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The Ergogenic Effects of Acute Citrulline Malate Supplementation on Weightlifting Performance in Trained Females

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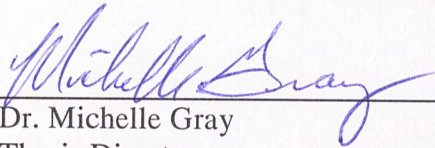
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Weightlifting Performance in Trained Females

A thesis submitted in partial fulfillment
of the requirements for the degree of
Bachelors of Science in Education

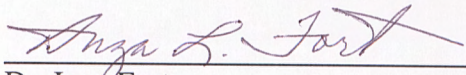
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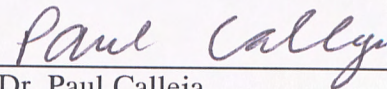
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The Ergogenic Effects of Acute Citrulline Malate Supplementation on
Weightlifting Performance in Trained Females

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Abstract

All previous investigations evaluating the effectiveness of citrulline malate (CM) as an ergogenic aid involved male subjects. The purpose of this investigation was to evaluate the ergogenic effects of CM supplementation on upper- and lower-body, submaximal, resistance exercise performance in trained females. Based on previous results, we hypothesized supplementation with CM would increase performance in females. This study utilized a randomized, double blind, crossover design. Testing trials took place within the Human Performance Laboratory and/or the University of Arkansas: Donna Axum Fitness Center. An *a priori* sample of 14 subjects were required, therefore this study included 15 female. Subjects reported for three visits. Visit one included demographic/body composition, and one-repetition maximum (1-RM) measurements. On subsequent visits, subjects consumed CM (8 g dextrose+8 g CM) or placebo (8 g dextrose) prior to beginning exercise. The protocol included: six sets of upper-body exercise and six sets of lower-body exercise at 80% 1-RM with one minute rest between each upper- and lower-body set and two minutes rest when transitioning from upper- to lower-body exercises. Repeated measures ANOVA determined trial differences. Paired-tests evaluated differences between total repetitions, initial and final halves, and the initial, middle, and final thirds of each exercise between trials. During the final half of upper-body exercise, subjects completed significantly ($p = .04$) more repetitions when consuming CM (12.13 ± 2.85) compared to placebo (11.13 ± 2.75). Similar results were observed during lower-body exercise. Total repetitions (66.73 ± 30.49 vs. 55.13 ± 20.64 , $p = .03$), repetitions completed during the middle third (18.36 ± 6.71 vs. 15.29 ± 5.78 , $p = .05$), final third (17.57 ± 7.19 vs. 14.21 ± 7.15 , $p = .02$), and final half (26.50 ± 10.70 vs.

21.71±9.76, $p = .04$) of exercise were significantly greater for CM compared to placebo, respectively. In trained females, CM supplementation increased performance during submaximal resistance exercise. These data have attractive implications for female athletes competing in sports with strength-based requirements.

Keywords: ergogenic aids, Citrulline malate, nitric oxide, and exercise performance in females, strength training, and all related derivatives.

Introduction

Ergogenic aids have become common practice in today's exercise regimens (Maughan, Depiesse, & Geyer, 2007) and there are many available supplements. One section of ergogenic aids which is gaining in popularity, are those documented to increase production of nitric oxide. Nitric oxide is best known as a vasodilator, which helps to regulate blood flow and mitochondrial respiration, particularly during exercise (Bescos et al., 2009; Bescos, Sureda, Tur, & Pons, 2012; Petrovic et al., 2008). Increasing nitric oxide in muscles can offer positive effects both before and after exercise including increased glucose uptake, muscle contractility, muscle blood flow, and repair via satellite cell activation (Petrovic et al., 2008). The combination of these effects makes nitric oxide an important area of study with regards to athletic performance.

Nitric oxide (NO) cannot be consumed in a supplement. NO has a short half-life in the blood stream and therefore, intermediates of the nitric oxide pathway must be supplemented instead. Citrulline malate (CM) is most commonly recognized for the role it plays in the nitric oxide pathway (Sureda et al., 2009). Citrulline malate (CM) is made from the combination of l-citrulline and malate and functions as the precursor to l-arginine in vivo (Rouge, Des Robert, Robins, Le Bacquer, Volteau, De La Cochetiere, &

Darmaun, 2007a). L-citrulline is a nonessential amino acid produced in the body, but unlike l-arginine, it can avoid being metabolized and is transported to the kidneys where it can be made into l-arginine (Bescos et al., 2012). 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 provide additional ergogenic effects (Bescos et al., 2012). L-arginine has been utilized as a method to increase nitric oxide production; however, orally supplementing l-arginine is ineffective (Bescos et al., 2009; Perez-Guisado & Jakeman, 2010). These mechanisms allow for an increase of nitric oxide in the blood and, therefore, positive ergogenic effects before and after exercise.

There is limited research available on the ergogenic effects of CM supplementation. Researchers suggest that CM increases athletic performance and helps reduce muscle soreness among males using an upper-body exercise routine when performing bench press repetitions to fatigue (Perez-Guisado & Jakeman, 2010). Recent data have evaluated similar dosing protocols in males when incorporating lower-body resistance exercise and has been found to reduce fatigue (Wax, Kavazis, Weldon, & Sperlak, 2014). In an upper-body resistance exercise protocol, CM was found to be useful in increasing athletic performance in high intensity anaerobic exercise as well as diminishing muscle soreness. CM has also been found to increase muscular force (Perez-Guisado & Jakeman, 2010). Additionally, CM has been implicated in increased aerobic energy production (Bendahan et al., 2002). There is some existing data available studying the effects of CM on exercise performance, but the findings only involve male subjects (Wax et al., 2014; Wax, Kavazis, & Lockett, 2015). Therefore, the purpose of

this study was to examine the ergogenic effects of acute oral CM supplementation on weightlifting performance in trained females.

Literature Review

Background

Ergogenic aids are becoming commonplace in today's fitness culture. An ergogenic aid is defined as a nutritional supplement that enhances an individual's exercise capacity (Perez-Guisado & Jakeman, 2010). Supplementing with ergogenic aids is gaining popularity among today's athlete population (Calfee & Fadale, 2006). In order to be able to use supplementation in a healthy and beneficial manner, it is imperative to understand how they work in the body and in different populations. There are many categories of supplements and different subsets among those categories, which can be effective depending on what each individual is looking to achieve. One common type of supplementation is the consumption of ergogenic aids prior to beginning a workout routine. A pre-workout supplement that is gaining popularity as a means to increase exercise performance is CM. CM is composed of l-citrulline and malate (Perez-Guisado & Jakeman, 2010; Wax et al., 2014; Wax et al., 2015).

