Effects of Citrulline Malate and Beetroot Juice Supplementation on Blood Flow, Energy Metabolism, and Performance During Maximum Effort Leg Extension Exercise

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[1]Department of Allied Health Sciences, Human Movement Science Curriculum, University of North Carolina, Chapel Hill, North Carolina; [2]Department of Exercise and Sport Science, Applied Physiology Laboratory, University of North Carolina, Chapel Hill, North Carolina; [3]Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina; and [4]Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina

Abstract

Trexler, ET, Keith, DS, Schwartz, TA, Ryan, ED, Stoner, L, Persky, AM, and Smith-Ryan, AE. Effects of citrulline malate and beetroot juice supplementation on blood flow, energy metabolism, and performance during maximum effort leg extension exercise. *J Strength Cond Res* 33(9): 2321–2329, 2019—Citrulline malate (CitMal) and beetroot juice (BEET) are increasingly popular ergogenic aids, but few studies have rigorously investigated their effects on resistance exercise performance and underlying mechanisms. The current randomized, double-blind, crossover study evaluated the effects of CitMal and BEET supplementation on blood flow, metabolic efficiency, and performance during maximal isokinetic leg extension exercise. After familiarization, 27 recreationally active men (age: 22 ± 4 years) completed 3 visits in which subjects ingested a treatment beverage (CitMal [8 g], BEET [400-mg nitrate], or placebo [PLA]), followed by a 2-hour rest period, warm-up, and 5 sets of 30 concentric leg extensions. Before and after exercise, ultrasound was used to measure diameter (aDIAM) and blood flow (aBF) of the superficial femoral artery, along with cross-sectional area and echo intensity of the vastus lateralis. Plasma analytes (lactate, nitrate/nitrite [NO_x], and urea nitrogen [BUN]) were also assessed at these times, and indirect calorimetry was used to measure energy expenditure and respiratory exchange ratio before and during exercise. Resting NO_x values were higher in BEET (233.2 ± 1.1 $\mu mol \cdot L^{-1}$) compared with CitMal (15.3 ± 1.1, $p < 0.0001$) and PLA (13.4 ± 1.1, $p < 0.0001$). Postexercise NO_x values, adjusted for resting differences, were higher in BEET (86.3 ± 1.2 $\mu mol \cdot L^{-1}$) than CitMal (21.3 ± 1.1, $p < 0.0001$) and PLA (18.1 ± 1.1, $p < 0.0001$). No other variables were affected by treatment (all $p > 0.05$). While BEET increased NO_x, neither treatment was found to enhance performance, blood flow, metabolic efficiency, nor the hormonal response to leg extension exercise.

Key Words: nitric oxide, strength, exercise hyperemia

Introduction

Nitric oxide (NO) is a multifaceted signaling molecule, influencing the activity of a wide range of physiological systems in the human body. In the context of exercise, NO is believed to improve physical performance by influencing exercise efficiency, mitochondrial respiration, sarcoplasmic reticulum calcium kinetics, glucose uptake, and muscle fatigue (2). In the NO synthase (NOS)-dependent pathway of NO production, arginine is the direct precursor to NO, yielding citrulline as a byproduct (2). Citrulline can then be recycled back into arginine, allowing for continuation of NO production. A second pathway of NO production functions independently of the NOS enzymes; in the NOS-independent pathway, nitrate is reduced to nitrite, which is further reduced to NO. Several studies have described potential mechanisms by which BEET and other sources of nitrate may enhance exercise performance through the NOS-independent pathway. Ferguson et al. (12) demonstrated that BEET supplementation increased blood flow to the active musculature during exercise in rats, and several studies have documented enhanced metabolic exercise efficiency during walking (24), running (24), cycling (26), and leg extension exercise (1). As such, BEET supplementation has been reported to enhance exercise capacity (measured as time to exhaustion) during both cycling (3) and treadmill running tests (24). Similarly, BEET supplementation resulted in a significant improvement in rowing ergometer performance in trained male rowers (5) and has also been shown to enhance cycling time trial performance in well-trained male cyclists (7). However, the extent to which these mechanisms specifically apply to resistance exercise performance remains unclear.

Kramer et al. (23) found 6 days of potassium nitrate supplementation to enhance peak Wingate power, but not a timed resistance exercise protocol. Mosher et al. (30) used a crossover design to compare the effects of a BEET supplement containing 400 mg of nitrate on muscular endurance, defined as bench press repetitions completed over 3 sets to failure, demonstrating improved volume (total weight lifted), with no effect on blood lactate. Although this finding has yet to be replicated, evidence

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suggests that the ergogenic effects of BEET may be pronounced in high-intensity activities that rely on the recruitment of type II muscle fibers, such as resistance exercise. For example, rodent research suggests that the effects of nitrate on blood flow (12), oxygen partial pressure (13), and muscle force production (19) are larger in type II muscle fibers than type I fibers, and the effects of nitrate on muscle contractile properties appear to be more pronounced under conditions of acute muscular fatigue (34). Furthermore, the NOS-independent pathway of NO production is stimulated in conditions of hypoxia and acidosis, as would be expected during exhaustive high-intensity sprint or resistance exercise.

