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ORIGINAL CONTRIBUTION

Acute citrulline malate supplementation improves upper- and lower-body submaximal weightlifting exercise performance in resistance-trained females

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Abstract
Purpose Citrulline malate (CM) is a nonessential amino acid that increases exercise performance in males. However, based on physiological differences between genders, these results cannot be extrapolated to females. Therefore, the purpose of this investigation was to evaluate effects of acute CM supplementation on upper- and lower-body weightlifting performance in resistance-trained females.
Methods Fifteen females (23 ± 3 years) completed two randomized, double-blind trials consuming either CM (8 g dextrose + 8 g CM) or a placebo (8 g dextrose). One hour after supplement consumption, participants performed six sets each of upper- (i.e., bench press) and lower-body (i.e., leg press) exercises to failure at 80 % of previously established one-repetition maximum. Immediately after each set, repetitions completed, heart rate and rating of perceived exertion (RPE) were recorded.
Results Repeated-measures analysis of variance indicated that subjects completed significantly ($p = .045$) more repetitions throughout upper-body exercise when consuming CM versus placebo (34.1 ± 5.7 vs. 32.9 ± 6.0, respectively). When consuming CM, similar significant ($p = .03$) improvements in total repetitions completed were observed for lower-body exercise (66.7 ± 30.5 vs. 55.13 ± 20.64, respectively). Overall RPE score was significantly lower ($p = .02$) in upper-body exercise when subjects consumed CM versus placebo (7.9 ± 0.3 and 8.6 ± 0.2, respectively). The supplement consumed exhibited no significant effects on heart rate at any time point.
Conclusions Acute CM supplementation in females increased upper- and lower-body resistance exercise performance and decreased RPE during upper-body exercise. These data indicate that athletes competing in sports with muscular endurance-based requirements may potentially improve performance by acutely supplementing CM.

Keywords Ergogenic aid · Sports nutrition · Amino acids · Resistance exercise · Women · Nitric oxide

Introduction

Athletes are continually searching for techniques with which to improve exercise performance [1–5], and multiple reports support the use of legal ergogenic aids as a method to increase resistance exercise performance to exhaustion in males [6, 7]. However, females have traditionally been understudied with regard to human research [8], and although many ergogenic aids have proven beneficial to males, *it cannot be assumed that males and females will experience similar responses to supplemental use due to physiological differences between genders* [9]. As of 2010, females represented 50.8 % of the US population [10], and 65 % of female athletes have reported use of nutritional supplements throughout their college careers [11]. As a result, it is becoming increasingly important to understand the ergogenic effects of supplemental aids on resistance exercise performance to exhaustion in females.

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Table 1 Subject demographic data

	Age (years)	Height (cm)	Body mass (kg)	Body fat (%)	Years weightlifting	Workouts per week	Bench press 1 RM (kg)	Leg press 1 RM (kg)	Bench press strength ratio	Leg press strength ratio
Mean	23	162.6	67.1	25.9	5.1	5.2	49.2	180.0	0.73	2.68
(SD)	(3)	(19.2)	(7.0)	(5.5)	(3.9)	(0.7)	(10.3)	(30.3)	(0.13)	(0.37)

Data are expressed as mean ± SD. Workouts per week refer specifically to resistance training workouts; *1RM* one-repetition maximum strength. Upper- and lower-body strength ratios were calculated by dividing 1RM weight lifted (kg) by body weight [23]

One particular subsection of ergogenic aids involves those documented to increase nitric oxide production. Nitric oxide is a potent vasodilator, which helps increase blood flow and mitochondrial respiration, particularly during exercise [12]. Increasing nitric oxide levels in muscles can offer positive effects both before and after exercises, including increased glucose uptake, muscle contractility, muscle blood flow, and muscle repair via satellite cell activation [13]. When taken together, these effects may create a positive environment in which resistance exercise performance to exhaustion may be augmented.

Recently, citrulline malate (CM), an intermediate in the nitric oxide pathway, has garnered interest for its role as an ergogenic aid based on the potential to increase nitric oxide production, resulting in enhanced exercise performance [14–16]. L-citrulline is a nonessential amino acid produced in the body and physiologically functions as the precursor to L-arginine (in vivo precursor to nitric oxide [17]). However, unlike L-arginine, it can bypass hepatic metabolism and is transported to the kidneys where it can be directly converted into L-arginine [12]. In order to be effective, L-citrulline must be combined with the amino acid malate, which acts as an intermediate of the tricarboxylic acid cycle and may increase the rate of adenosine triphosphate production [12]. Recent investigations involving the use of CM as a supplemental aid have demonstrated positive ergogenic effects with regard to submaximal resistance exercise performance to exhaustion. After taking an acute dose (8 g) of CM, resistance-trained males increased resistance exercise performance to exhaustion for the upper- [16] and lower-body [15] musculature when lifting at 80 and 60 % of their one-repetition maximum (1RM), respectively. These strength improvements were also present when performing body weight exercises after a similar acute dose [14], indicating that CM supplementation may benefit strength performance from a multitude of intensities.

