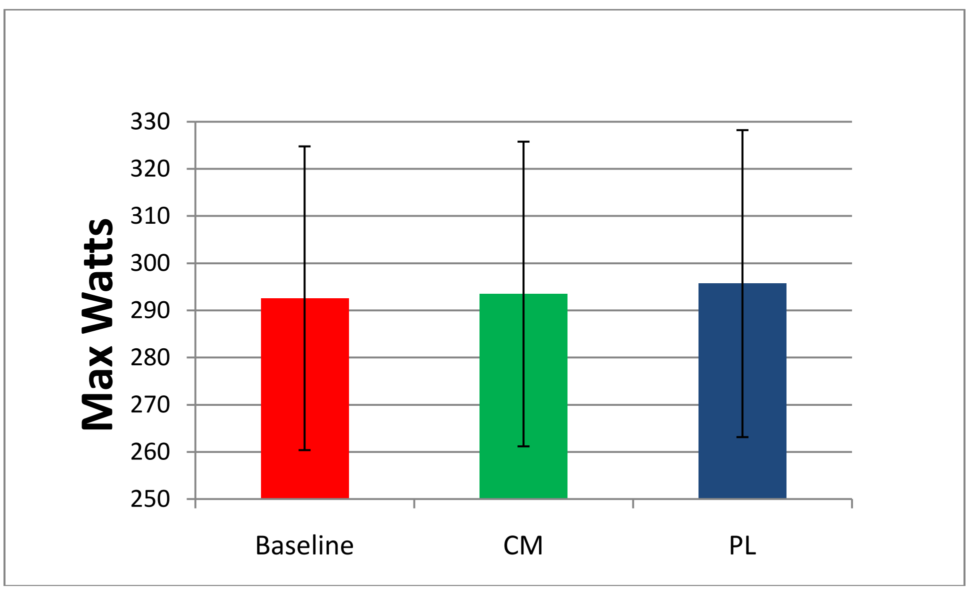

Figure 4. Maximum watts attained for all three conditions.

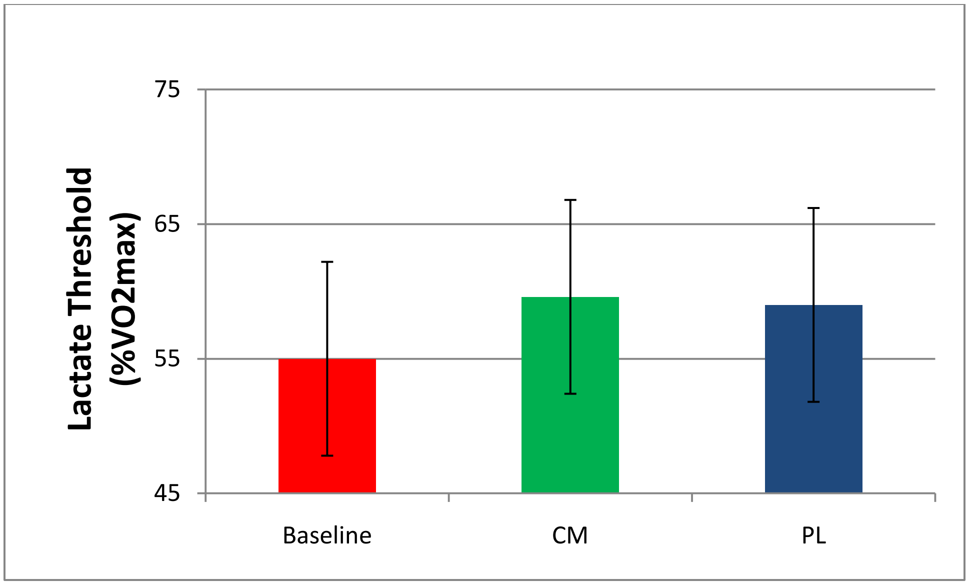

Figure 5. Lactate threshold (as % of VO2max) for all three conditions.