Preliminary studies have reported promising results for the ergogenic potential of citrulline-based supplements, which use the NOS-dependent pathway. In 2010, Perez-Guisado and Jakeman (31) evaluated the effects of CitMal (8 g) on resistance training performance. Results showed a statistically significant difference between CitMal and placebo conditions, with subjects completing more repetitions before failure after the consumption of CitMal. In recent years, more positive findings have emerged; Wax et al. (38) documented significant improvements in upper-body and lower-body (39) repetitions to fatigue (RTF) in resistance-trained men after consumption of CitMal, in comparison with a placebo treatment. Glenn et al. found acute CitMal ingestion to enhance upper- and lower-body RTF in resistance-trained women (16) and also found CitMal to improve multiple indices of strength and power in female masters' tennis players (15). Most commonly, these studies have shown NO precursors to most notably benefit fatigue resistance, as demonstrated by outcomes such as repetitions completed over the course of multiple fatigue-inducing sets taken to concentric failure.

Despite these promising findings regarding the ergogenic potential of citrulline supplementation, several recent studies have also reported null findings. Chappell et al. (8) evaluated the effects of an 8-g CitMal dose in comparison with a placebo treatment and found no ergogenic effect over 10 sets of leg extension exercise. Gonzalez et al. (17) investigated the same dose, finding no significant improvement in bench press RTF. Furthermore, the exact mechanism driving the purported ergogenic effect of CitMal is not yet fully understood. Performance effects of CitMal may indeed be NO-mediated, but may also relate to citrulline's role in ammonia clearance through the urea cycle, or malate's role in aerobic energy production through the tricarboxylic acid cycle. If substantial alterations occur in urea cycle function or aerobic energy production, such effects would likely be reflected by differences in postexercise urea and lactate accumulation, respectively. With only 1 study to date assessing traditional resistance training outcomes after the consumption of dietary nitrate (BEET) (30), its effects remain uncertain in the absence of replication.

The purpose of the current study was to evaluate the effects of citrulline malate and beetroot juice supplementation on parameters of blood flow, metabolic efficiency, blood biomarkers (urea, lactate, and nitric oxide metabolites), and strength performance in the context of maximal leg extension exercise. To assess indices of both peak force production and fatigue resistance, performance measurements included peak torque, average torque, and total work across multiple sets completed with maximal effort. It was hypothesized that CitMal and BEET would increase leg extension performance, NO_x levels, blood flow, and metabolic efficiency of exercise in comparison with PLA, with no difference between treatments.

Methods

Experimental Approach to the Problem

The current study used a randomized, double-blind, placebo-controlled, crossover design to investigate the performance effects of citrulline malate (CitMal) and beetroot juice (BEET) supplementation. Subjects initially completed an enrollment visit, in which they were familiarized with the leg extension exercise protocol. Between 2 and 10 days later, they returned to complete a series of 3 testing visits, separated by 5–10 days. On arrival for testing sessions, height and body mass were measured using a stadiometer (PE-AIM-101; Perspective Enterprises, Portage, MI, USA) and calibrated electronic scale (2101 KL; Health-O-Meter, McCook, IL, USA). Measurements were taken in light athletic clothing, with the shoes removed. At each testing visit, subjects ingested 1 of 3 treatment beverages (CitMal, BEET, or placebo), followed by a 2-hour rest period. Resting measurements were obtained before the onset of exercise, including femoral artery blood flow parameters (vessel diameter [aDIAM] and blood flow [aBF]), vastus lateralis (VL) cross-sectional area (CSA) and echo intensity (EI), and blood analytes (plasma lactate, nitrate/nitrite [NO_x], and blood urea nitrogen [BUN]). Subjects completed a leg extension warm-up, followed by an isokinetic leg extension protocol consisting of 5 sets of 30 maximal concentric muscle actions, for which peak torque, mean torque, and total work were measured. Indirect calorimetry was used to measure energy expenditure (EE) and respiratory exchange ratio (RER) at rest and throughout the exercise test. After exercise, subjects laid supine, and post-test measurements were obtained. The testing visit timeline is presented in Figure 1. Artery blood flow, aDIAM, CSA, and EI were collectively measured to assess the effects of supplementation on the control of blood flow and fluid accumulation in the active musculature. Whole-body EE, RER, and plasma lactate were measured to assess energy efficiency and fuel utilization during exercise. Plasma NO_x was measured to assess nitric oxide metabolism, and BUN was measured to assess ammonia clearance through the urea cycle. These assessments of blood flow, EE, substrate utilization, and blood analytes were selected to evaluate several potential mechanisms by which acute NO precursor supplementation may influence resistance exercise performance.

Subjects were encouraged to maintain their normal diet and exercise habits throughout the study. Three-day diet logs were used to assess habitual dietary habits; The Food Processor software (ESHA Research, Salem, OR, USA) was used to calculate typical macronutrient intakes, and habitual dietary nitrate intake was calculated using reference values reported by Jonvik et al. (22). All testing visits occurred at the same time of day (±1 hour), and subjects were required to fast for at least 4 hours preceding each visit. Within 24 hours of testing, subjects were instructed to avoid strenuous exercise and alcohol consumption, and caffeine was restricted within 12 hours of test visits. Subjects were instructed to avoid mouthwash use on the day of testing and to avoid chewing gum or brushing their teeth within 8 hours of visits.

