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ORIGINAL RESEARCH

Acute and Chronic Citrulline Malate Supplementation on Muscle Contractile Properties and Fatigue Rate of the Quadriceps

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This study compared the acute and chronic impact of citrulline malate (CM) supplementation on muscle contractile properties and fatigue rate of the quadriceps. Eighteen recreationally trained males consumed both a placebo (PL) and CM treatment for two separate dosing periods. The first experimental testing session for each dosing period was considered the baseline day, the second session the acute day, and the third session the chronic day, which followed seven consecutive days of supplementation. All testing sessions included exercising on a cycle ergometer at 50%–60% of their max power output for 30 min followed by performing the Thorstensson test on an isokinetic dynamometer. A two-way (Supplement × Time) analysis of variance with repeated measures resulted in no significant interactions ($p > .05$) (PL: baseline day, acute day, chronic day vs. CM: baseline day, acute day, chronic day) for peak power (in watts) (469 ± 81, 490 ± 97, 502 ± 99 vs. 464 ± 85, 480 ± 103, 501 ± 81); peak torque (in newton meters) (150 ± 26, 157 ± 32, 161 ± 31 vs. 149 ± 27, 156 ± 33, 161 ± 26); fatigue rate (in percentage) (57 ± 9, 57 ± 10, 58 ± 9 vs. 57 ± 10, 56 ± 9, 58 ± 9); and heart rate (in beats per minute) (156 ± 17, 146 ± 13, 146 ± 9 vs. 155 ± 11, 146 ± 11, 146 ± 9). The results of this study suggest that neither acute nor chronic supplementation of CM had an effect on recovery or fatigue rate of the quadriceps.

Keywords: power, force, blood flow, sports performance, ergogenic aid

Citrulline malate (CM) is a commonly sold over the counter ergogenic aid that has been gaining popularity due to its proposed ability to aid in reducing fatigue by increasing blood flow (Sureda & Pons, 2012). CM is composed generally in the ratio of 2:1 of L-citrulline, a nonessential amino acid, and malate, a Krebs cycle intermediate, respectively (Curis et al., 2005; Trexler, Keith, et al., 2019). A majority of studies have suggested that consuming citrulline and malate together with this ratio increases the effectiveness because of the combined ability to enhance oxidative energy turnover, improve acid–base balance, augment muscle force output, and lower energy cost for muscular force production (Bendahan et al., 2002; Farney et al., 2019; Perez-Guisado & Jakeman, 2010). However, the exact mechanisms of how CM alleviates fatigue are not fully understood. One proposed theory is that CM is able to facilitate the clearance of ammonia and lactate through its role as an intermediate in the urea cycle (Curis et al., 2005). In addition, CM is thought to increase nitric oxide concentrations through the nitric oxide synthase-dependent pathway by converting L-citrulline into L-arginine via the intestinal–renal axis of the kidneys (Curis et al., 2005; Morris, 2004). It is speculated that this process will enhance the recovery processes, exercise performance, mitochondrial respiration, muscle contractility, muscle repair, sarcoplasmic reticulum calcium handling, and glucose uptake (Glenn et al., 2017; Sureda et al., 2010; Trexler, Keith, et al., 2019).

The promoted acute effects of CM supplementation have been widely researched over the past several years with the majority of acute investigations utilizing around 8 g of CM (Farney et al., 2019; Glenn et al., 2017; Trexler, Keith, et al., 2019; Wax et al., 2015). Farney et al. (2019) concluded that an acute ingestion of 8 g of CM had no statistical significance on isokinetic leg dynamometer peak power, peak torque, fatigue rate, and lactate accumulation following a high-intensity exercise protocol. Despite the lack of statistical significance, it was speculated within the Farney investigation that chronic supplementation may promote a benefit. Others have also shown that neither 8 g of CM nor 400 mg of nitrate via beetroot juice had a statically significant impact on leg extension torque, blood flow, metabolic efficiency, or hormonal response (Trexler, Keith, et al., 2019). Conversely, studies have found CM to have an ergogenic benefit when applying maximal and submaximal strength exercise protocols. For example, supplementing with 8 g of CM was shown to significantly increase repetitions to failure for both the bench press and leg press among female participants (Glenn et al., 2017) as well as for the leg press, hack squat, and leg extension (Wax et al., 2015).