It is important to note that there are only two studies evaluating the effects of CM on resistance exercise performance to exhaustion and both studies included male participants [15, 16]. The only study involving L-citrulline supplementation including females utilized a mixed gender subject sample (males and females) [18] and found nonsignificant results when using an absolute dose (6 g) of L-citrulline (independent of malate) which is lower than what has been deemed effective in males. There are important physiological differences between males and females [19], perhaps most notable are the differences in reproductive hormones. One hormone present in significantly higher levels in females versus males is estrogen [20]. *Estrogen up-regulates nitric oxide production* [21, 22], *and based on CM's role in the nitric oxide pathway, females may have augmented responses to CM supplementation compared to males*. However, before comparing CM effects between sexes, it is important to first determine whether CM indeed improves females' resistance exercise performance to exhaustion. Therefore, the purpose of this investigation was to evaluate the ergogenic effects of an 8 g acute dose of exogenous CM supplementation on upper- and lower-body, submaximal resistance exercise performance to exhaustion in trained female weightlifters. Based on previously reported results in males, we hypothesized that 8 g of acute CM supplementation would increase submaximal resistance exercise performance to exhaustion during both upper- and lower-body exercises when compared to a placebo.

Materials and methods

Participants

An a priori power analysis was conducted (G*Power v. 3.1.9) for an *F* test (repeated measures, within factors for six time points). To yield a power of 0.8 with a moderate Cohen's *f* effect size of 0.25, a correlation between repeated measures of 0.7 and an alpha of 0.05, 12 total subjects were required. To account for potential attrition, 15 females were recruited from the southern region of the USA (Table 1), and all subjects successfully completed the intervention. Inclusion criteria consisted of the following: 18–30 years of age, classified low risk as categorized by the American College of Sports Medicine [23], participation in resistance training a minimum of two times per week for ≥1 year, and refrained from CM supplementation within

the last year. To confirm training status, the ratio of weight lifted to body weight in kg [23] was calculated, and for the purposes of this investigation, subjects were required to be in the 60th percentile or greater for both upper- and lower-body strength. Anyone reporting certain lifestyle factors (i.e., smoking) and diseases (i.e., diabetes) that decrease nitric oxide production was excluded from participation. In addition, subjects consuming any supplements within the last year including branched-chain amino acids, protein, L-arginine, and/or L-citrulline were excluded from participation. To ensure subjects met inclusion criteria, all individuals completed a health history questionnaire before testing began. The university's Institutional Review Board prior to testing approved all measures and procedures, and each subject was required to sign a statement of informed consent.

Food logs were distributed to all participants to record their food and fluid intake for 24 h prior to each trial. Participants were asked to replicate the first trial's dietary intake for the subsequent trial. At the baseline testing visit, a list of instructions was provided to participants which included examples of portion sizes within categories of meats/cheeses, starches/breads, fruits/vegetables, combination dishes (e.g., casseroles), beverages/fluids, and miscellaneous (e.g., condiments), as well as written instructions with how to record meals. Subjects were provided a blank sheet with columns to record the amount and type of food (including brand names), along with the time of day the food was consumed. A new copy of this information was provided to subjects before the subsequent trial, along with a copy of the diet log from the first trial (in order to replicate the diet). The returned logs were reviewed with subjects, and any questions/clarifications were confirmed before the trials began. If subjects cooked multi-ingredient dishes, they were asked to provide recipes so individual breakdowns could be established. If subjects ate outside the house (restaurant, fat food, etc.), they were asked to provide the exact name of the item and location purchased. Finally, subjects were specifically instructed to record dietary logs immediately after each meal/snack (opposed to the end of the day), to ensure accuracy. All diet logs were analyzed for total kilocalorie and macronutrient intakes (Nutritionist Pro, Redmond, WA) to ensure that dietary intake was similar between trials (see Table 2). To further account for dietary intake affecting outcome measures on testing days, participants fasted 3 h prior to each trial. All participants were instructed to refrain from vigorous exercise and alcohol for 24 h prior to testing. They were also instructed to abstain from consuming caffeine 12 h prior to each trial. Participants replicated the same attire for all trials and wore clothes/shoes in which they normally exercised. Since high-intensity exercise performance is decreased during the follicular phase of menstruation [24] and estrogen levels fluctuate significantly between phases of the menstrual cycle [20], all testing took place outside of the follicular phase (minimum of 48 h after the termination of menses) in order to avoid any confounding effects due to hormonal fluctuations [25]. Both non-oral and oral contraceptive users were included in the study, as no differences in estrogen levels have been reported between these groups [26]. Subjects verbally confirmed adherence to all controls prior to the start of each supplementation trial.