Subjects

Healthy, recreationally active, male nonsmokers between the ages of 18 and 35 years were recruited for the current study. A priori power analysis indicated that 21 subjects would be required to ensure 80% power to detect a difference in leg extension torque between treatments with an alpha level of 0.05, assuming a correlation of 0.7 among repeated measures. To account for

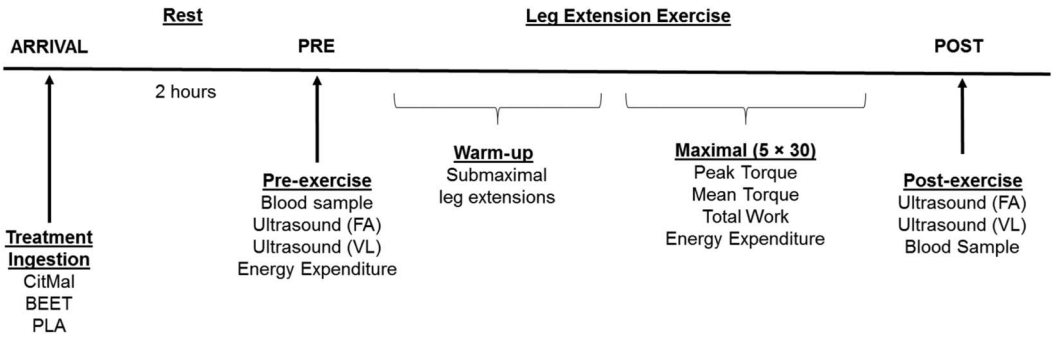

Figure 1. Timeline of each testing visit. CitMal = citrulline malate; BEET = beetroot juice; PLA = placebo; FA = femoral artery; VL = vastus lateralis.

anticipated subject dropout and maintain a counterbalanced design, 30 total subjects were enrolled. Subjects were recreationally active, as defined by at least 2 hours of exercise (such as resistance exercise, endurance exercise, or recreational sport) per week for at least 8 weeks preceding enrollment. Subjects were free from any injuries or medical conditions that would influence blood flow, exercise tolerance, or the ability to perform leg extensions to failure. To qualify for inclusion, subjects could not have used medications or supplements with potential to influence study outcomes in the 8 weeks preceding enrollment. Supplements warranting exclusion include those believed to have ergogenic effects, such as creatine, beta-alanine, nitric oxide precursors, and beta-hydroxy beta-methylbutyrate, among others. Subjects were not excluded for whey protein use due to its similarity to conventional food sources of dietary protein, and subjects were not excluded for a history of caffeine use due to its common presence in conventional foods and beverages. Subjects were questioned about the previous 8 weeks to ensure that sufficient time was provided for supplements that require washout periods lasting several weeks. Subjects were excluded if they had used recreational drugs in the past month, or if they had lost or gained ≥4.5 kg, participated in a clinical trial altering exercise or nutrition habits, or consumed more than 3 alcoholic drinks per day. All study procedures were approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill, and all subjects were informed of the benefits and risks of the investigation and provided written informed consent before participation.

Procedures

Familiarization Visit. A single familiarization visit was completed before the first testing visit. After written informed consent, subjects completed a health history questionnaire and received an explanation of previsit guidelines. Subjects were then positioned on the isokinetic dynamometer (Humac Norm; Computer Sports Medicine Inc., Stoughton, MA, USA) with a hip angle of 110°, and seat settings were recorded to enable replication at future visits. Leg extension maximal voluntary contraction (MVC) torque was measured as the highest of 3 attempts, with the knee fixed at 90° of flexion. Subjects then completed a familiarization protocol consisting of 3 sets of 30 concentric leg extension repetitions, with 1 minute of rest between sets. With each repetition, the leg was extended from a 90° knee angle to full extension at a speed of $180°{\cdot}s^{-1}$, and the leg passively returned to the flexed position at a speed of $90°{\cdot}s^{-1}$. All dynamometer testing occurred with the arms folded across the chest, with subjects fastened to the chair by a seatbelt crossing the chest and waist. Subjects returned for the first testing visit 2–10 days later.

Supplementation. Subjects consumed 1 of 3 treatment beverages in a randomized order: a 70-ml shot of beetroot juice containing 400-mg nitrate (BEET; Beet It Sport, James White Drinks Ltd., Ipswich, United Kingdom); a placebo drink of blackcurrant juice (70 ml; Ribena; Lucozade Ribena Suntory Ltd., Uxbridge, United Kingdom); or 8 g of citrulline malate (CitMal; 2:1 ratio, BulkSupplements.com, Henderson, NV, USA) mixed into the placebo beverage. Blackcurrant juice is a commonly used placebo treatment in previous beetroot juice studies, due to similar macronutrient content and negligible content of nitrate and citrulline. A recent study found that the measured nitrate content of beetroot juice product used in the current study effectively matched the amount listed on the label (14). Chappell et al. (8) tested 5 commercially available citrulline malate products, with results indicating that such products contain 4.21–5.26 g of citrulline per 8-g serving, which are consistent with previous citrulline doses in the literature (36). To enhance the uniformity of treatments, additional lemon juice and sweetener (Crystal Light; Kraft Foods, Chicago, IL, USA) were used to mask flavors. Treatments were mixed in opaque containers by an individual who was not present for supplement ingestion or testing. Peak blood levels of NO precursors are achieved approximately 1.4–2.3 hours after citrulline ingestion (32) and 2–3 hours after BEET ingestion (41); as such, treatments were ingested 2 hours before testing. Treatment sequence was randomly assigned using Random Allocation Software (Isfahan, Iran).