A search of Medline (PubMed), and Google Scholar was conducted using the keywords *ergogenic aids, Citrulline malate, nitric oxide, and exercise performance in females, strength training*, and all related derivatives. This review included randomized control studies that assessed the influence of CM on exercise performance in women. No restriction was placed on the year or language in which the article was originally published. All intervention trials were placebo controlled. After thorough search, 87

articles were initially observed and then the list was edited down to 41 relevant articles to be included in the review.

Exercise Performance in Women

Physical activity is a staple in achieving overall health at any age. Exercise has been implicated in chronic disease prevention and may enhance the immune system (Feigenbaum & Pollock, 1999). There are many ways to accomplish better fitness; one in particular is resistance training. This type of training can be catered to an individual's specific goals by adjusting the frequency and volume of training as well as the mode of training (Pollock et al., 1998). In college-aged women, weight training shows significant gains in muscle strength as well as significant increases in bone density. Bone density is an important measure and risk factor of osteoporosis (Lohman, 1982). In another study, weight training specifically was shown to have a positive effect on body image (Henry, Anshel, & Michael, 2006). Also, in trained females, while maintaining total body mass, resistance training has also been implicated in increasing fat-free mass and decreasing overall body fat percentage. Additionally, resistance training has been shown effective in preventing additionally injury (Kraemer, Fleck, & Deschenes, 2012).

Supplementation in Women

Supplementation research is a relatively new field in exercise science, and supplementation studies geared toward women in exercise science are lacking. Some available research covers creatine-monohydrate (Zuniga et al., 2012), caffeine (Goldstein, Jacobs, Whitehurst, Penhollow, & Antonio, 2010), beta-alanine (Culbertson, Kreider, Greenwood, & Cooke, 2010), and glutamine (Rouge, Des Robert, Robins, Le Bacquer, Volteau, De La Cochetiere, & Darmaun, 2007b). In one study, creatine-monohydrate

supplementation significantly improved upper-body strength gain and decreased the body fat percentage as assessed by skinfold in female athletes participating in a resistance-training program (Brenner, Rankin, & Sebolt, 2000). Caffeine supplementation has been implicated in enhancing strength performance in resistance-trained women (Goldstein, Jacobs, Whitehurst, Penhollow, & Antonio, 2010). Beta-alanine has been implicated in the delayed onset of neuromuscular fatigue.

Citrulline Malate

L-citrulline is a nonessential amino acid involved in part of the urea cycle and malate is an intermediate in the tricarboxylic acid cycle (Bendahan et al., 2002). The name citrulline is derived from the latin word “Citrullus” which means watermelon. Citrulline was first isolated from watermelon and it is the primary dietary source. Citrulline is also found in smaller quantities in seafood, some nuts and seeds, algae, meat, rice protein and soy protein isolate (King, Mainous, & Geesey, 2008; Rimando & Perkins-Veazie, 2005). Citrulline malate can be used as an ergogenic aid because it is a precursor of l-arginine, which is an intermediate in the nitric oxide (NO) cycle. Citrulline malate is hypothesized to be successful as an ergogenic aid because it assists in the removal of ammonium which is said to contribute to fatigue; malate is part of the Krebs cycle and can avoid blocking the oxidative pathway due to ammonia and can curb the accumulation of lactic acid by redirecting it toward other uses (Perez-Guisado & Jakeman, 2010). Nitric oxide can regulate physiological functions of skeletal muscle such as glucose uptake and oxidation, mitochondriogenesis, contractile functions, blood flow and fatty acid oxidation, and muscle repair (Petrovic et al., 2008). All of these factors contribute to the effectiveness of citrulline malate as an ergogenic aid.

L-arginine was originally used to help boost nitric oxide production during exercise, but different from l-arginine, citrulline can avoid hepatic metabolism and is not catabolized by arginase enzymes. It can successfully travel to the kidneys where it can be turned into l-arginine and then used in the nitric oxide pathway (Wax et al., 2014). L-citrulline can be converted to l-arginine. Nitric oxide is synthesized by nitric oxide synthase which uses l-arginine as a substrate, but because l-arginine is susceptible to catabolism in the liver, supplementation of citrulline has been shown to increase levels of l-arginine more effectively than supplementation of l-arginine.

Research regarding CM in women is lacking at best. From studies performed with males, research indicates that CM supplementation in men has contributed to decreased soreness (Wax et al., 2014). Citrulline malate is documented to allow the individual to perform more repetitions than without supplementation, leading to larger gains (Wax et al., 2015). CM has been shown to significantly reduce fatigue and allows for faster recovery and may allow for training benefits. We hypothesize that CM will enhance upper-and lower-body exercise performance by increasing the number of repetitions the subject is able to complete.

Materials and Methods

Subjects

Based on previous literature (Wax et al., 2014; Wax et al., 2015), an *a priori* sample of 14 subjects were required for this investigation. As a result, this study included 15 females from the southern region of the United States (Table 1).

Inclusion criteria included: 18-30 years of age, classified as low-risk individuals as categorized by the American College of Sports Medicine (American College of Sports Medicine, 2013); participation in weight training a minimum of two times per week over the duration of at least one year; and refrained from CM supplementation within the last year. Before testing began, each subject completed a health history questionnaire and signed a statement of informed consent. The University's Institutional Review Board prior to testing approved all measures and procedures.

Table 1

Subject demographic data

	<u>Age</u> (Years)	<u>Height</u> (cm)	<u>Weight</u> (kg)	<u>Body Fat</u> (%)	<u>Years</u> <u>Training</u>	<u>Workouts</u> <u>Per Week</u>
Mean	22.7	162.64	67.06	22.85	5.1	5.2
(SD)	(2.9)	(19.17)	(6.96)	(5.49)	(3.9)	(0.7)

Food logs were distributed to all participants to be completed during the 24-hours leading up to each trial. To account for dietary consumption on testing days, participants fasted 3-hours prior to each trial. All participants had never ingested exogenous, supplementary CM and were instructed to refrain from vigorous exercise and alcohol for 24-hours and caffeine 12-hours prior to each trial. Subjects replicated the same attire for all trials and wore clothes/shoes in which they normally exercised. All trials began a minimum of 48-hours after the completion of their self-reported menstrual cycle in order to avoid any confounding effects due to hormonal irregularities.