Research on chronic supplementation is much less extensive than acute investigations with results being even more inconsistent in comparison. Stanelle et al. (2020) reported that L-citrulline supplementation of 6 g daily for 7 days improved performance in the 40-km time trial with an increase in average heart rate, rating of perceived exertion, and average power output. However, L-citrulline supplementation did not alleviate fatigue during the supramaximal sprint repeat task. Another chronic investigation conducted by Bailey and colleagues reported that during moderate-intensity exercise, supplementing with 3.4 g of citrulline over 16 days enhanced muscle oxygenation and increased blood nitrate levels in participants (Bailey et al., 2016). Taken together, these findings suggest that chronic CM supplementation may promote aerobic energy production during exercise.

To the best of our knowledge, no study has investigated simultaneously the acute (1 day) and chronic (>1 day) effects of CM supplementation on muscle contractile properties (peak torque and peak power) and fatigue rate of the quadriceps. Therefore, the purpose of this study was to investigate the acute effects and the chronic effects of CM on muscle contractile properties of the

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quadriceps along with changes in heart rate when performing a 30-min steady-state cycling bout of exercise. We hypothesized that supplementing with CM both acutely and chronically would help attenuate quadriceps fatigue while maintaining contractile efficiency levels through an improved blood flow to the quadriceps. In addition, based upon CM's ability to be converted into nitric oxide, we hypothesized that CM would help lower HR during the 30-min steady-state cycling bout.

Methods

Subjects

Eighteen recreationally trained males (mean ± *SD*: 24 ± 5 years, 83 ± 14 kg, 174 ± 6 cm, 28 ± 5 kg/m^2) participated in this study. To qualify to participate within this investigation, each participant had to have been involved in a structured exercise training program (including both aerobic and anaerobic) for the last 6 months and be free from any knee/hip injuries. At the time of testing, none of the participants had any recent surgeries or knee/hip injuries, and none were smokers. After explanation of the experimental procedures, all participants gave their written informed consent before study commencement, which was approved by the Texas A&M University—Kingsville Institutional Review Board. All participants underwent a health screening procedure according to the American College of Sports Medicines' Guidelines for Exercise Testing and Prescription (10th edition). Prior to the beginning of the testing sessions, all participants were fully familiarized with the laboratory exercise testing procedures.

Study Design

The purpose of this investigation was to determine whether supplementing with 8 g of CM for both an acute consumption period and 7-day consumption period would help to attenuate quadriceps fatigue following a 30-min steady-state cycling exercise bout (Figure 1). The 8 g of CM was chosen based upon the most common dosage consumed in the literature. To test our hypothesis, participants engaged in seven separate sessions with the first testing session being the familiarization testing session and the subsequent six sessions being the experimental testing sessions. All participants were blinded to supplementation and were assigned in a randomized, counterbalanced order with a crossover design to consume a placebo (PL) and CM treatment for two separate dosing periods. The six experimental testing sessions included two baseline testing sessions with no supplement consumption and four supplemental testing sessions. For each dosing period, participants exercised on three separate occasions with 7 days between each session. In addition, there was a one week (7 days) "wash-out" period following the first supplement dosing period. The first experimental testing session for each dosing period was considered the baseline day (BL), the second session was the acute day (D1), and the third session was the chronic day (D2). Separate baselines were performed for each dosing period to minimize any form of a "training effect." For the chronic supplementation, all participants consumed each treatment for seven consecutive days. All experimental testing sessions included participants cycling on standard cycle ergometer for 30 min followed by completing the Thorstensson isokinetic leg extension (THOR) test.

Preparticipation Screening/Testing

The first laboratory visit was considered the familiarization testing session and encompassed preparticipation screening and performing a graded exercise test (GXT). Initial screening consisted of measuring participants' resting heart rate and blood pressure; those who were cleared to engage in moderate- to vigorous-intensity exercise based on ACSM's guidelines were allowed to participate. Following screening, all participants performed a GXT to determine their maximal wattage on the stationary bike followed by a familiarization on the Biodex dynamometer (Shirley, New York, NY).