Blood Analytes. A 10-ml venous blood sample was obtained from the antecubital region of the arm before (PRE) and after (POST) exercise, using lithium heparin-coated tubes. Samples were centrifuged immediately, and aliquots of plasma were frozen at −80° C for batch analysis. Blood was sampled to evaluate the effects of supplementation on urea cycle function (BUN), anaerobic metabolism (lactate), and NO activity (nitrate/nitrite; NO_x). Commercially available, enzyme-linked assays were used to quantify BUN (Kit MBS9305638; MyBioSource.com, San Diego, CA, USA), total lactate (Kit MBS755961; MyBioSource.com, San Diego, CA, USA), and total NO_x (Kit KGE001; R&D Systems, Minneapolis, MN, USA). All assays were performed in duplicate and averaged for analysis. Assay coefficients of variation ranged from 3.6 to 7.6%.

Ultrasound: Superficial Femoral Artery Blood Flow and Vastus Lateralis Imaging. Doppler-mode ultrasound (Logiq-e; GE Healthcare, Chicago, IL, USA) was used to assess vessel diameter and

blood flow. The probe (12LRS, 5–13 MHz) was placed over the superficial branch of the femoral artery, 1–3 cm distal to the point at which the common femoral artery bifurcates into deep and superficial branches. Duplex mode was used to allow for simultaneous imaging of the superficial femoral artery and the spectral waveform velocity profile. A minimum of 4 cardiac cycles were recorded per captured image; a perpendicular line was drawn across the artery to measure its diameter, and the measure function within the device's default software was used to obtain values of artery diameter and blood flow. Within each image, velocity values were averaged among up to 4 consecutive cardiac cycles; at least 2 images were captured per time point, with values averaged for analysis. Test-retest reliability values for aDIAM (intraclass correlation coefficient [ICC] = 0.82, *SEM* = 0.03 cm) and aBF (ICC = 0.86, *SEM* = 5.92 $ml \cdot min^{-1}$) for our laboratory have previously been reported using this methodology to assess the brachial artery.

Ultrasound was also used to assess VL CSA and EI, to assess muscle belly swelling as a result of exercise hyperemia. A panoramic scan was performed at the midpoint of the VL, and images were analyzed offline using ImageJ software (National Institute of Health, Bethesda, MD, USA, Version 1.37). The polygon tool was used to trace the VL border to encapsulate the entire muscle, without including the surrounding fascial border. The area of the resultant polygon and the relative brightness of its constituent pixels (EI) were then calculated. To account for the influence of subcutaneous fat thickness on EI values, fat thickness was measured at the medial, middle, and lateral aspects of each image, and the average fat thickness was used to calculate fat-corrected EI values, as described by Young et al. (42). Muscle CSA and EI values were averaged among 2 images obtained at each time point. Test-retest reliability values for VL CSA (ICC = 0.87, *SEM* = 2.12 cm^2) and EI (ICC = 0.74, *SEM* = 4.58 arbitrary units [AU]) for our laboratory have previously been reported using this methodology. Resting scans were obtained after at least 5 minutes of supine rest. Postexercise scans were taken in the supine position, with vessel scans obtained 5 minutes after the conclusion of the exercise bout (27).

Indirect Calorimetry. To evaluate energy efficiency, respiratory gases were collected for 15 minutes before exercise (PRE) and throughout the maximal leg extension test. A mouthpiece and hose were used to connect subjects to an indirect calorimeter (TrueOne 2,400; ParvoMedics, Inc., Sandy, UT, USA), and data were collected continuously and averaged every 15 seconds. Expired gases were used to calculate EE ($KCal \cdot d^{-1}$) and RER (AU) by the device's default software, at rest and during maximal exercise, using the following equations:

$$\mathrm{EE}\left(\frac{\mathrm{kcal}}{\mathrm{day}}\right) = \left[\left(3.9 \times \left(\dot{\mathrm{V}}\mathrm{o}_2 \ \left(\mathrm{L} \times \mathrm{min}^{-1}\right)\right)\right) + \left(1.1 \times \left(\dot{\mathrm{V}}\mathrm{Co}_2 \ \left(\mathrm{L} \times \mathrm{min}^{-1}\right)\right)\right)\right] \times 1{,}440 \ \ \mathrm{min},$$

$$\mathrm{RER} = \frac{\dot{\mathrm{V}}\mathrm{Co}_2 \ \left(\mathrm{L} \times \mathrm{min}^{-1}\right)}{\dot{\mathrm{V}}\mathrm{o}_2 \ \left(\mathrm{L} \times \mathrm{min}^{-1}\right).}$$

For resting values, the first 5 minutes of resting data was discarded, and the remaining 10 minutes was used for analysis; exercise values were calculated from gases collected during the 8-minute exercise period. The rate of EE ($KCal \cdot d^{-1}$) was calculated for each span of time, and values for EE were then converted from $KCal \cdot d^{-1}$ to KCal burned in 8 minutes by dividing by 180. Statistics from our laboratory pertaining to test-retest reliability for resting EE (ICC = 0.94, *SEM* = 125.6 $kcal \cdot d^{-1}$) and RER (ICC = 0.83, *SEM* = 0.03 units) using similar methodology have been reported previously.