Table 2 24-h dietary intake values between supplementation trials

	CM	PLA	*p* value
Total kilocalories	2259.2 ± 1146.3	2125.1 ± 676.0	.40
Carbohydrates	255.5 ± 140.8	269.5 ± 97.3	.57
Fats	92.9 ± 61.1	78.4 ± 37.6	.38
Protein	144.5 ± 107.7	126.2 ± 60.1	.50

Data are expressed as mean ± SD. All macronutrient amounts are expressed in grams

CM citrulline malate, *PLA* placebo

Procedure

This study utilized a randomized, double-blind, crossover design where participants served as their own controls. Each participant reported to the Human Performance Lab for a total of three visits. All trials were scheduled at the same time (±1 h) to ensure chronobiological control; trials were also separated by at least 1 week to allow for sufficient recovery/washout between visits [14–16]. The initial visit included signing an informed consent and completion of a health history questionnaire (to ensure all participants met inclusion criteria), demographic and body composition measurements, and establishment of 1RM for the bench press (upper-body) and leg press (lower-body) exercises. Body mass was assessed with a balance beam and height with a stadiometer (Detecto, Webb City, MO). Body fat and lean mass were measured by dual-energy X-ray absorptiometry (DXA, General Electric, Fairfield, CT). For the DXA, proper calibration procedures and quality assurance analysis were followed as previously described [27].

Next, 1RM was determined for the upper and lower body using a plate-loaded (Iron Grip, Santa Ana, CA), flat barbell bench press (Hammer Strength, Rosemont, IL) and a plate-loaded (Iron Grip, Santa Ana, CA), leg press (Hammer Strength, Rosemont, IL). Prior to 1RM testing, each participant completed a self-selected warm-up protocol, and all warm-up protocols were recorded and replicated during each subsequent visit. The 1RM protocol was carried out as previously specified [28]. To begin, the individual completed a warm-up set of 8–10 reps at 50 % of their body mass. After a 60-s rest interval, a second warm-up

set of 3–5 reps was completed at a 5–10 kg increase from the initial set. After another 60-s rest interval, the participant then completed 2–3 reps with a 5–10 kg increase from the second warm-up set and rested for an additional 120 s. Each subsequent one-repetition attempt increased resistance an additional 5–10 kg, each followed by a 120-s rest period. This final step was repeated as necessary until the participant could no longer complete one unassisted repetition with additional weight. This value was assumed to be their 1RM and was utilized to calculate exercise intensity for the two subsequent, supplementation trials.

On the following two visits, each participant consumed either the placebo (8 g dextrose, Now Foods, Bloomingdale, IL) or CM (8 g dextrose + 8 g CM, Powder City, Philadelphia, Pennsylvania) in randomized order. The same amount of dextrose was used during both testing trials in order to ensure that the CM was the only variable different between visits. As is standard practice in supplementation research, the CM provided for this investigation was third party lab tested for supplement purity and authenticity. An outside researcher mixed all supplements in a sealed shaker (blender bottle, Lehi, UT) combined with a cherry flavoring (Mio™, Northfield, IL). To ensure preservation of the double-blind design, subjects also 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-h seated rest period [14–16].

After the 1-h rest period, participants performed the same warm-up as was used during their 1RM testing and then began the weightlifting protocol (resistance exercise performance to exhaustion). Each participant first completed six sets of bench press followed by six sets of leg press at 80 % of their previously established 1RM (Fig. 1). Specifics for this protocol were determined via extensive piloting in subjects with fitness background similar to the subjects utilized in the investigation. For each set, subjects were instructed to complete as many repetitions as possible until they could no longer complete a repetition without assistance. Researchers recorded the number of repetitions participants completed during each set. A timed 60-s rest was completed after each set of the bench and leg press exercises. After completion of all six bench press sets, a 120-s rest interval was allotted before beginning the six leg press sets. Heart rate via a Polar™ (Lake Success, NY) heart rate monitor and rating of perceived exertion (RPE) via the OMNI weightlifting scale [29, 30] were also recorded through the intervention trials. Heart rate and RPE were collected at baseline, immediately after the completion of each set and after the timed 60-s rest (before the next set began).

Upon completion of the supplement intervention, participants were asked which supplement they thought they had consumed. Participants were also asked whether they experienced any side effects throughout the course of the study related to the supplement ingested.

Statistical analyses

Statistical Package for the Social Sciences (SPSS, version 20) was used to conduct all analyses. Normal distribution of data was assessed via histograms and boxplots. Descriptive statistics (mean, standard deviation, and standard error) were calculated for all data. Independent variables included the supplementation protocol (i.e., CM vs. placebo) and time (i.e., each set). Dependent variables included repetitions completed during each set of upper- and lower-body exercise (bench press and leg press, respectively), heart rate, and RPE.

A 2 (trial) × 6 (set) repeated-measures analysis of variance (RM-ANOVA) was utilized to determine differences between supplements during each set for repetitions lifted, HR, and RPE. For any significant F scores, post hoc tests with the applied Bonferroni correction were utilized. Statistical significance was set at $\alpha = .05$.

Finally, Fisher's exact test was used to indicate the ability of each participant to accurately determine which supplement they had consumed during the intervention trials ($\alpha = .05$). All values are reported as mean ± SD.