Graded Exercise Test

Testing for maximal wattage was done by having participants perform a GXT on a rate-independent cycle ergometer (Lode Corival, Groningen, The Netherlands). The GXT began with a 5-min warm-up on the stationary cycle at a resistance of 50 W. Following the warm-up, participants began pedaling at a cadence of 100 revolutions per minute (RPMs) at a resistance of 100 W. Upon completion of the first minute, the wattage was increased by 25 W each minute. This continued until participants were unable to maintain a cadence of 100 RPMs for more than 10 consecutive seconds. The highest wattage achieved for an entire minute was determined as their maximal power output. Following the GXT, participants were then familiarized with the Biodex dynamometer by performing the THOR test.

Experimental Testing Procedures

The subsequent six laboratory visits were deemed the experimental testing sessions. All participants began by performing a standardized

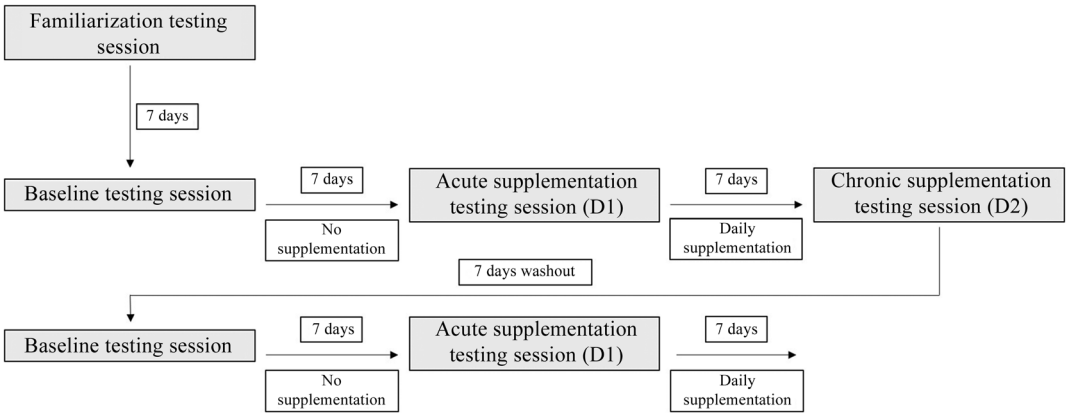

Figure 1 — Timeline of study protocol. D1 = acute day; D2 = chronic day.

warm-up for 5 min on a stationary bike at 50 W. Following the warm-up, participants began cycling for 30 min above 70 RPMs at 50%–65% of each participant's maximum wattage from the GXT. Heart rate was measured every 5 min throughout the cycling period as a measure of intensity of activity and averaged for data analysis. Upon completion of the cycle set, participants cooled down for 5 min by pedaling at a decreased wattage of 25 W. Following the 5-min recovery period, participants performed the THOR test. There were no diet restrictions within this protocol; however, participants were asked to complete a diet log for the day prior to the first experimental testing session along with the day of the first experimental testing session. A copy of the diet log was made for each participant so they could mimic dietary history for the subsequent five experimental testing sessions. In addition, all participants were asked to refrain from any additional supplementation throughout the duration of the study.

Isokinetic Leg Extension Test

The THOR test was used to determine peak power, peak torque, and rate of fatigue in the dominant quadriceps (Thorstensson & Karlsson, 1976). The THOR test was performed following the 30-min cycling test and subsequent 5-min recovery period. Following the 5-min recovery period, participants were seated on the Biodex dynamometer with straps across the upper body and hips to ensure total isolation of the dominant quadriceps. Adjustments were made so that the starting position for each participant was with the knee joint flexed, and the medial condyle of the working leg was centered with the attachment arm of the Biodex. All trials, including both the familiarization trial and experimental trials, had the participants begin the isokinetic test with no resistance for 10 repetitions. Upon completion of the warm-up set and following a 10-s rest, participants performed 50 maximal leg extensions. The maximal leg extensions were performed at a constant rate of 180°/s. Upon completion of the test, the results for the three highest and the three lowest values for power, torque, and fatigue rate from the 50 repetitions were recorded and averaged, respectively, for data analysis. Fatigue rate was displayed as a percentage and was derived by the difference between peak torque and lowest torque, divided by peak torque, and finally multiplied by 100 to get into a percentage.