Exercise Test. Before maximal exercise testing, a leg extension warm-up was completed with the right leg. Subjects completed approximately 8 minutes of isotonic concentric leg extensions with 25% of MVC torque, with 1 repetition completed every 4 seconds, as described elsewhere (35). Subjects then completed a single set of 5 isokinetic leg extensions with escalating effort; the first repetition was completed at 50% effort, escalating up to 100% effort on the fifth. After the warm-up, subjects completed 5 sets of 30 maximal-effort, concentric leg extensions. Repetitions were completed from a 90° knee angle to full extension at a speed of $180° \cdot s^{-1}$, with passive leg flexion ($90° \cdot s^{-1}$) between repetitions, and 1 minute of passive rest between sets. Verbal encouragement was provided to motivate subjects to provide consistent maximal effort for all testing sessions. The dynamometer's default software was used to calculate gravity-adjusted values for peak torque, average torque, and total work for each set, with a sampling rate of 100 Hz. Peak torque was calculated as the highest torque value obtained during any single repetition during the set, whereas average torque was the average of the peak torque values obtained for all repetitions in a given set. Total work for each set was calculated as the sum of the total area under the torque-position curve for each repetition of a set. For each variable, values from all 5 sets were summed for analysis. Immediately after the final set, subjects were laid supine in preparation for postexercise measurements.

Statistical Analyses

A series of general linear-mixed models were used for data analysis. Random intercept models were fitted, with subject identified as a random effect. Preliminary models were fitted to confirm separately that carryover, sequence, period × treatment, and habitual nitrate × treatment interaction effects were nonsignificant. Leg extension outcomes (peak torque, average torque, and total work; summed across all 5 sets) were assessed by fitting mixed models with fixed effects including period and treatment. All other outcomes were analyzed with fixed effects including treatment as the predictor variable, and period and resting values as covariates. Data missing due to technician or equipment error were assumed to be missing completely at random, with all omnibus tests including at least 77 of 79 possible observations. One observation was missing for hormone analyses and arterial ultrasound outcomes, and 2 were missing for indirect calorimetry outcomes. Significant overall treatment effects were followed by pairwise comparisons, using the Tukey-Kramer adjustment for multiplicity. Model residuals were visually assessed to confirm the absence of heteroscedasticity and non-normal distributions. As a secondary analysis, baseline NO_x values were compared to assess the effects of treatment on resting NO metabolites. All analyses were performed using SAS PROC MIXED (SAS Software, Cary, NC, USA); the criterion for statistical significance was set a priori at $\alpha = 0.05$. Descriptive demographic data are presented as mean ± *SD*; mixed-model results are presented as adjusted least squares mean ± *SE*. For pairwise comparisons, 95% confidence intervals are presented for the difference (Δ [lower bound, upper bound]).

Results

Subjects

Thirty subjects enrolled in the current study. Three withdrew before the first testing visit, and 1 withdrew after visit 1; those providing

a reason for withdrawal cited schedule constraints or injuries unrelated to the study. Individuals withdrawing from the study did not seem to differ from those completing the study with regard to any identifiable distinguishing characteristics. Twenty-seven subjects completed at least 1 testing visit (age: 22 ± 4 years; height: 178.4 ± 6.8 cm; body mass: 78.9 ± 12.5 kg). As noted elsewhere (35), average dietary intakes of returned food logs (n = 25) were 2,558 ± 699 KCal·d^{-1}, 280 ± 90 g·d^{-1} carbohydrate, 101 ± 34 g·d^{-1} fat, 131 ± 42 g·d^{-1} protein, and 115 ± 98 mg·d^{-1} nitrate.

Blood Analytes

Model residuals for BUN and NO_x were not normally distributed; so, natural log transformations were performed for both. Confidence intervals are presented on the logarithmic scale; least square mean values and *SE*s are back-transformed and reported on their original scale to facilitate interpretation. Treatment had a significant effect on resting baseline NO_x levels (F[2, 47.8] = 303.68, $p < 0.0001$), with higher values in BEET (233.2 ± 1.1 μmol·L^{-1}) compared with CitMal (15.3 ± 1.1, $p < 0.0001$; logΔ = −2.72 [−3.04 to −2.41]) and PLA (13.4 ± 1.1, $p < 0.0001$; logΔ = −2.85 [−3.17 to −2.54]). The effect of treatment on postexercise NO_x levels was also significant (F[2, 56.5] = 19.60, $p < 0.0001$), with significantly higher values in BEET (86.3 ± 1.2 μmol·L^{-1}) than CitMal (21.3 ± 1.1, $p < 0.0001$; logΔ = −1.40 [−1.99 to −0.81]) and PLA (18.1 ± 1.1, $p < 0.0001$; logΔ = −1.56 [−2.16 to −0.96]). The effect of treatment on postexercise BUN values was not significant (F[2, 47.4] = 2.13, p = 0.13; CitMal: 1.91 ± 1.03, PLA: 1.94 ± 1.03, BEET: 1.81 ± 1.03 mmol·L^{-1}). One subject presented at all 3 testing visits with resting lactate values over 4 *SD*s from the group mean; all of this subject's data were excluded from lactate analyses. The effect of treatment on postexercise lactate was nonsignificant, but a trend was observed (F [2, 44.8] = 2.53, p = 0.09), with CitMal (0.46 ± 0.03 mmol·L^{-1}) trending lower than PLA (0.54 ± 0.03 mmol·L^{-1}, p = 0.08; Δ = −0.08 [−0.17 to 0.01]), but not BEET (0.49 ± 0.03 mmol·L^{-1}, p = 0.62; Δ = −0.04 [−0.13 to 0.06]). Blood analyte outcomes are presented in Figure 2.