Results

Based on the weight lifted to body weight ratio [23], the participants in the current investigation were within the 70th and 90th percentiles for upper- and lower-body

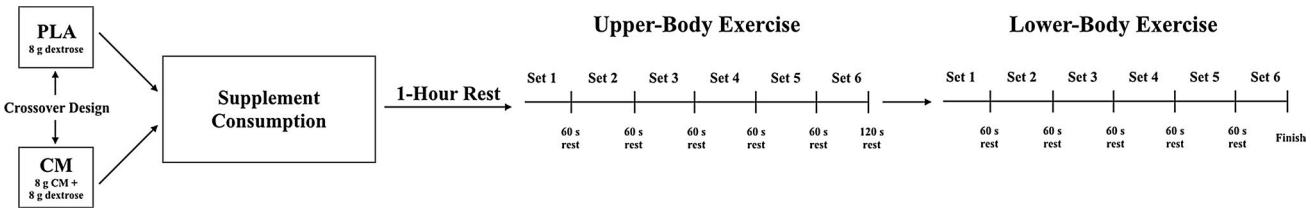

Fig. 1 Visual depiction of the exercise protocol and overall study design. *PLA* placebo, *CM* citrulline malate

strength, respectively (Table 1). This confirmed our participant's exercise habits and resistance training experience. No significant differences were observed for total kilocalorie or individual macronutrient (carbohydrates, fats, proteins) intake between trials (Table 2), indicating that dietary intake had no effect on overall results. To ensure there was no order effect associated with supplementation trials, the sequence of conditions was randomized and counterbalanced between visits (trial 1: CM, trial 2: placebo vs. trial 1: placebo, trial 2: CM).

Repetitions completed

Upper-body resistance exercise performance to exhaustion was determined via the bench press exercise (Fig. 2a). Although RM-ANOVA indicated no significant interaction between CM and PLA trials ($F = 0.85$, $p = 0.52$), significant main effects were observed for time ($F = 58.39$, $p < 0.01$; Fig. 2a) and supplementation trial ($F = 4.94$, $p = 0.045$). This indicates that when consuming CM, subjects were able to complete more repetitions overall compared to placebo (34.1 ± 5.7 and 32.9 ± 6.0, respectively).

Lower-body resistance exercise performance to exhaustion was determined via the leg press exercise (Fig. 2b). Similar to the bench press exercise, there was no significant interaction between exercise sets and supplement consumed ($F = 0.48$, $p = .79$). However, independent of supplement consumed, there was a significant main effect of time ($F = 26.43$, $p < .01$; Fig. 2b). Independent of set, there was a main effect of supplementation trial ($F = 6.06$, $p = .03$), where subjects lifted significantly more total repetitions throughout the leg press exercise versus placebo (66.73 ± 30.49 vs 55.13 ± 20.64, respectively).

Rating of perceived exertion

For upper-body exercise, immediately after completion of the bench press exercise, overall RPE (independent of set) was significantly lower ($F = 7.22$, $p = .02$) when consuming CM versus placebo (7.9 ± 0.3 and 8.6 ± 0.2, respectively; Fig. 3a). This specifies that although the subjects completed more overall bench press repetitions when consuming CM, their overall feelings of exertion were significantly lower than when consuming the placebo. Independent of supplement ingested, during upper-body exercise, RPE immediately after each exercise set (Fig. 3a) and RPE before each exercise set (i.e., after the 60-s rest interval; Fig. 3b) significantly increased over time ($F = 11.63$, $p < .01$ and $F = 12.20$, $p < .01$, respectively).

Although CM resulted in significantly greater total amount of repetitions completed during the leg press, RPE was identical between trials ($p > 0.05$). Independent