Supplementation Procedures

The CM treatment consisted of 8 g of CM mixed into 20 oz of sugar-free flavored water. The PL treatment contained 20 oz of sugar-free flavored water alone. The baseline testing sessions involved the participants consuming no beverage. For the four supplemental testing sessions, participants first consumed either the PL or CM drink, then began the exercise protocol 1 hr after finishing the drink to allow adequate digestion. Following the first supplemental testing day for both treatments, participants reported to the Human Performance Laboratory daily to consume their beverage. In the case of weekends or other special circumstances, participants were given bags of the treatment to consume on their own. All consumption of the treatments on these special exceptions were monitored by having the participants confirm via email that they had consumed their treatment each day. To our knowledge, participants were 100% compliant in consuming their treatment every day during both supplementation periods.

Data Analysis

Two-way (Supplement × Time) analysis of variances with repeated measures were used to analyze for differences in the dependent variables between trials (PL and CM) across time (BL and D1 for acute supplementation; BL and D2 for chronic supplementation). If needed, appropriate post hoc tests were used to make all pairwise comparisons for specific differences across the four experimental trials. The experiment-wise error rate ($\alpha = .05$) was maintained throughout all post hoc tests for specific differences. Finally, a partial eta squared was used for effect size determination with the characterization of .02 as small, .13 as medium, and .26 as large (Bakeman, 2005).

Results

There were no significant main effects for supplement consumption (Table 1) observed from BL to D1 (acute supplementation) in peak power ($p = .634$; $\eta_p^2 = .014$), peak torque ($p = .838$; $\eta_p^2 = .003$), fatigue rate ($p = .862$; $\eta_p^2 = .002$), or heart rate ($p = .968$; $\eta_p^2 = .0001$).

In addition, there were no significant main effects for supplement consumption (Table 1) observed from BL to D2 (chronic supplementation) in peak power ($p = .836$; $\eta_p^2 = .003$), peak torque ($p = .883$; $\eta_p^2 = .001$), fatigue rate ($p = .978$; $\eta_p^2 = .0001$), or heart rate ($p = .842$; $\eta_p^2 = .002$).

There were no significant Supplement × Time interactions from BL to D1 (acute supplementation) in peak power ($p = .759$; $\eta_p^2 = .006$) (Figure 2), peak torque ($p = .940$; $\eta_p^2 = .0001$) (Figure 3), fatigue rate ($p = .631$; $\eta_p^2 = .014$) (Figure 4), or heart rate ($p = .754$; $\eta_p^2 = .006$) (Figure 5).

Finally, there were no significant Supplement × Time interactions from BL to D2 (chronic supplementation) in peak power ($p = .818$; $\eta_p^2 = .003$) (Figure 2), peak torque ($p = .829$; $\eta_p^2 = .003$) (Figure 3), fatigue rate ($p = .723$; $\eta_p^2 = .008$) (Figure 4), or heart rate ($p = .961$; $\eta_p^2 = .0001$) (Figure 5).

Discussion

To our knowledge, this is the first study to investigate simultaneously the acute and chronic effects of CM supplementation on muscle contractile properties and fatigue rate of the quadriceps. There are numerous investigations on the acute effects of CM with limited investigations on the chronic effects of CM. Collectively, results are mixed with CM supplementation and performance; therefore, this provided a rationale to examine the acute and chronic effects of CM at the same time. The primary findings from this investigation were that within recreationally trained men, no significant interactions occurred for peak power, peak torque, or fatigue rate of the quadriceps when supplementing with CM either

Table 1 Supplement Main Effects

	Acute supplementation		Chronic supplementation	
Variable	PL	CM	PL	CM
Peak power (W)	479 ± 87 [436, 522]	472 ± 91 [427, 517]	485 ± 86 [442, 528]	483 ± 82 [442, 523]
Peak torque (N·m)	154 ± 28 [140, 168]	153 ± 30 [138, 167]	156 ± 28 [142, 169]	155 ± 26 [142, 168]
Fatigue rate (%)	57 ± 8 [53, 61]	57 ± 8 [53, 61]	58 ± 8 [54, 62]	57 ± 9 [53, 62]
Heart rate (bpm)	151 ± 14 [144, 158]	151 ± 10 [146, 156]	151 ± 12 [145, 157]	151 ± 9 [146, 155]

Note. Values are mean ± *SD* [95% confidence interval]. No statistical significance was reached for any variable. bpm = beats per minute; CM = citrulline malate; PL = placebo.