Leg Extension Performance

Values for leg extension peak torque, average torque, and total work were each summed across all 5 sets for analysis. Peak torque was not significantly affected by treatment (F[2, 48.2] = 0.08, p = 0.92; CitMal: 646.0 ± 21.6, PLA: 648.9 ± 21.6, BEET: 645.8 ± 21.6 N·m). Average leg extension torque was also unaffected by treatment (F[2, 48.2] = 0.49, p = 0.62; CitMal: 567.8 ± 20.6, PLA: 577.4 ± 20.5, BEET: 572.4 ± 20.6 N·m). Similarly, treatment did not have a significant effect on total leg extension work (F[2, 48] = 0.09, p = 0.91; CitMal: 12,969 ± 414, PLA: 12,993 ± 413, BEET: 13,042 ± 414 N·m). Leg extension outcomes are presented in Figure 3. To investigate the possibility of set-specific effects, additional models were constructed to assess the effect of supplementation at each individual set of leg extension (stratified by set number). These exploratory analyses confirmed that supplementation did not significantly affect peak torque, average torque, or total work for any set (all $p > 0.05$).

Ultrasonography

The effect of treatment on muscle CSA was not significant, but a trend was observed (F[2, 44.1] = 3.15, p = 0.053), with CitMal (35.4 ± 0.4 cm^2) tending to be lower than PLA (36.2 ± 0.4 cm^2, p = 0.06; Δ = −0.84 [−1.72 to 0.04]), but not BEET (36.1 ± 0.4 cm^2, p = 0.13; Δ = −0.72 [−1.61 to 0.16]). Treatment did not have a significant effect on fat-corrected muscle EI (F[2, 48.3] = 2.24, p = 0.12; CitMal: 94.0 ± 0.78, PLA: 93.3 ± 0.76, BEET: 95.2 ± 0.78 AU). Muscle ultrasound outcomes are presented in Figure 4. Treatment did not significantly affect aDIAM (F[2, 44.1] = 1.67, p = 0.20; CitMal: 0.72 ± 0.01, PLA: 0.71 ± 0.01, BEET: 0.73 ± 0.01 cm). Similarly, aBF values were not significantly affected by treatment (F[2, 46.8] = 0.71, p = 0.50; CitMal: 457 ± 34, PLA: 458 ± 34, BEET: 486 ± 34 ml·min^{-1}). Visual inspection of residuals suggested that log transformation may be warranted for aBF, but log transformation also yielded a nonsignificant treatment effect (F[2, 47.6] = 0.34, p = 0.72).

Indirect Calorimetry

The effect of treatment on EE was nonsignificant, but a trend was observed (F[2, 44.6] = 2.50, p = 0.09). Values tended to be higher in BEET compared with PLA (37.8 ± 1.5 vs. 35.4 ± 1.5 kcal, p = 0.08; Δ = −2.4 [−5.0 to 0.2]), but not CitMal (36.2 ± 1.5 kcal, p = 0.32; Δ = −1.5 [−4.1 to 1.0]). Treatment did not have a significant effect on RER (F[2, 47.5] = 0.23, p = 0.80; CitMal: 1.04 ± 0.01, PLA: 1.03 ± 0.01, BEET: 1.04 ± 0.01 AU).

Discussion

Nitric oxide precursor supplements, such as CitMal and BEET, have previously been shown to have ergogenic effects, such as improved time trial performance, time to exhaustion, and RTF, through a number of potential mechanisms, including improvements in blood flow, energy efficiency, ammonia clearance, and aerobic ATP production (21,31). In the current study, although BEET increased plasma NO_x, this effect did not translate to improvements in any of the blood flow, energy efficiency, or performance parameters measured. Results of the current study suggest that neither CitMal nor BEET significantly enhance leg extension performance, energy efficiency, exercise hyperemia, or hormonal responses to isokinetic leg extension exercise in recreationally active men.

The most notable finding of the current study is the absence of an ergogenic effect on leg extension exercise. Citrulline malate has previously been shown to enhance bench press RTF in resistance-trained men (31). This finding was later supported by studies showing improved upper-body RTF (38) and lower-body RTF (39) in resistance-trained men and women (16). A single study has previously evaluated the effects of BEET on traditional dynamic constant external resistance exercise, with a significant increase in bench press RTF observed in resistance-trained men (30). By contrast, the current study found no such benefit on isokinetic leg extension, despite causing a marked increase in pre-exercise plasma NO_x levels. Several methodological factors may explain the lack of ergogenic effects in the current study, such as the type of exercise test used, the dosing strategy of supplementation, and the characteristics of the population sampled.