Procedures

This study utilized a randomized, double blind, crossover design where subjects served as their own controls. Each subject reported to the Human Performance

Laboratory for a total of three visits. Although visits were not required to be at the same time between subjects, all visits were at the same time \pm 1 hour within subject trials to ensure chronobiological control. 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 one-repetition maximum (1-RM) for the bench press and leg press exercises. Body mass was assessed with a balance beam and height with a stadiometer (Detecto, Webb City, MO). Body fat and lean mass were also measured by dual-energy x-ray absorptiometry (DXA; General Electric, Fairfield, CT) on the initial visit. For the DXA, proper calibration procedures and quality assurance analysis were followed as previously described (Glenn, Gray, & Vincenzo, 2014).

Next, 1-RM 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 1-RM testing, each subject completed a self-selected warm-up protocol and all warm-up protocols were recorded so they could be replicated during each subsequent visit. The 1-RM protocol was carried out as specified by the American College of Sports Medicine (American College of Sports Medicine, 2013). To begin, the individual completed a warm-up set of 8-10 reps at 50% of their body weight. 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 attempt increased resistance an additional 5-10 kg and subjects were

instructed to complete one repetition 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.

On the following visits, each subject consumed the following in randomized order: placebo (8 g dextrose, NowFoods, Bloomingdale, IL), Citrulline-Malate (8 g dextrose + 8 g CM; Powder City, Philadelphia, Pennsylvania). All supplements were mixed by an outside researcher 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 discrepancies. After consuming the provided supplement, participants underwent a 1-hour rest period (Wax, 2014; Wax, 2015, Perez-Guisado, 2010).

After a 1-hour rest period, participants performed the same warm-up as was used during their 1RM testing, and then began the weightlifting regimen. Each subject completed six sets of bench press followed by six sets of a leg press at 80% of their previously established 1-RM. 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 leg press sets. Researchers recorded the number of repetitions the participants completed during each set. Heart rate via a Polar™ (Lake Success, NY) heart rate monitor and rating of perceived exertion (RPE) via the Omni scale (Lagally & Robertson, 2006; Robertson et al., 2003) 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 rest period (before the next set began).

Upon completion of the supplementation intervention, subjects were asked as to which supplement they thought they had consumed. Subjects were also asked if 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 using histograms and boxplots. Descriptive statistics were calculated for demographic data and performance during each intervention trial.

Upper- and lower-body strength ratios were calculated as follows: strength ratio = weight lifted/body weight (American College of Sports Medicine, 2013). Independent variables included the supplementation protocol followed during each trial. Dependent variables included repetitions completed during each set of exercise (bench press and leg press), heart rate, and rating of perceived exertion.

Repeated measures analysis of variance (RMANOVA) was utilized to determine differences in dependent variables between supplementation trials for each set of resistance exercise. When significance was observed within the repeated measures, follow-up univariate analyses were utilized to determine where the differences existed. Within the RMANOVA, statistical significance was set at $\alpha < .05$ for all initial analyses; however, when conducting follow-up univariate analyses within the repeated measures, the Bonferroni correction was applied to account for multiple comparisons ($.05/2 = .025$).

Total number of repetitions completed during each trial, total repetitions completed during the initial (sets 1-2), middle (sets 3-4), and final (sets 4-5) thirds of exercise, and total repetitions completed during the initial (sets 1-3) and final (sets 4-6) halves of exercise were calculated and evaluated via paired t-test. The percent of repetitions completed in reference to previous sets (i.e. the number of repetitions completed in set 5, compared as a percentage of the number of repetitions completed in set 1) were also calculated and compared between trials. Statistical significance was set at $\alpha < .05$ for all paired t-test analyses.

Finally, Fishers Exact Test was used to indicate the ability of each subject to accurately determine which supplement they had consumed during the intervention trials ($\alpha < .05$). All values are reported as mean \pm SD.

Results

Exercise performance was measured using bench press and leg press for the upper- and lower-body protocols respectively. The exercise protocols involved six sets to failure, which were performed at 80% of the subject's previously established 1-RM value. Overall, when consuming CM, subjects completed significantly ($p = .03$) more total repetitions during both exercise protocols as compared to placebo (101.9 ± 29.1 and 88.3 ± 21.4 repetitions, respectively, see Figure 1). There was a trend in the upper-body exercise for total repetitions completed, but the results were not significant ($p = .08$, see Figure 2). Results indicate that for the leg press exercise, supplementing with CM allowed subjects to complete significantly ($p = .03$) more repetitions than when supplementing with placebo (66.7 ± 30.5 and 55.1 ± 20.6 repetitions, respectively, see

Figure 3).