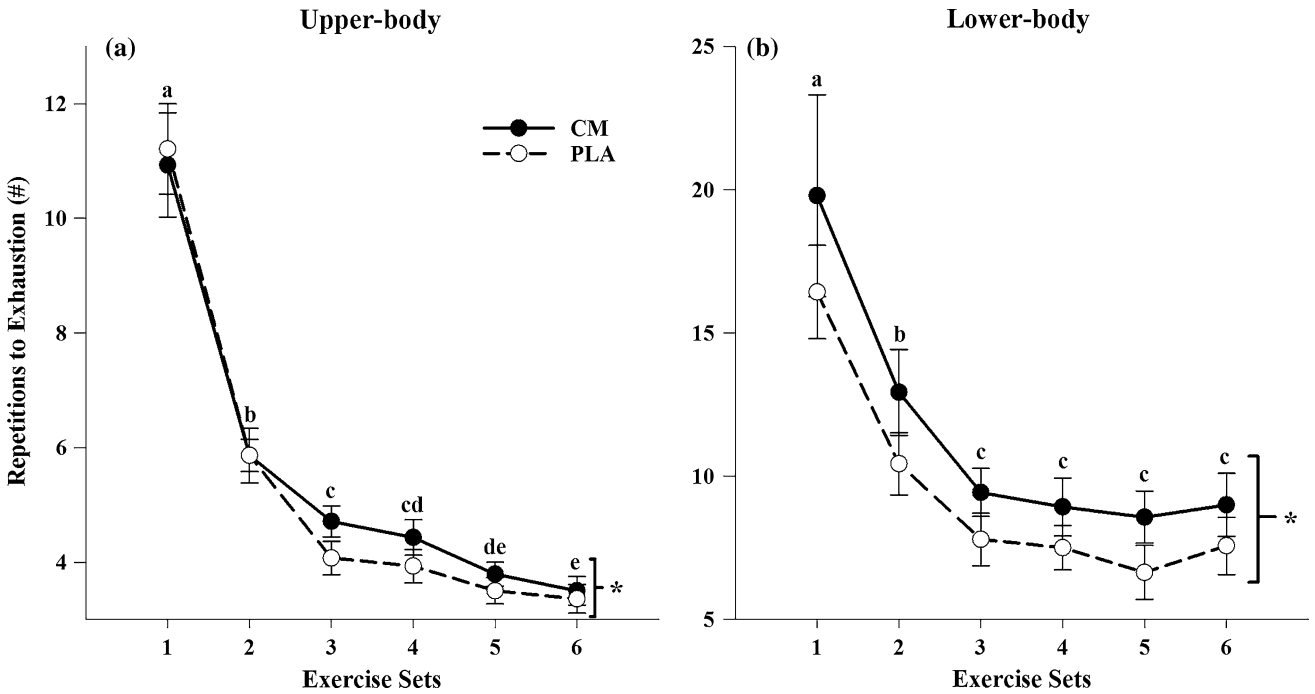

Fig. 2 Repetitions completed during **a** upper-body (i.e., bench press) and **b** lower-body (i.e., leg press) exercise. *PLA* placebo, *CM* citrulline malate. *Significantly greater overall effect of supplement trial for CM when compared to PLA ($p < .05$). *Letters* indicate significant effects of time (i.e., differences in overall repetitions completed between bench press sets). Time points with different letters are significantly different after adjusting for multiple comparisons via the Bonferroni correction. Data are expressed as mean ± SE