Rather than open-endpoint tests of dynamic constant external resistance exercise with multijoint exercises, the current study implemented an isokinetic, single-joint, range-of-motion controlled assessment of torque production. Chappell et al. (8) used a similar, 10-set isokinetic leg extension test and also found no performance benefit from CitMal. Both ex vivo (19) and in vivo (18) studies have shown nitrate to enhance muscle contractile properties in a manner that increases the rate of force production and shortening velocity (10), which may be attenuated in exercise tests in which the velocity of muscle actions is constrained. Coggan and Peterson (10) suggest that acute effects of NO precursors on muscle contractile function

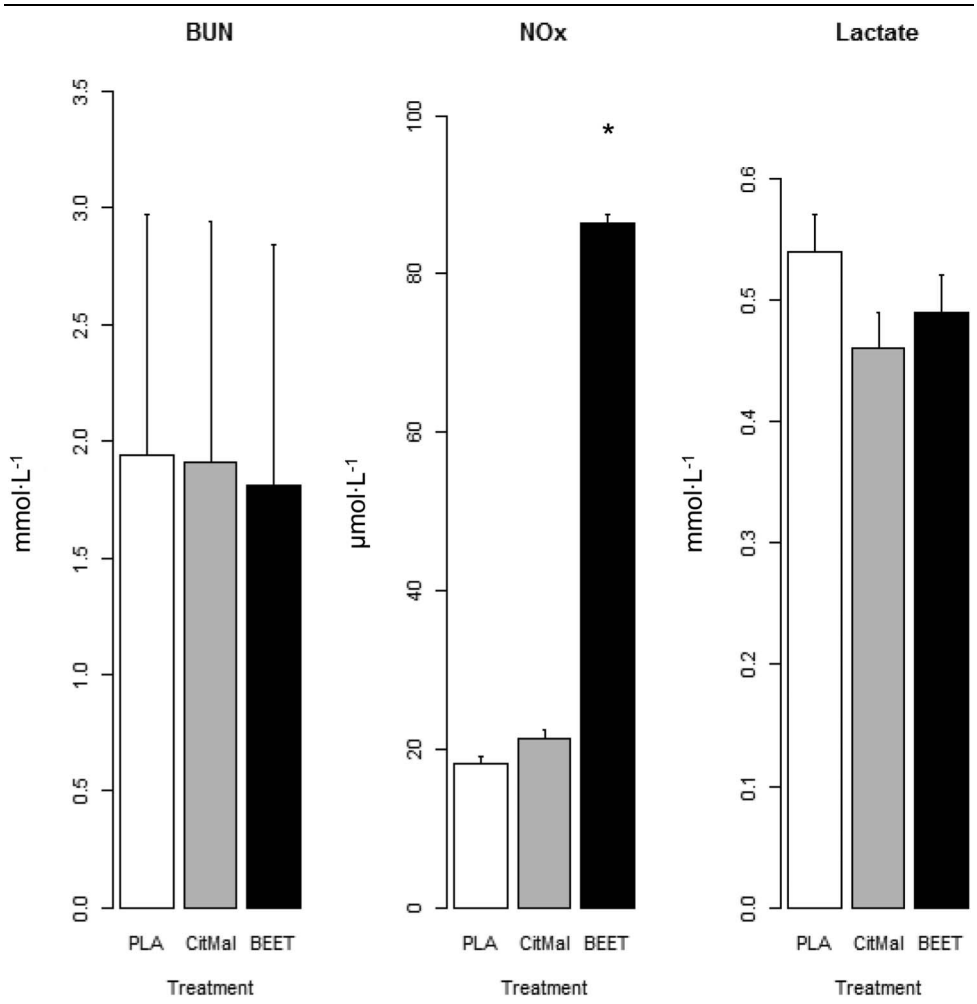

Figure 2. Postexercise plasma levels of blood urea nitrogen (BUN), nitrate/nitrite (NO_x), and lactate. Data are presented as adjusted least squares mean + *SE*. *Significantly greater than CitMal and PLA ($p < 0.0001$).

are attributable to increased calcium release in the sarcoplasmic reticulum and enhanced myofibrillar calcium sensitivity, thereby increasing twitch force, contraction velocity, rate of force development, and maximal power. This is supported by recent experimental findings (9), in which acute BEET supplementation enhanced leg extension torque and power at an angular velocity of $360°·s^{-1}$, but not at velocities ranging from 90 to $270°·s^{-1}$. To replicate these observations, future studies should investigate the effects of BEET and CitMal supplementation within a wide range of contraction velocities, with a particular emphasis on high-velocity muscle actions.

In addition, differences in dosing strategies should be noted. Peak blood levels of NO precursors are achieved approximately 1.4–2.3 hours after citrulline ingestion (32) and 2–3 hours after BEET ingestion (41). As such, the current study provided treatments 2 hours before exercise, whereas most previous citrulline studies have tested outcomes 1 hour after supplement ingestion. Cutrufello et al. (11) investigated the performance effects of L-citrulline provided 1 or 2 hours before chest press testing; although neither timing strategy significantly improved performance, the 1-hour condition yielded 3 additional repetitions compared with placebo, whereas the 2-hour condition resulted in completion of 1 less repetition than placebo. Mosher et al. (30) provided a 6-day supplementation protocol to increase plasma NO_x values over time, rather than a single, acute dose before exercise. Although the current study adopted a single-dose approach that has previously been shown to enhance leg extension power (9), multiple-day BEET supplementation interventions enhance performance more reliably than acute, single-dose interventions (21), which may explain these discrepant findings. In addition, a dose-response relationship for BEET has been observed in other exercise modalities, with more favorable effects documented after 8.4 mmol nitrate in comparison with 4.2 mmol (41). As the current study administered a dose of only 6.4 mmol (400 mg), further research is needed to explore the possibility that ergogenic effects may be observed at higher doses.