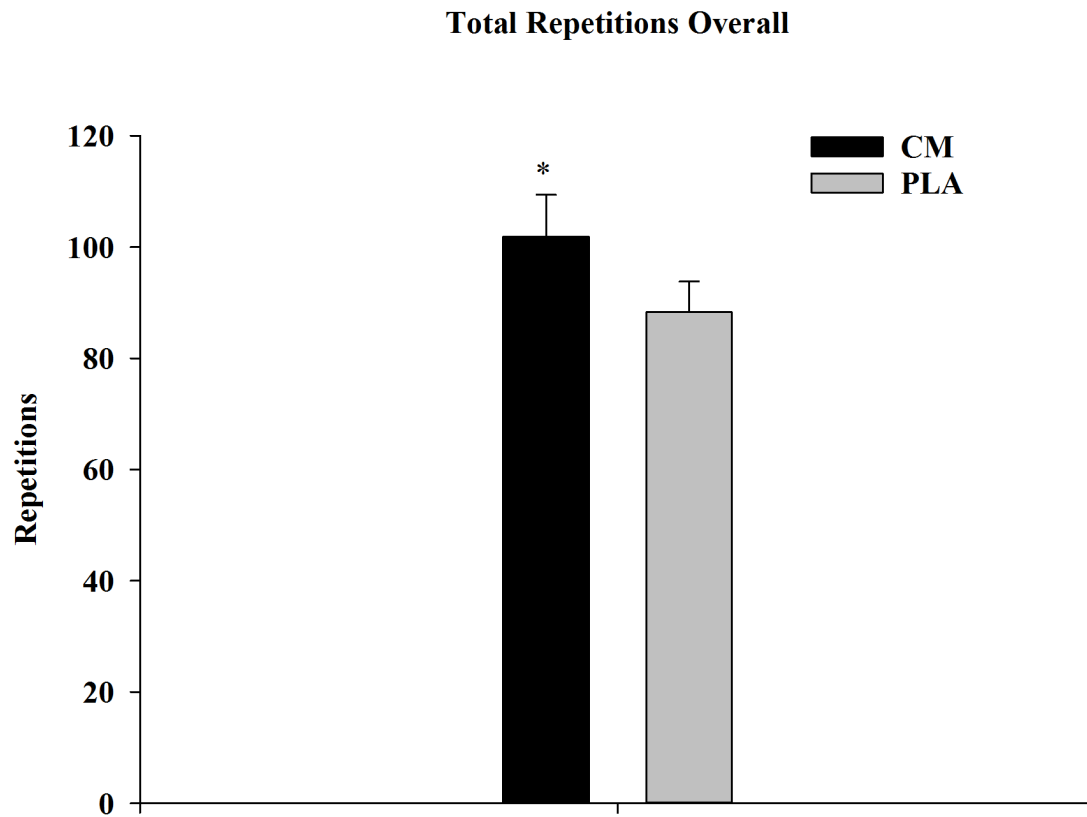


Figure 1. Mean values of total repetitions overall for each trial. Subjects completed significantly more repetitions when supplementing with CM than without. Standard errors are represented in the figure by error bars attached to each column.

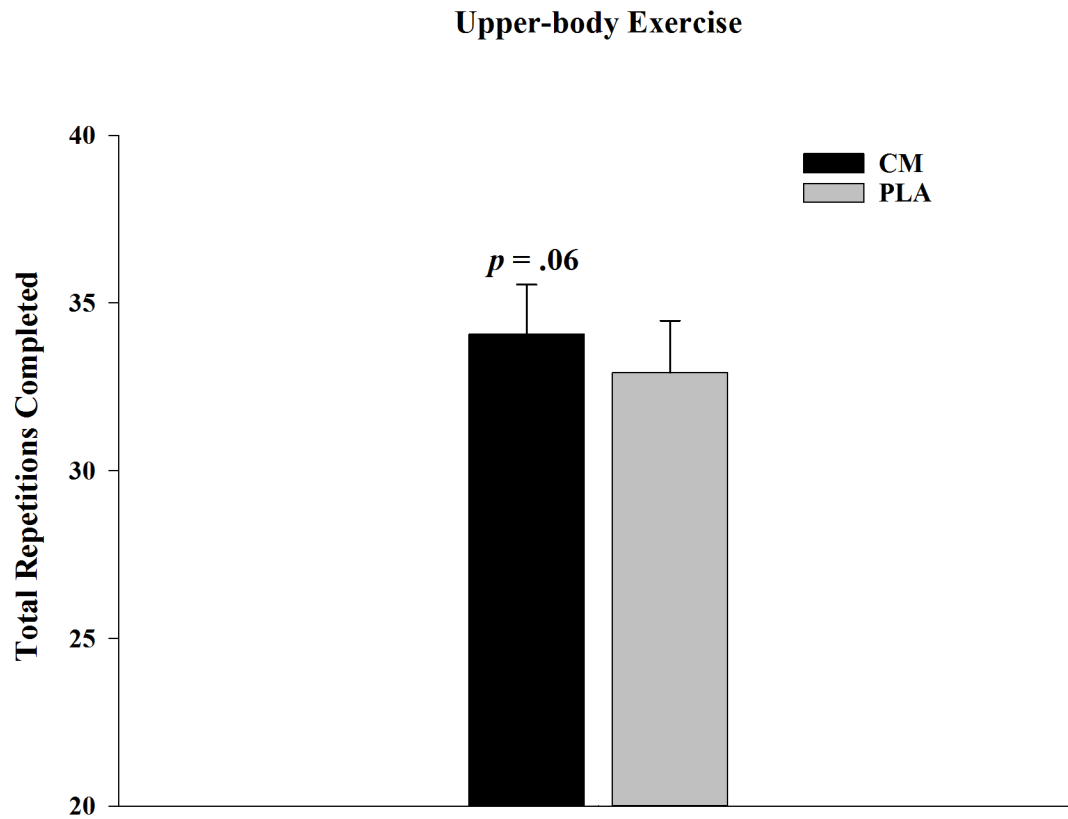


Figure 2. Mean values for total repetitions in the upper-body segment of the exercise protocol. Standard errors are represented in the figure by the bars attached to each column.

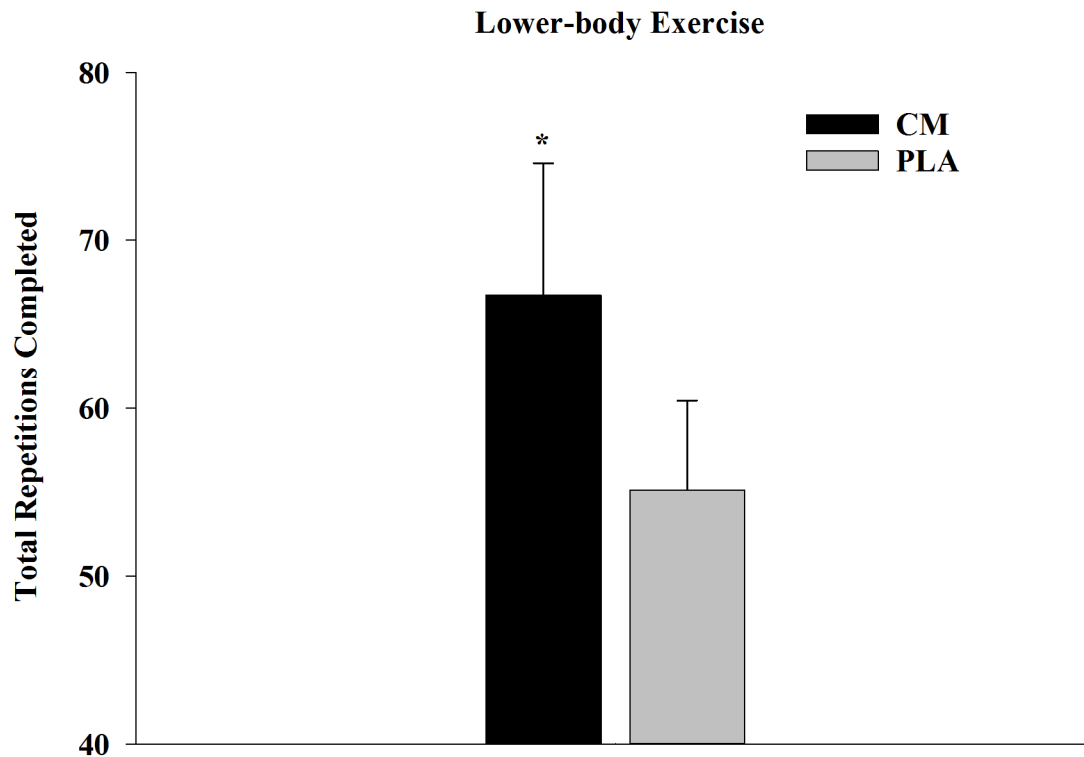


Figure 3. Mean values for total repetitions in the lower-body segment of exercise the protocol. Significance is indicated by the asterisk (*), and standard errors are represented in the figure by the error bars attached to each column.