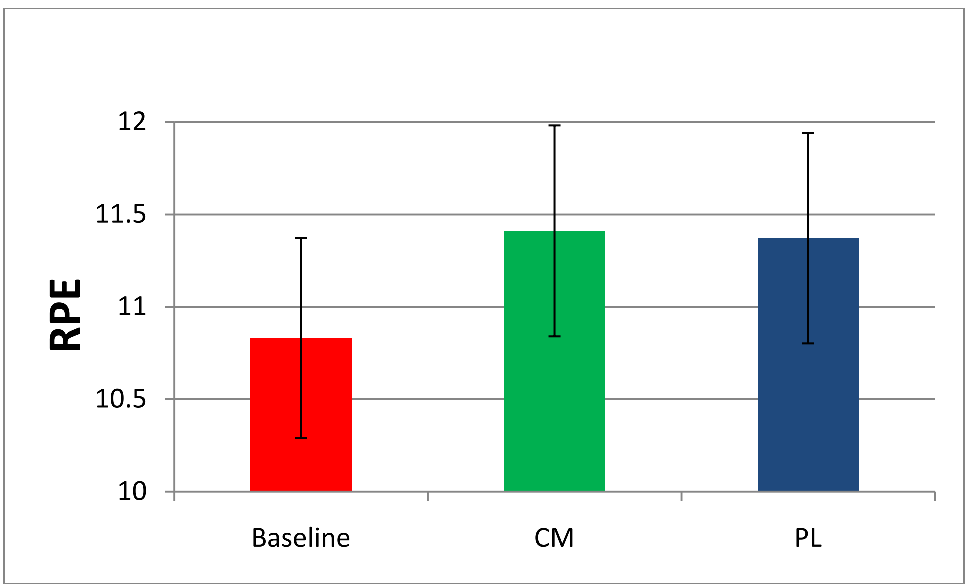

Figure 6. Mean ratings of perceived exertion for all three conditions.

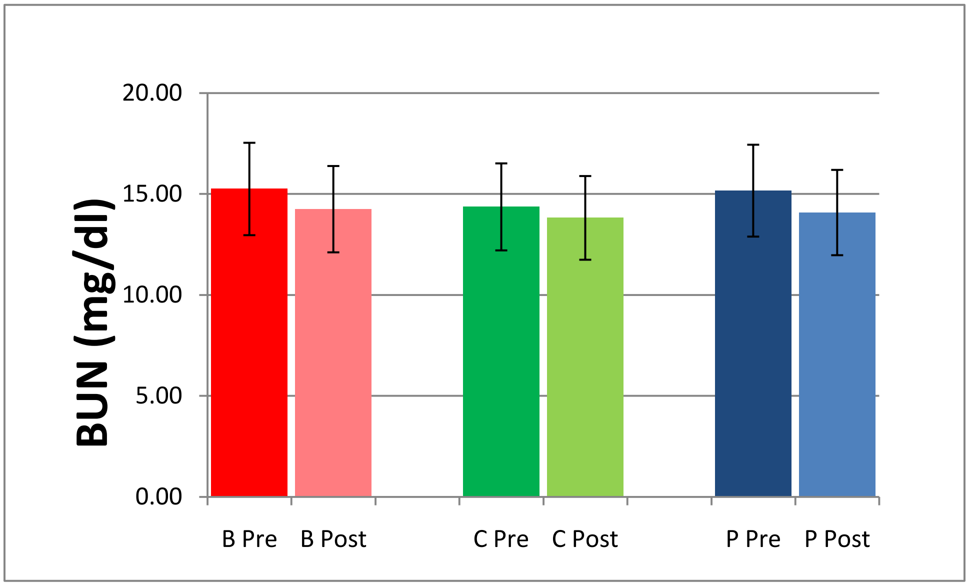

Figure 7. Pre-exercise and post-exercise BUN levels for all conditions.

DISCUSSION

The results of this study do not support the hypothesis that CM ingestion can improve aerobic performance. Specifically, CM ingestion did not improve VO_2max, lactate threshold, or time-to-exhaustion during incremental cycle ergometry. Although a few studies have demonstrated a positive influence of CM ingestion on exercise performance (Bendahan et al., 2002; Perez-Guisado and Jakeman, 2010), the specific exercise modes and intensities that are affected are not yet clear nor is the mechanism by which CM ingestion could improve performance. There are several mechanisms through which CM ingestion could potentially have an ergogenic influence.

Malate is the tricarboxylic acid (TCA) cycle intermediate that shows the largest change in concentration during exercise (Gibala et al., 1998). The TCA cycle is a key metabolic pathway for the oxidative production of protons for the electron transport system. Increased available malate could potentially increase the $NADH^+ + H^+$ production of the TCA cycle, which could increase ATP turnover. The ability to supercharge the TCA cycle by boosting various intermediate levels has been examined by several researchers with equivocal results (Brown et al, 2004; Sahlin et al., 1990). Malate is also a key carrier of protons across the mitochondrial membrane in the malate-aspartate shuttle. Thus, increases in available malate could potentially increase transport of protons into the mitochondria to feed the electron transport chain (Wu et al., 2006), which could also positively influence ATP turnover. Bendahan et al. (2002) presented a compelling case for the ability of CM ingestion to supercharge the TCA cycle by using ^{31}P

nuclear magnetic resonance spectroscopy to show that phosphate kinetics were positively influenced by 15 days of CM ingestion. However, their subjects were sedentary males who complained of chronic fatigue, and the exercise bout they utilized was only three minutes of sub-maximal finger flexion. The results of the current study do not support the ability of CM ingestion to enhance oxidative metabolism in healthy human subjects.