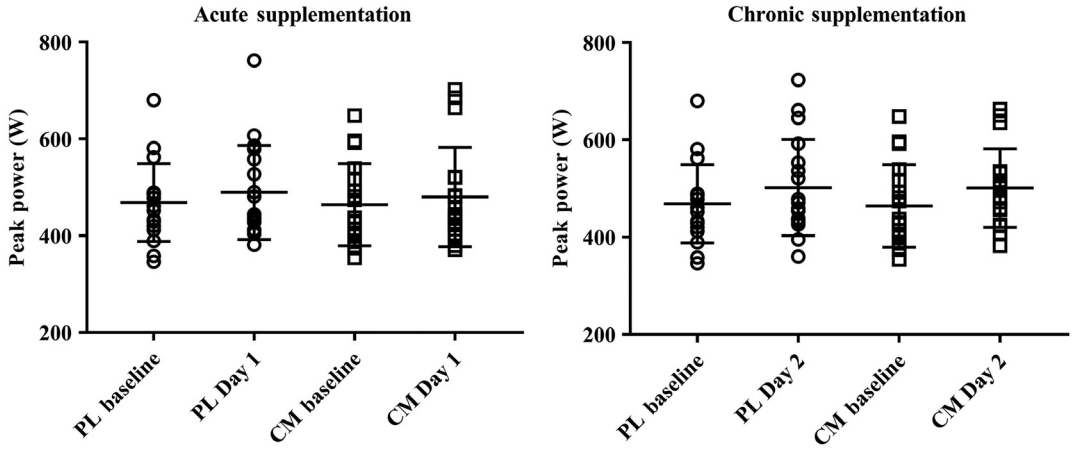

Figure 2 — Supplement × Time interaction for peak power. Values are mean ± *SD* [95% confidence interval]; no statistical significance was reached. Acute: *PL baseline* 469 ± 81 [429, 509], *PL Day 1* 490 ± 97 [441, 538] versus *CM baseline* 464 ± 85 [422, 506], *CM Day 1* 480 ± 103 [429, 531]; chronic: *PL baseline* 469 ± 81 [429, 509], *PL Day 2* 502 ± 99 [453, 551] versus *CM baseline* 464 ± 85 [422, 506], *CM Day 2* 501 ± 81 [461, 541]. CM = citrulline malate; PL = placebo.