When comparing the initial half of upper-body exercise (sets 1-3) there were no significant differences between conditions ($p = .76$). However, when supplementing with CM, subjects performed more repetitions in the latter half (sets 4-6) of exercise ($p = .038$) when compared to the placebo (12.13 ± 2.85 vs. 11.13 ± 2.75 , respectively, see Figure 4). When evaluating the initial, middle, and final thirds of exercise there were no significant differences between trials. Although it is important to note that in the middle ($p = .06$) and final third ($p = .07$), trends for significance were observed (see Figure 5).

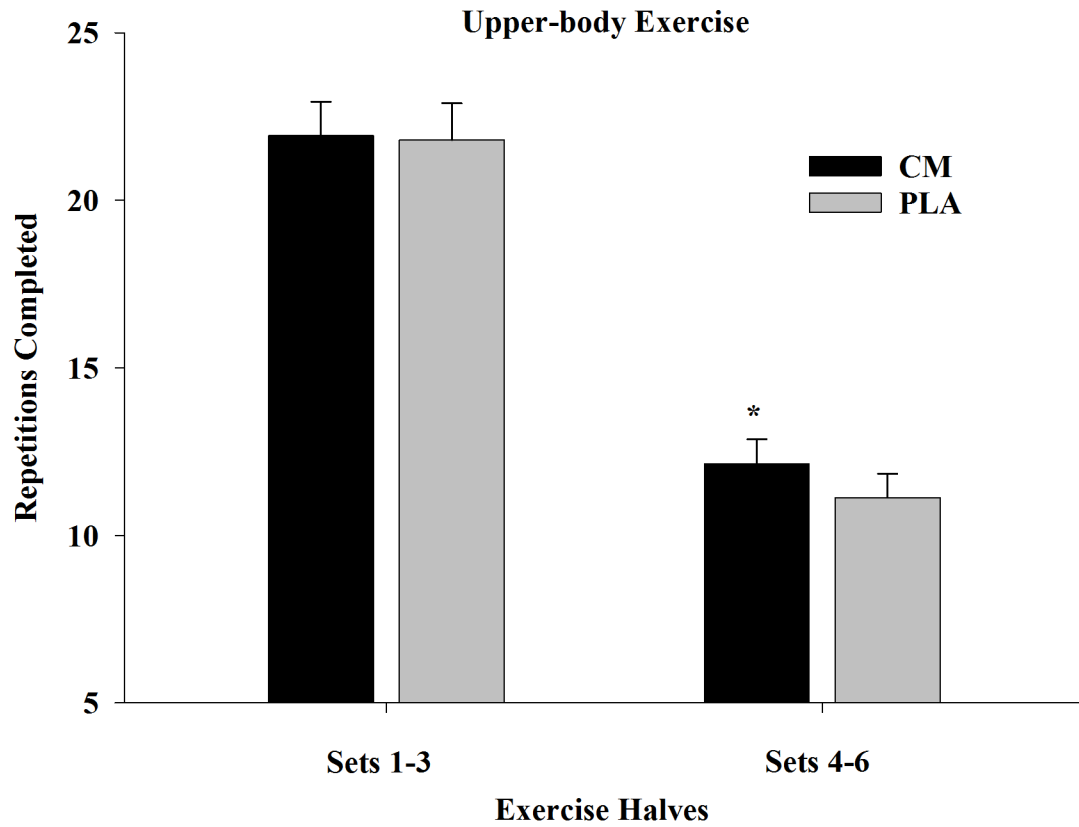


Figure 4. Mean values representing the halves (sets 1-3 and 4-6) of upper-body exercise. Significance is indicated by the asterisk (*). Standard errors are represented in the figure by the error bars attached to each column.

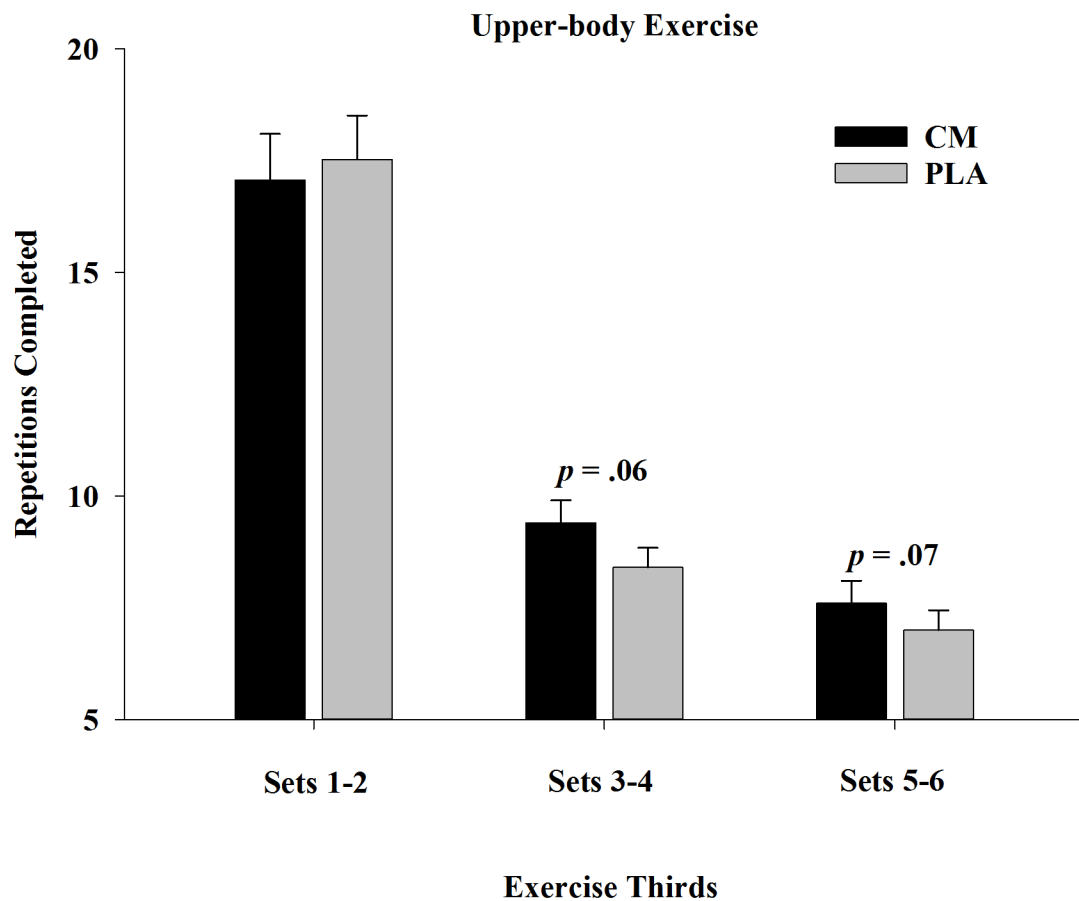


Figure 5. Mean values representing thirds (sets 1-2, 3-4, 5-6) of upper-body exercise. Standard errors are represented in the figure by the error bars attached to each column.