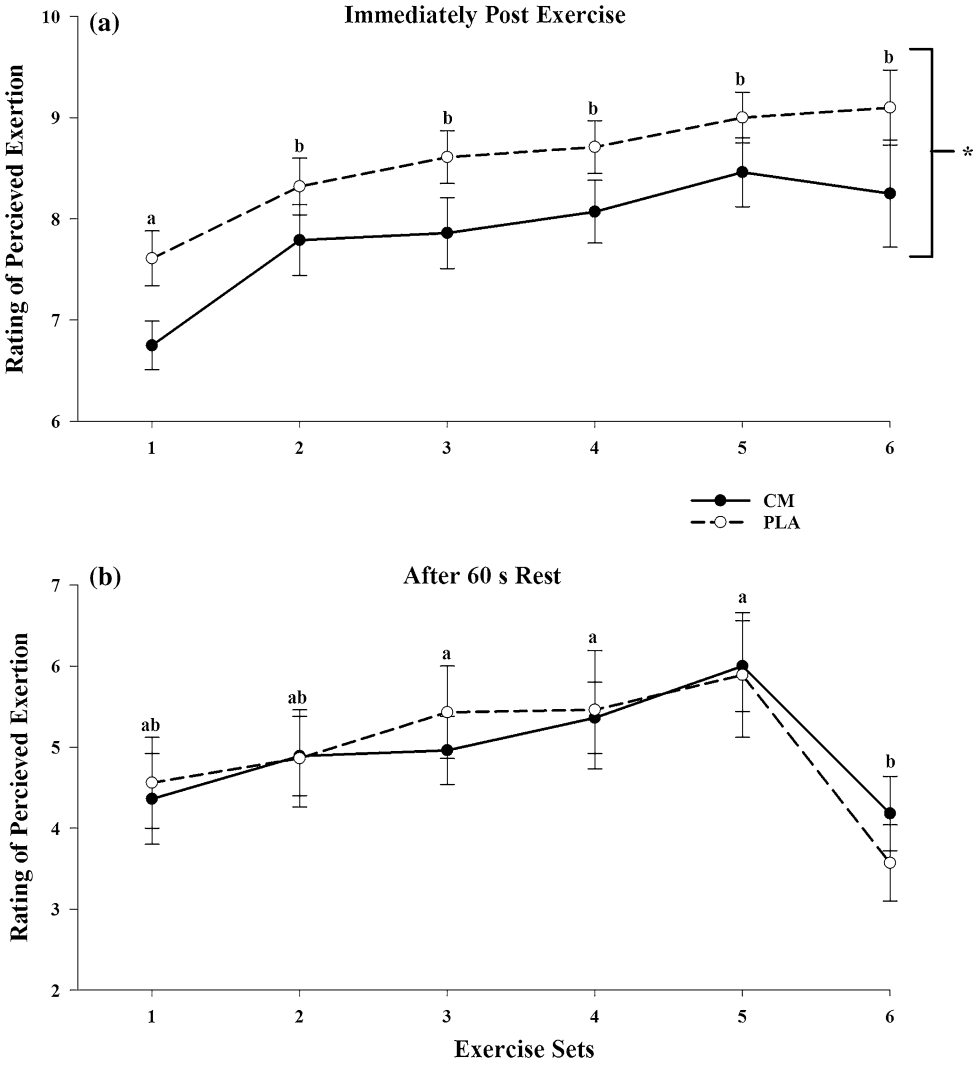

Fig. 3 Rating of perceived exertion (RPE) during upper-body exercise (i.e., bench press) **a** immediately after completion and **b** after 60-s rest interval of each exercise set. *PLA* placebo, *CM* citrulline malate. *Significantly greater overall effect of supplement trial for CM when compared to PLA ($p < .05$). *Letters* indicate significant effects of time (i.e., differences in overall RPE). Time points with *different letters* are significantly different after adjusting for multiple comparisons via the Bonferroni correction. Data are expressed as mean ± SE

of supplement, however, RPE immediately after each set of the leg press (Fig. 4a) and before each exercise set (after the 60-s rest interval; Fig. 4b) significantly increased with each set ($F = 15.18$, $p < .01$ and $F = 13.68$, $p < .01$, respectively).

Heart rate

During upper-body exercise, heart rate after the 60-s rest period was similar between sets ($F = 1.01$, $p = .42$) and trials ($F = 1.75$, $p = .21$; trial grand mean ± SD for CM versus placebo = 99 ± 17 versus 102 ± 18 bpm, respectively). When evaluating heart rate immediately after completion of each set of bench press exercise, a significant main effect of time ($F = 4.02$, $p < .01$) indicated that after set five, heart rate was significantly ($p = .02$) lower than after set one (131 ± 19 and 138 ± 20 bpm, respectively); however, there were no other differences in heart rate between sets. The lower heart rate may be due to the decreased number of repetitions performed during the later exercise sets as fatigue becomes more prevalent.

In the leg press, the supplement consumed did not affect the heart rate responses after each 60-s rest period ($F = 0.44$, $p = .52$). However, there was a significant main effect of time ($F = 3.78$, $p < .01$), where heart rate was significantly greater in set five compared to set six (136 ± 21 and 129 ± 21 bpm, respectively). Similar to the bench press exercise, the lower heart rate in set six may be due to the lower number of repetitions performed during the later exercise sets. Finally, heart rate immediately after the completion of each set was not different between sets ($F = 0.83$, $p = .54$) or trials ($F = 1.91$, $p = .20$) for lower-body exercise (grand means of heart rate through all six sets = 135 ± 21 and 133 ± 19 bpm).

Fisher's exact test indicated that the participants were unable to accurately assess which supplement they had consumed based on a 2 (supplement guess) × 2 (accuracy) analysis ($p = .50$). Accurate guesses for the trials were