It is possible that the beneficial effects of malate on aerobic performance were negated by the influence of citrulline. In healthy adults, Hickner et al. (2006) observed a reduction in treadmill TTE following citrulline ingestion with no difference in VO_2max between citrulline and placebo trials. They also observed a suppressed insulin response to exercise following citrulline ingestion, leading them to speculate that citrulline ingestion may reduce nitric oxide-mediated pancreatic insulin secretion typically seen with high-intensity exercise. This seems unlikely as citrulline is a powerful precursor to arginine which increases nitric oxide (NO) levels. NO has been demonstrated to stimulate insulin release (Laffranchi et al., 1995). Further, Sureda et al. (2009) observed a normal insulin response to exhaustive exercise following ingestion of the combination of citrulline and malate. They also observed significant increases in arginine and NO following CM-supplemented exercise. Such an increase could be ergogenic. Although the role of endothelium-derived NO in acute exercise has not been fully resolved, the ability of NO to induce vasodilation could potentially lead to increased work capacity (Maiorana et al., 2003). Studies investigating such an increase have produced mixed results (Chen et al., 2010; Kanaya et al., 1999; Wennmalm et al., 1995). Although we did not specifically measure NO, our results do not support the notion of a CM driven increase in NO leading to increased work capacity.

Citrulline is a key intermediate in the urea cycle, a pathway in the liver that converts toxic ammonia into non-toxic urea and eliminates it from the body. During prolonged and/or intense exercise, ammonia production rises due to increased protein metabolism and the conversion of AMP to IMP. Increases in available citrulline could potentially increase hepatic ureogenesis, providing a protective effect against ammonia poisoning (Briand et al., 1992; Callis et al., 1991). In a microbial model, Briand et al. (1992) found that CM accelerated ammonium elimination and facilitated the consumption of lactate. Furthermore, citrulline has been demonstrated to increase renal re-absorption of bicarbonate (Callis et al., 1991) that could mitigate the effects of exercise-induced acidosis. Acidosis stimulates the conversion of AMP to IMP, contributing to hyperammonemia so there is potential within this system for a prospective buffering agent like citrulline to have a substantial effect. Perez-Guisado and Jakeman (2010) recently demonstrated a powerful positive affect of CM ingestion on multi-set bench press performance that they attributed to these effects of citrulline. Subjects were able to complete 52% more repetitions following a single 8 g dose of CM taken one hour prior to exercise than with placebo ingestion. Unfortunately, they did not measure any serum metabolites that would indicate enhanced buffering to acidosis or hyperammonemia. They did however, note that subject experienced less post-exercise muscle soreness after the CM trial and theorized that may have been due to a buffering effect of CM on acidosis or hyperammonemia. In the current study, we measured resting and post-exercise BUN in an attempt to determine if the CM ingestion influenced ammonia production or clearance and failed to observe a difference between conditions. This could be due to mitigation of the effect of CM after two consecutive weeks of use in the current study versus the single application in the Perez-Guisado study. However, it is more likely our 8-12 minute VO_2max test was not a powerful enough stressor to significantly drive the urea cycle or influence BUN levels. Nevertheless, based on the most recent literature, we suspect that the potential ability of CM to buffer acidosis and/or hyperammonenia is the most likely mechanism for ergogenic action. Future investigations should include a work bout stressful enough to induce acidosis and hyperammonemia while measuring both serum pH and nitrogen levels during exercise and recovery.

CONCLUSIONS

Although well tolerated, CM ingestion at 6 g/d for 14 d did not influence aerobic performance in any observable way. Therefore we cannot recommend it as a practical ergogenic aid to Battlefield Airmen at this time. However, based upon our review of the relevant literature, we believe CM ingestion may possibly enhance performance during multiple bouts of intense exertion. Further study of its potential is recommended.

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LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

^{31}P – thirty-one phosphate isotope
711 HPW – 711 Human Performance Wing
AFRL – Air Force Research Laboratory
ACSM – American College of Sports Medicine
AMP – adenosine monophosphate
ANOVA – analysis of variance
ATP – adenosine triphosphate
bpm – beats per minute
BUN – blood urea nitrogen
CM – citrulline malate
cm – centimeters
Et al. – "and others" (Latin)
HR – heart rate
IMP – inosine monophosphate
kg – kilograms
$L \cdot min^{-1}$ – liters per minute
m – meters
microL – microliters
mL – milliliters
$mL \cdot kg^{-1} \cdot min^{-1}$ – milliliters per kilogram per minute
$mmol \cdot L^{-1}$ – millimoles per liter
NO – nitric oxide
n – number of subjects
PL – placebo
RER – respiratory exchange ratio
RPE – rating of perceived exertion
rpms – revolutions per minute
s or secs – seconds
SD – standard deviation
TCA – tricarboxylic acid
TTE – time to exhaustion
U.S. – United States
VO_2max – maximal oxygen uptake
W – watts