Considering the initial half of lower-body exercise (sets 1-3), a significant trend was observed ($p = .05$). However when supplementing with CM, there was a significant difference in the second half of exercise ($p = .04$) when compared to placebo (26.5 ± 10.704 vs. 21.7 ± 9.77 respectively, See Figure 6). When considering the initial, middle, and final thirds of exercise, there were significant differences in the middle ($p = .048$) and

final ($p = .02$) thirds of exercise when supplementing with CM as compared to placebo (18.4 ± 6.71 vs. 15.3 ± 5.784 and 17.57 ± 7.19 vs. 14.21 ± 7.15 respectively see Figure 7)

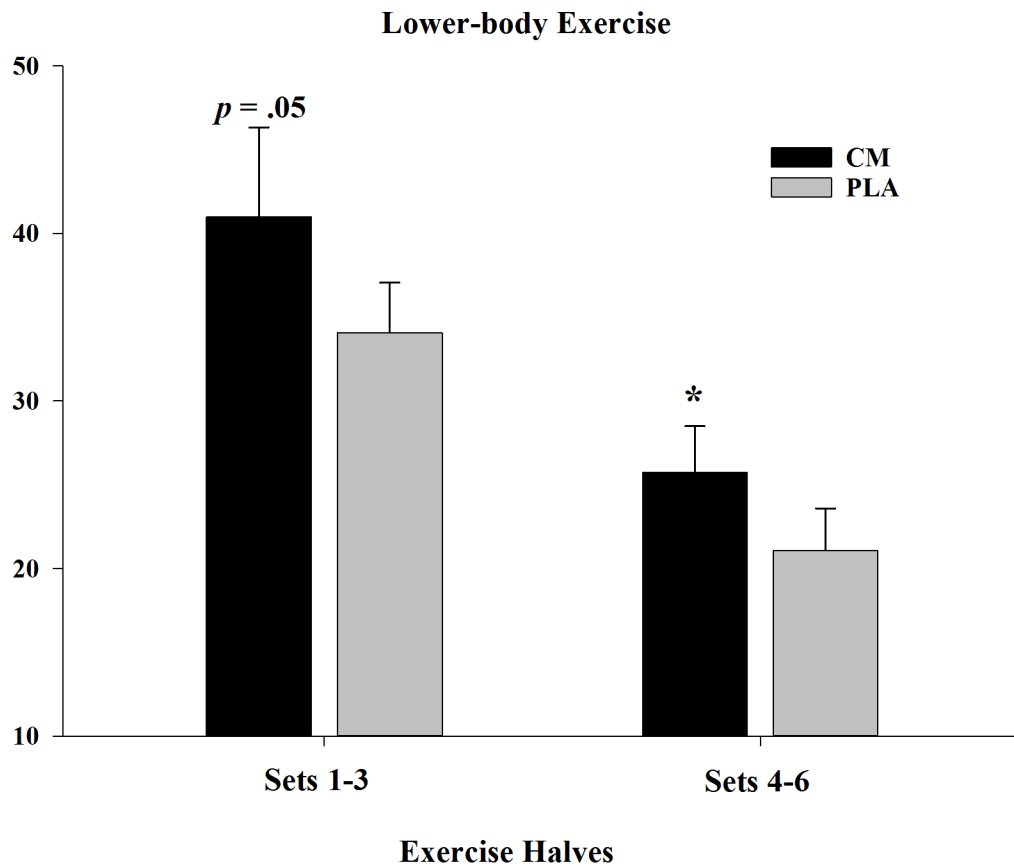


Figure 6. Mean values representing halves (sets 1-3, 4-6) of lower-body exercise. Significance is indicated by an asterisk (*). Standard errors are represented in the figure by the error bars attached to each column.