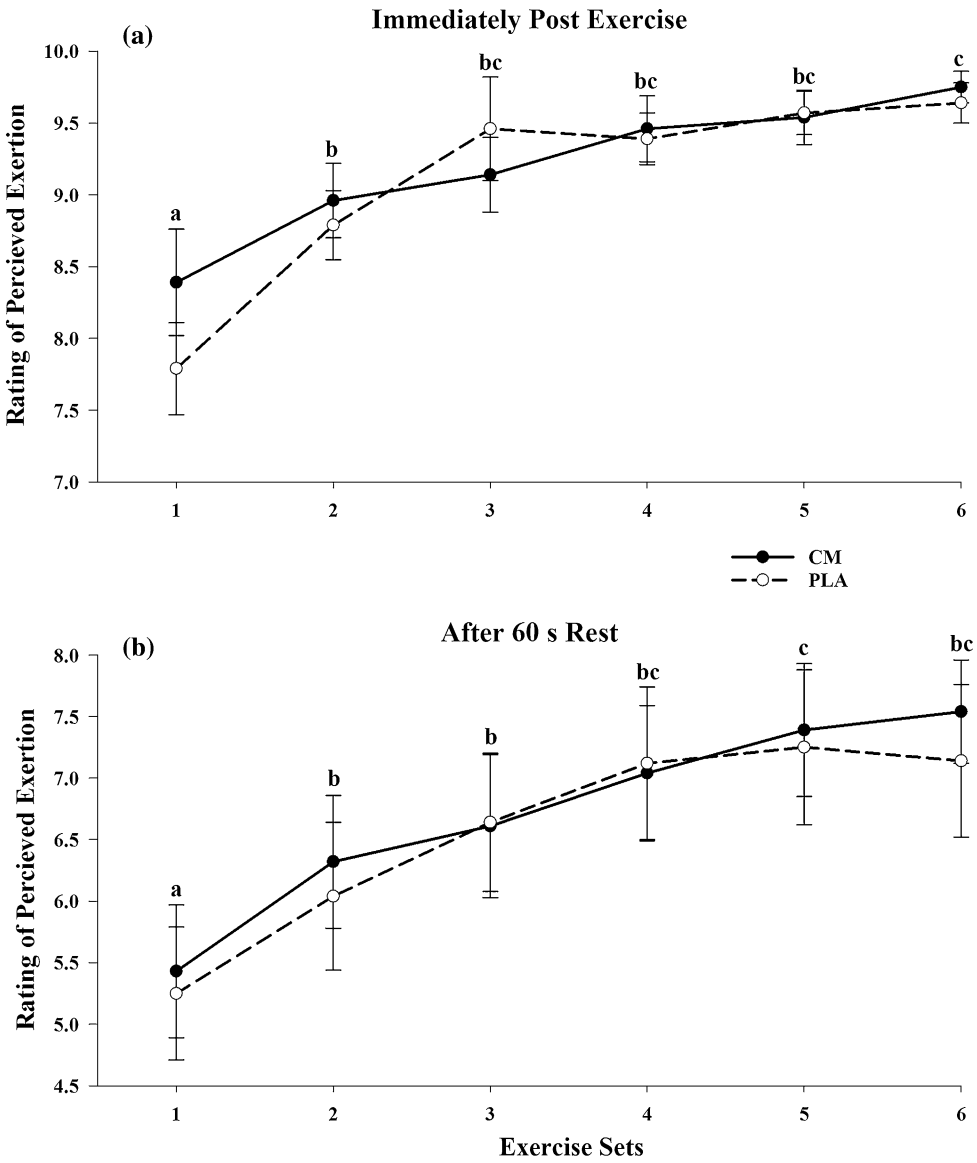

Fig. 4 Rating of perceived exertion (RPE) during lower-body exercise (i.e., leg press) **a** immediately after completion and **b** after 60-s rest interval of each exercise set. *PLA* placebo, *CM* citrulline malate. *Letters* indicate significant effects of time (i.e., differences in overall RPE). Time points with *different letters* are significantly different after adjusting for multiple comparisons via the Bonferroni correction. Data are expressed as mean ± SE

recorded at 53 %. One participant reported feelings of gastrointestinal discomfort throughout the course of the intervention. However, this was reported during the CM and placebo trials so it is unlikely that the CM was responsible for the self-reported discomfort.

Discussion

The purpose of this investigation was to evaluate the ergogenic effects of an 8 g acute dose of exogenous CM supplementation on upper- and lower-body, submaximal resistance exercise performance to exhaustion in trained female weightlifters. *To our knowledge, this is the first study evaluating the effects of an acute 8 g CM dose on submaximal resistance exercise performance to exhaustion in females*. Our a priori hypothesis was that CM supplementation would increase resistance exercise performance to exhaustion (repetitions completed) during both upper- and lower-body exercises when compared to a placebo. Regarding upper-body exercise, our initial hypothesis was supported as subjects completed significantly more total repetitions throughout the bench press exercise sets at 80 % 1RM when consuming CM. For lower-body exercise, our hypothesis was also supported as subjects completed significantly more total repetitions throughout the leg press sets at 80 % 1RM when consuming CM compared to the placebo.

During the bench press, CM increased performance throughout the exercise sets (Fig. 2a). Although these data are in contrast to the results in females by Cutrufello et al. [18], the previous investigation utilized a lesser acute dose

(6 g), which may not have been sufficient to elicit performance benefits (compared to the 8 g dose used in the current study). When expressed relatively by body mass, Cutrufello et al. [18] supplemented 0.09 g/kg compared to 0.12 g/kg in the current investigation. It is also important to note that Cutrufello et al. [18] supplemented with L-citrulline independent of malate which may have also been the reason for the lack of performance increases. However, the current results are similar to previous data collected in male participants similarly performing the bench press at 80 % 1RM [16], demonstrating that an acute dose (8 g) of CM improves submaximal resistance exercise performance to exhaustion (i.e., the number of repetitions that can be completed during repeated sets of submaximal upper-body resistance exercise to fatigue). In practice, these results have external application such as determining draft status in elite prospects. For example, the National Football League uses the 225-lb bench press test (submaximal repetitions to failure) to help predict level of professional success, which, in turn, directly effects an athletes earning potential [31, 32]. Moreover, collegiate basketball athletes demonstrating greater levels of upper-body submaximal strength collected significantly more blocks per game over the course of two consecutive seasons [33]. As a result, the ability to increase submaximal strength can have important implications for predicting sport performance and ultimately determining success.

Interestingly, the females appeared to experience greater benefits from CM supplementation during lower-body resistance exercise performance to exhaustion when compared to upper-body exercise (3 vs. 17 % increase in repetitions completed when taking CM, respectively). The discrepancies in upper- and lower-body performance may be due to differences in females' muscle mass distribution. Females have a larger proportion of their total body skeletal muscle mass in the lower-body musculature [34, 35], and this may be indicative of the performance differences between the bench press and leg press exercises. Regardless of the type of training regimen (i.e., upper- and/or lower-body resistance exercise), if CM supplementation acutely increases work completed during a single exercise bout, longitudinal consumption could ultimately demonstrate greater strength gains; however, longitudinal training effects of CM have yet to be elucidated.