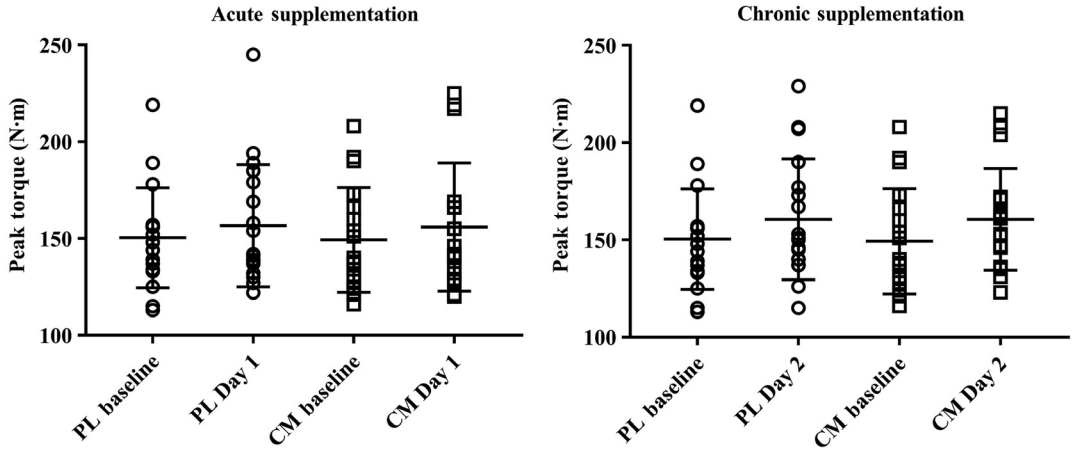

Figure 3 — Supplement × Time interaction for peak torque. Values are mean ± *SD* [95% confidence interval]; no statistical significance was reached. Acute: *PL baseline* 150 ± 26 [138, 163], *PL Day 1* 157 ± 32 [141, 172] versus *CM baseline* 149 ± 27 [136, 163], *CM Day 1* 56 ± 33 [139, 172]; chronic: *PL baseline* 150 ± 26 [138, 163], *PL Day 2* 161 ± 31 [145, 176] versus *CM baseline* 149 ± 27 [136, 163], *CM Day 2* 161 ± 26 [148, 174]. CM = citrulline malate; PL = placebo.

acutely or chronically. In addition, both supplementation periods of CM did not have a statistically significant impact on heart rate during a 30-min steady-state cycling test. It must be noted that one major limitation to the current investigation was the low sample size. However, based upon the lack of statistical significance along with small effect size (all effect sizes were <0.02 and were considered small based upon the Bakeman guidelines), we are confident that the risk of reporting a Type II error was minimal (Bakeman, 2005). Nonetheless, the current investigation follows similar results as those found by Farney et al. (2019) when assessing peak torque, peak power, and fatigue rate following a high-intensity exercise protocol when supplementing with 8 g of CM.

It is known that recovery during exercise is aided through the production of nitric acid with one major impact being the ability to maintain muscle contractile properties (Glenn et al., 2017; Sureda et al., 2010; Trexler, Persky, et al., 2019). This ability to maintain muscle contractile properties was the basis to our hypothesis of peak power and peak torque not being diminished along with a fatigue rate being alleviated following both CM supplementation periods. However, this was not the case. Our rationale was based upon CM's ability to increase oxidative energy turnover and lower pH to power ratio, which has been shown to aid in performance by enhancing skeletal muscle power (Bendahan et al., 2002). In addition, CM has been shown to attenuate fatigue through aiding in ammonia detoxification and improving utilization of pyruvate (Gonzalez & Trexler, 2020). Maintaining the integrity of the cell is an important role for CM's potential as an ergogenic aid because prolonged high-intensity activity results in increased ammonia concentrations in the blood. Unfortunately, this accumulation will then lead to an increased rate of glycolysis and accumulation of lactate, which will ultimately lead to fatigue (Mutch & Banister, 1983). The impact of CM on metabolic by-products on muscle contractile properties was the purpose of the Farney investigation, which had participants perform three rounds of four exercises at 20 s per exercise (Farney et al., 2019). Along with the current