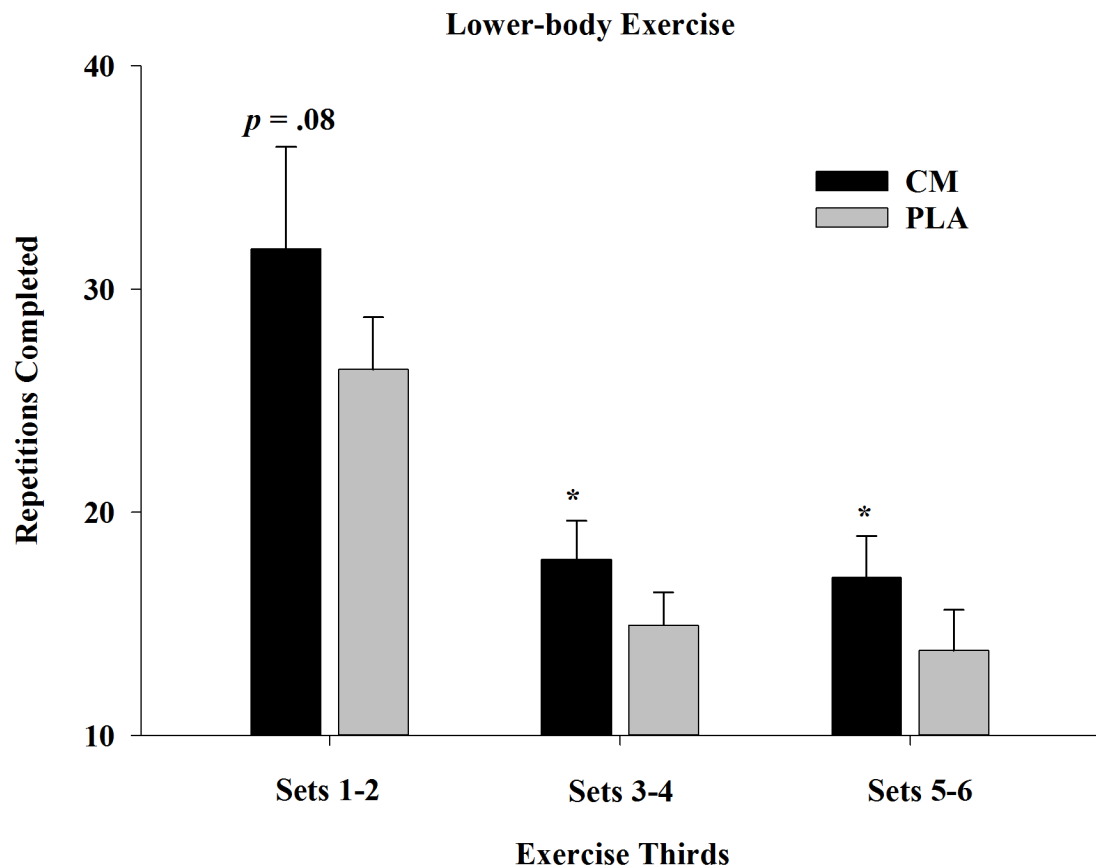


Figure 7. Mean values representing thirds (sets 1-2, 3-4, 5-6) of lower-body exercise. Significance is indicated by an asterisk (*). Standard errors are represented in the figure by the error bars attached to each column.

RPE and heart rate were also measured through the six set protocol. During upper-body exercise, although there was a significant effect of time ($F = 55.37, p < .01$) for heart rate during the supplementation trials, however, there was no significant interaction between the groups ($F = 0.52, p = .90$). With regards to time there was a similar significant effect for RPE ($F = 58.22, p < .01$) with no interaction present between the groups ($F = .66, p = .78$).

RPE and heart rate were measured throughout the six set lower-body protocol as well. There was a significant effect of time ($F = 39.393, p < .01$) for heart rate during supplementation, however, there was no significant interaction between the groups ($F = .03, p = .98$). Regarding time, there was a similar effect for RPE ($F = 41.45, p < .01$) with no interaction existing between the groups ($F = .17, p = .99$).

Discussion

In the current study, we hypothesized that citrulline malate supplementation prior to upper-and lower-body resistance protocols would enhance resistance exercise performance in resistance-trained females. This is the first study evaluating the effects of CM in women. Our results suggest that CM supplementation may augment performance in latter parts of the exercise regimen in both upper-and lower- body exercise, allowing the subjects to perform more repetitions when compared to placebo supplementation.

Subjects who supplemented with CM performed significantly more repetitions in the second half of upper-body exercises as compared to the first and there was a trend for significance in the middle and final thirds of upper-body exercise. Existing data suggest that in adult males, there was a significant increase in the number of repetitions performed when consuming CM as compared to placebo (Perez-Guisado & Jakeman, 2010). Additionally, results of another study indicate that while there were no significant differences in the individual sets within each exercise, there were significant findings that subjects performed more repetitions overall (Wax et al., 2015). These results indicate that CM has positive effects as an ergogenic aid and may be beneficial for use in females.

Overall, subjects performed significantly more repetitions in the lower-body exercise routine when consuming the CM supplement as compared to the placebo. More specifically, there were significant differences between the number of repetitions completed in the latter half and middle and final thirds of exercise when consuming CM when compared to the placebo. These findings are consistent with existing findings in male subjects that CM supplementation lessens fatigue, allowing the subjects to perform more repetitions during a lower-body resistance-training regimen (Wax et al., 2014).

Additionally, existing data point to a direct relationship between increasing heart rate and increasing intensity of exercise. (Keisler & Armsey II, 2006). There were no significant differences in resting heart rates prior to the exercise bout. The results of the present study support these data, which indicate that CM does not produce a stimulant-like response, which is seen in performance enhancing substances such as caffeine. Stimulants, such as caffeine, are known to enhance exercise performance. While the specific mechanism behind the benefits of CM supplementation are not well understood.

The first of its kind, this study also evaluated each subject's rating of perceived exertion (RPE) during the resistance training protocols. RPE follows a similar trend to heart rate in that it was not found to be significantly different between the two trials. This shows that CM supplementation does not play a role in the perceived exertion or perceived difficulty of the resistance-training task. During this study, participants produced more repetitions but reported no significant differences in RPE. This finding is particularly informative in regards to rest periods. CM makes the rest time more effective, either allowing subjects to perform more repetitions with the same amount of rest or to perform the same amount of repetitions with less rest.

These significant differences are believed to be due to the role of CM in the nitric oxide pathway. Nitric oxide (NO) is important in exercise because it increases blood flow during exercise. An increase in blood flow during exercise has important implications regarding recovery time and the sensations of soreness as well as yielding higher exercise performance (Bescos et al., 2012; Perez-Guisado & Jakeman, 2010). For these reasons, nitric oxide was thought to be useful as an ergogenic aid. In an effort to increase NO in the body, l-arginine was used as an ergogenic aid. When cellular conditions are acceptable, l-arginine is oxidized to NO in the body. The body naturally produces l-arginine in the kidneys and the liver, and it was believed that by supplementing with additional l-arginine, there would be an increase in NO. However, l-arginine is also involved in other reactions in the body, namely metabolic ones, being a crucial intermediate in the ammonia cycle (Bescos et al., 2012). However, when used as a supplement alone, the data were inconclusive. It showed some gains in acute supplementation but could not be linked to NO, and another study showed no gains. L-Citrulline is relevant because it is a precursor to l-arginine. Different than l-arginine, l-citrulline can avoid hepatic metabolism and is not degraded by arginase enzymes. This unique aspect of citrulline may make it a more effective way of increasing nitric oxide concentration than l-arginine. L-citrulline supplementation alone has not been deemed effective (Hickner et al., 2006), but when combined with malate leads to significant increases in oxidative ATP production. Individuals who supplement with CM have seen an increase in the rate of recovery and an increase in performance in resistance training exercise (Bescos et al., 2012). This is believed to be especially valuable information because of how CM affects rest periods. CM allows the body to capitalize on the time

allotted for rest either allowing subjects to rest for shorter amounts of time and achieve the same gains or allowing subjects to complete more repetitions given the same amount of rest. Both of these options could allow for increased resistance trained exercise performance.

The results of the present study are consistent with the existing data (Wax et al., 2014; Wax et al., 2015) However, the link between citrulline malate and increases in blood flow has not been established. Based on previous results in males, future research needs to be conducted to establish that connection. Further studies should include measuring blood levels with finger sticks or using a Flow Mediated Dilation ultrasound to measure the blood flow directly.