In this investigation, we also present the first data evaluating the effects of CM on RPE. When examining RPE immediately after the completion of upper-body exercise sets (Fig. 3b), subjects reported lower overall feelings of exertion (8 %) when consuming CM. These results are important because subjects also completed more total work during the bench press exercise sets. The ability to demonstrate lower perceived exertion for a greater work output has attractive implications for performance. It is proposed that exercise is terminated when feelings of discomfort outweigh the potential rewards [36], and CM appears to decrease feelings of discomfort which might allow for increased reward (i.e., increased number of repetitions completed). As exercise is concluded when exertional stress reaches a certain threshold, CM supplementation may increase the threshold so that more work can be completed before reaching the maximal limit of exercise termination.

It has been previously proposed that CM does not act as a stimulant [15] and this is supported by the fact that resting heart rates were not different between CM and placebo trials. There were also no differences in heart rates between trials at any point during the exercise interventions. This is similar to previous data demonstrating that supplements designed to increase nitric oxide production do not affect heart rate during rest [37] or exercise [15].

Considerations and limitations

The results of the current investigation are consistent with previous data reporting that acute supplementation of CM increases upper- and lower-body submaximal resistance exercise performance to exhaustion [14–16]. Neither nitric oxide production nor blood flow was directly measured during the trials; therefore, discussion about the proposed mechanisms of action is theoretical. Future research is required to develop mechanistic support for the observed increases in performance. The exact mechanisms with which exogenous CM affects performance have yet to be confirmed; however, multiple theories have been proposed. A well-supported hypothesis revolves around L-citrulline's role in the nitric oxide pathway [12]. Unlike its downstream counterpart, L-arginine, L-citrulline can bypass hepatic metabolism and therefore may be a more efficient method of increasing levels of extracellular L-arginine [38], ultimately leading to increased nitric oxide production. When combined with malate (intermediate component of the tricarboxylic acid cycle), L-citrulline also appears to increase the rate of adenosine triphosphate production [39], mitochondrial respiration, and muscle blood flow during exercise [40]. Taken together, it may be these physiological changes leading to enhanced physical ability. Finally, the amino acid creatine is synthesized from L-arginine at a rate of about 1–2 g/d, and L-arginine has been suggested to increase creatine delivery to skeletal muscle based on the ability to increase muscle blood flow [41–43]. As CM is a precursor to L-arginine, this may also be a potential mechanism by which CM supplementation may increase physical ability; however, before these hypotheses can be confirmed, more mechanistic research is required to determine how CM enhances resistance exercise performance to exhaustion.

Reports have indicated that some participants may experience gastrointestinal discomfort associated with exogenous ingestion of supplemental CM. In the work by Perez-Guisado and Jakeman [16], 15 % of male participants (6 out of 41) reported stomach discomfort after consumption of an 8 g acute dose. In contrast, another investigation reported no side effects or discomfort with the same dosage [15]. Our results correspond with the work by Wax and colleagues, as the only participant experiencing gastrointestinal discomfort reported similar feelings during both trials. This indicates that it was the dextrose and/or the artificial flavoring causing the discomfort. Nevertheless, the safety associated with long-term consumption of CM is unknown, and supplements available to consumers are not required to be tested for banned substances and/or exact amounts of the active ingredient. Therefore, care should be taken when purchasing over-the-counter CM and when considering continual use.

As these data were only collected in female participants with resistance training experience, these data cannot be extrapolated to untrained populations. Subjects were not familiarized to the testing procedures; however, based on similar previous methodologies [15, 16] and the counterbalanced nature of the research design, it is not expected that a learning effect altered the results. The fact that subjects were unable to correctly guess which supplement they consumed during each trial also supports the absence of a trial effect associated with the final outcomes. It is also important to note that the females in this study were tested outside the follicular phase of the menstrual cycle (high concentrations of estrogen and progesterone), and results may differ if tested during the follicular phase (when estrogen and progesterone are lowest). However, evaluation outside the follicular phase provides a broader clinical relevance as females spend only ~25 % in the follicular phase [20]. Future research should investigate CM supplementation during the follicular versus luteal phases to determine whether estrogen levels modify the effects of CM on performance.

Conclusion

In conclusion, the results from this investigation provide preliminary evidence, suggesting that acute intake of CM may increase upper- and lower-body submaximal resistance exercise performance to exhaustion in young resistance-trained females; however, these benefits appear to be greater when performing lower-body exercise. When consuming CM, RPE was attenuated during upper-body exercise while exercise volume increased. These outcomes have appealing implications for athletes, as acutely consuming CM before exercise may increase training volume during a single workout. As such, acute increases in volume with each workout may longitudinally elicit enhanced strength gains; however, future investigations are necessary to evaluate the long-term training effects of CM.

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