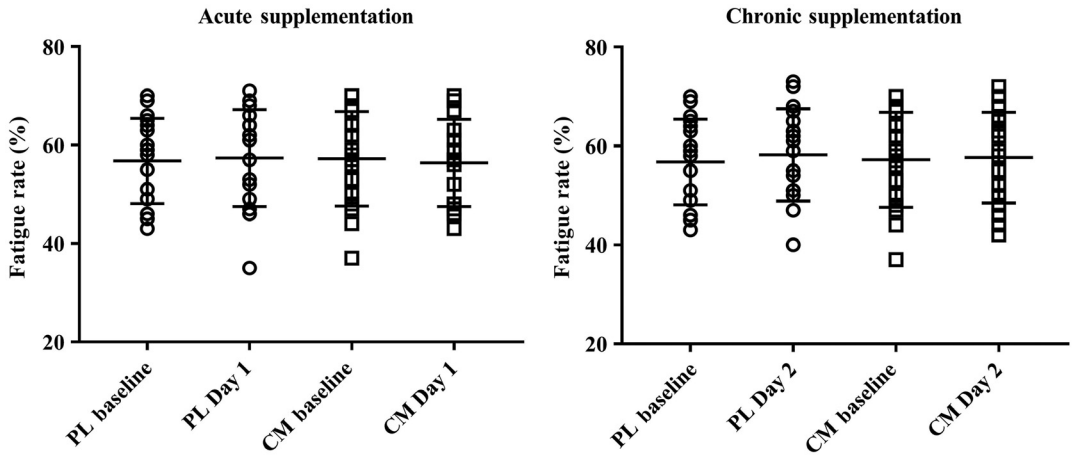

Figure 4 — Supplement × Time interaction for fatigue rate. Values are mean ± *SD* [95% confidence interval]; no statistical significance was reached. Acute: *PL baseline-57* ± 9 [53, 61], *PL Day 1* -57 ± 10 [53, 62] versus *CM baseline*-57 ± 10 [52, 62], *CM Day 1* -56 ± 9 [52, 61]; chronic: *PL baseline* 57 ± 9 [53, 61], *PL Day 2* 58 ± 9 [54, 63] versus *CM baseline* 57 ± 10 [52, 62], *CM Day 2* 58 ± 9 [53, 62]. CM = citrulline malate; PL = placebo.

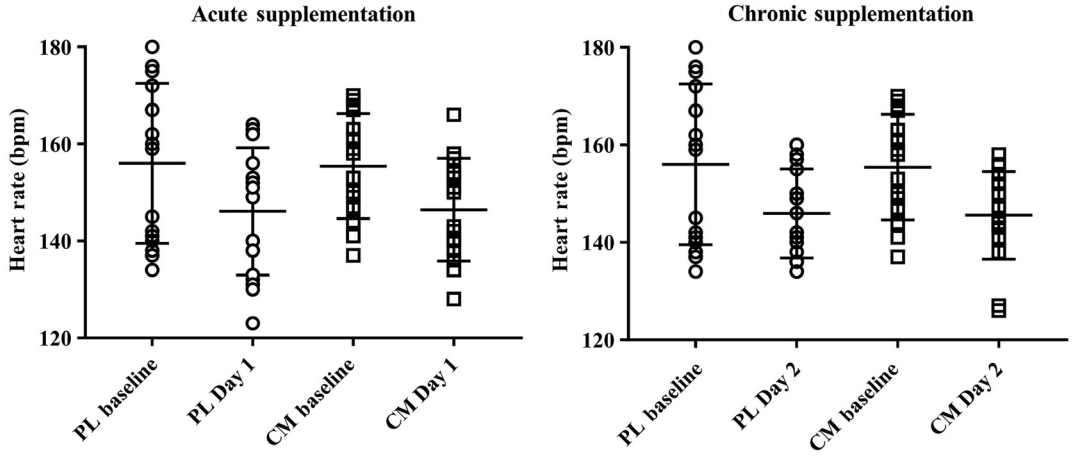

Figure 5 — Supplement × Time interaction for heart rate. Values are mean ± *SD* [95% confidence interval]; no statistical significance was reached. Acute: *PL baseline* 156 ± 17 [148, 164], *PL Day 1* 146 ± 13 [140, 153] versus *CM baseline* 155 ± 11 [150, 161], *CM Day 1* 146 ± 11 [141, 152]; chronic: *PL baseline* 156 ± 17 [148, 164], *PL Day 2* 146 ± 9 [141, 151] versus *CM baseline* 155 ± 11 [150, 161], *CM Day 2* 146 ± 9 [141, 150]. CM = citrulline malate; PL = placebo; bpm = beats per minute.

investigation, the Farney investigation had participants perform the same isokinetic leg extension test with their main variables of interest being peak power, peak torque, fatigue rate, total repetitions, and lactate accumulation. In agreement with the current investigation, Farney et al. (2019) found that CM had no statistically significant differences in peak power, peak torque, fatigue rate, total repetitions, or lactate accumulation. The high-intensity exercise protocol used in the Farney investigation was successful in accumulating lactate; however, CM was unable to combat the rise in lactate. Therefore, the actual purported ability of CM to maintain metabolic by-product accumulation remains to be fully elucidated.