We present the first data evaluating the ergogenic effects of CM in trained women. Results found that with CM supplementation, the subjects performed more overall repetitions than when supplementing with the placebo. The results of this study are important because they are the first data collected in females, and they provide information for practical use. CM supplementation could be a valuable tool for athletes, particularly between the ages of 18-30, to increase exercise performance in sports or events that require a resistance-training component.

References

- American College of Sports Medicine. (2013). *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins.
- Bendahan, D., Mattei, J. P., Ghattas, B., Confort-Gouny, S., Le Guern, M. E., & Cozzone, P. J. (2002). Citrulline/malate promotes aerobic energy production in human exercising muscle. *British Journal of Sports Medicine, 36*(4), 282-289.
- Bescos, R., Gonzalez-Haro, C., Pujol, P., Drobnic, F., Alonso, E., Santolaria, M. L., . . . Galilea, P. (2009). Effects of dietary L-arginine intake on cardiorespiratory and metabolic adaptation in athletes. *International Journal of Sport Nutrition, 19*(4), 355-365.
- Bescos, R., Sureda, A., Tur, J. A., & Pons, A. (2012). The effect of nitric-oxide-related supplements on human performance. *Sports Medicine, 42*(2), 99-117.
- Brenner, M., Rankin, J. W., & Sebolt, D. (2000). The effect of creatine supplementation during resistance training in women. *The Journal of Strength & Conditioning Research, 14*(2), 207-213.
- Calfee, R., & Fadale, P. (2006). Popular ergogenic drugs and supplements in young athletes. *Pediatrics, 117*(3), 577-589.
- Culbertson, J. Y., Kreider, R. B., Greenwood, M., & Cooke, M. (2010). Effects of beta-alanine on muscle carnosine and exercise performance: A review of the current literature. *Nutrients, 2*(1), 75-98.

Feigenbaum, M. S., & Pollock, M. L. (1999). Prescription of resistance training for health and disease. *Medicine and Science in Sports and Exercise*, 31, 38-45.

Goldstein, E., Jacobs, P. L., Whitehurst, M., Penhollow, T., & Antonio, J. (2010). Research article caffeine enhances upper body strength in resistance-trained women. *J Int Soc Sports Nutr*, 7(18), 1-6.

Henry, R. N., Anshel, M. H., & Michael, T. (2006). Effects of aerobic and circuit training on fitness and body image among women. *Journal of Sport Behavior*, 29(4), 281.

Hickner, R. C., Tanner, C. J., Evans, C. A., Clark, P. D., Haddock, A., Fortune, C., . . . McCammon, M. (2006). L-citrulline reduces time to exhaustion and insulin response to a graded exercise test. *Medicine and Science in Sports and Exercise*, 38(4), 660-666. doi:10.1249/01.mss.0000210197.02576.da [doi]

Keisler, B. D., & Armsey II, T. D. (2006). Caffeine as an ergogenic aid. *Current Sports Medicine Reports*, 5(4), 215-219.

King, D. E., Mainous, A. G., & Geesey, M. E. (2008). Variation in L-arginine intake follow demographics and lifestyle factors that may impact cardiovascular disease risk. *Nutrition Research*, 28(1), 21-24.

Kraemer, W. J., Fleck, S. J., & Deschenes, M. R. (2012). *Exercise physiology: Integrating theory and application* (First ed.). Baltimore, MD: Lippincott Williams & Wilkins.

- Lagally, K. M., & Robertson, R. J. (2006). Construct validity of the OMNI resistance exercise scale. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 20*(2), 252-256.
- Lohman, T. (1982). Body composition methodology in sports medicine. *The Physician and Sportsmedicine, 10*(12), 47-58.
- Maughan, R. J., Depiesse, F., & Geyer, H. (2007). The use of dietary supplements by athletes. *Journal of Sports Sciences, 25*(S1), S103-S113.
- Perez-Guisado, J., & Jakeman, P. M. (2010). Citrulline malate enhances athletic anaerobic performance and relieves muscle soreness. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 24*(5), 1215-1222.
- Petrovic, V., Buzadzic, B., Korac, A., Vasilijevic, A., Jankovic, A., Micunovic, K., & Korac, B. (2008). Antioxidative defence alterations in skeletal muscle during prolonged acclimation to cold: Role of L-arginine/NO-producing pathway. *The Journal of Experimental Biology, 211*(1), 114-120.
- Pollock, M. L., Gaesser, G. A., Butcher, J. D., Després, J., Dishman, R. K., Franklin, B. A., & Garber, C. E. (1998). ACSM position stand: The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. *Med Sci Sports Exerc, 30*(6), 975-991.

- Rimando, A. M., & Perkins-Veazie, P. M. (2005). Determination of citrulline in watermelon rind. *Journal of Chromatography A*, 1078(1), 196-200.
- Rouge, C., Des Robert, C., Robins, A., Le Bacquer, O., Volteau, C., De La Cochetiere, M. F., & Darmaun, D. (2007a). Manipulation of citrulline availability in humans. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 293(5), 1061-1067.
- Rouge, C., Des Robert, C., Robins, A., Le Bacquer, O., Volteau, C., De La Cochetiere, M. F., & Darmaun, D. (2007b). Manipulation of citrulline availability in humans. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 293(5), G1061-7. doi:00289.2007 [pii]
- Sureda, A., Córdova, A., Ferrer, M. D., Tauler, P., Perez, G., Tur, J. A., & Pons, A. (2009). Effects of L-citrulline oral supplementation on polymorphonuclear neutrophils oxidative burst and nitric oxide production after exercise. *Free Radical Research*, 43(9), 828-835.
- Wax, B., Kavazis, A. N., & Lockett, W. (2015). Effects of supplemental citrulline-malate ingestion on blood lactate, cardiovascular dynamics, and resistance exercise performance in trained males. *Journal of Dietary Supplements, Early Online*, 1-14.
- Wax, B., Kavazis, A. N., Weldon, K., & Sperlak, J. (2014). Effects of supplemental citrulline malate ingestion during repeated bouts of lower-body exercise in advanced weight lifters. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association*, 29(3), 786-792.

Zuniga, J. M., Housh, T. J., Camic, C. L., Hendrix, C. R., Mielke, M., Johnson, G. O., . . .

Schmidt, R. J. (2012). The effects of creatine monohydrate loading on anaerobic performance and one-repetition maximum strength. *Journal of Strength & Conditioning Research*, 26(6), 1651-1656.