Our findings are in agreement with the results of Bailey et al. (2016), who concluded that 3.4 g of citrulline per day for 16 days did not improve endurance exercise performance in healthy adults. It should be noted that citrulline supplementation within the Bailey investigation did increase plasma nitrite levels and muscle oxygenation during moderate-intensity exercise; however, the relevance of these improvements is negated due to the lack of increase in time to exhaustion during severe-intensity exercise. One major difference between the current investigation and the Bailey article was that 8 g of CM was consumed, whereas 3.4 g of citrulline was consumed, respectively. The Bailey investigation may not have supplied enough treatment to combat the stress placed on the body during severe-intensity exercise. However, based upon Farney et al. (2019), which had participants perform a high-intensity exercise protocol while consuming 8 g of CM, the possibility of CM having a positive impact while consuming chronically remains unsupported.

One of the theorized ergogenic aid benefits of supplementing with L-citrulline is the promotion of blood flow along with an increase in exercise efficiency, calcium handling, mitochondrial respiration, and glucose uptake and a reduction in fatigue through enhancing nitric acid production (Bailey et al., 2015). Despite our protocol involving a metabolically demanding 30-min cycle bout, supplementation did not have an impact on decreasing fatigue rate of the quadriceps during the THOR test. CM has previously been shown to improve performance by aiding in adenosine triphosphate production via maintaining acid–base balances within the malate–aspartate

shuttle (Rodgers et al., 2014). Wax et al. (2016) focused on muscular power and endurance by administering 8 g of CM to determine the effect on work performance during intense upper body resistance exercise in resistance-trained males. Within the 2016 Wax investigation, participants performed the following exercises to exhaustion: chin-ups, reverse chin-ups, and push-ups. The investigation found no significant differences in blood lactate or heart rate response; however, the CM condition resulted in a significantly greater number of total repetitions performed before reaching exhaustion. The results follow many others' that support CM's ability to attenuate fatigue and improve muscular power, work, and endurance during high-intensity strength and power exercise protocols (Buckinx et al., 2018; Glenn et al., 2016; Wax et al., 2016). Wax et al. (2015) reported similar results with 8 g of CM in that the number of repetitions performed at 60% of one repetition maximum increased for the leg press, hack squat, and leg extension. However, it was also noted that there were no statistically significant differences in heart rate, blood pressure, or lactate between the CM and PL groups (Wax et al., 2015). Despite the positive findings with CM supplementation, the results of our investigation do not support the efficacy of CM as an ergogenic aid.

Finally, heart rate was measured because CM has been gaining attention within clinical populations through its effect on vasodilation in adults with various cardiovascular diseases, such as heart failure and vasospastic angina (Kim et al., 2015; Morita et al., 2013). In addition, studies have seen, when supplementing with L-citrulline, a reduction in both systolic and diastolic blood pressure in participants who were normotensive, prehypertensive, hypertensive, obese, and experiencing heart failure with preserved ejection fraction when taken for periods of 2–8 weeks (Figueroa et al., 2016; Figueroa et al., 2013; Gonzales et al., 2017; Orozco-Gutierrez et al., 2010). These investigations support the possible efficacy of chronic L-citrulline supplementation in promoting cardiovascular functioning within clinical populations but are in disagreement with our findings. The current investigation demonstrates that within young, healthy individuals, both acute and chronic supplementation of L-citrulline had no statistically significant impact on heart rate. It is highly likely that this population may not be impacted through supplementation because of their adequate blood vessel compliance and well-functioning regulatory mechanisms. Therefore, further research should be directed toward detrained or clinical populations in which the effect of CM supplementation can be more impactful (Gonzalez & Trexler, 2020).

Conclusion

This investigation was the first, to our knowledge, to examine simultaneously the acute and chronic effects of CM on muscle contractile properties of the quadriceps. Our results show that 8 g of CM was ineffective in altering peak power or peak torque or reducing fatigue rate of the quadriceps when consumed either acutely or chronically. One possible reasoning behind the failure to reach statistical significance might be due to citrulline's role as a nonessential amino acid and malate's role as a metabolic precursor. Within healthy individuals, both components might be produced in sufficient amounts in the body; thus, increasing the levels of both citrulline and malate may not significantly enhance the utilization of CM as an ergogenic aid that has a purported benefit to alleviate fatigue. In addition, despite our protocol being metabolically demanding, the 30-min cycle bout may not have been a strong enough stimulus to elicit a response from CM. Based upon our findings, the consumption of CM acutely or chronically to help improve performance or lessen the onset of fatigue is not supportive within healthy individuals.

Acknowledgments

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