Given the lack of ergogenic effect in the current study, it is intuitive that indices pertaining to blood flow, energy efficiency, ammonia clearance, and lactate accumulation were largely unaffected by the experimental treatments. Previous research in adults with self-reported fatigue has indicated that CitMal enhances the efficiency of aerobic metabolism in exercising muscle after several days of supplementation (4). Although the current study found no effect of CitMal or BEET on lactate values, the observed postexercise lactate levels suggest that the exercise test did not elicit a large lactate response. Nonetheless, previous studies noting the ergogenic effects of BEET (30) and CitMal (38,39) have documented increased RTF in the absence of effects on lactate, casting doubt on reduced lactate accumulation as a primary ergogenic mechanism. Similarly, postexercise BUN values in the current study were not elevated beyond typical resting levels. Studies have shown

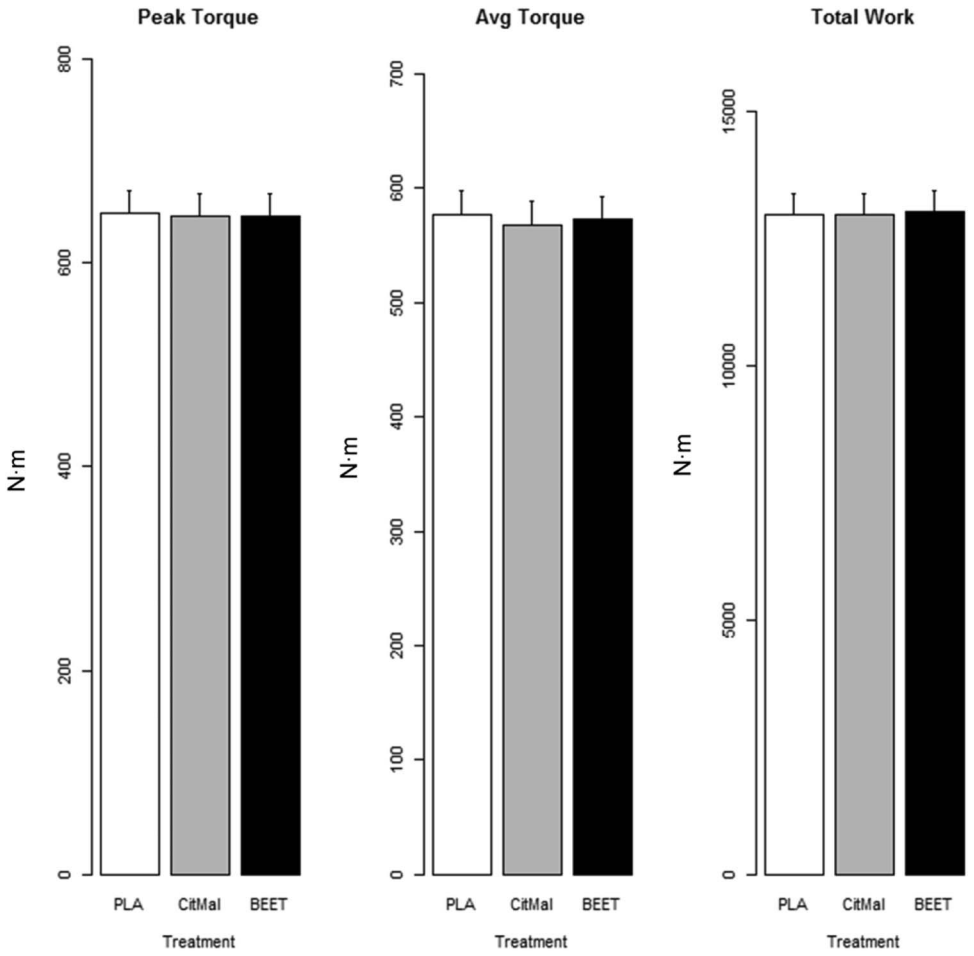

Figure 3. Leg extension outcomes. Data are presented as adjusted least squares mean + *SE*. N·m = Newton meters; avg = average.

no effect of BEET (37) on urinary urea levels, and citrulline supplementation has been shown to preserve postrace jump height performance in the absence of any effect on blood urea levels (28). CitMal did not increase plasma NO_x to a greater extent than PLA, whereas BEET caused a marked increase. Previous research has suggested that citrulline supplementation increases postexercise changes in plasma NO_x to a great extent than PLA, but this effect was not observed in the current study. This discrepancy may relate to the form and dosing strategy of supplementation; McKinley-Barnard et al. (29) provided 2 $g \cdot d^{-1}$ of L-citrulline for 7 days and also noted that coingestion of the antioxidant glutathione enhanced this effect. However, Suzuki et al. (33) used a similar dosing protocol with L-citrulline and found no significant impact on plasma NO_x changes after a 4-km cycling time trial, despite observing a performance improvement.

Several studies have reported reduced energy cost of walking (24), running (24), cycling (26), and bilateral leg extension (1) exercise after BEET supplementation. Although the exercise protocol in the current study elevated EE beyond normal resting levels, neither CitMal nor BEET significantly influenced EE during exercise. Similarly, RER was elevated in response to exercise, but was unaffected by either supplement. Notably, previous studies documenting enhanced energy efficiency of exercise have typically provided nitrate supplements for multiple (3–6) days before testing. Nitric oxide may enhance exercise efficiency by altering mitochondrial function through reductions in uncoupling protein-3 (UCP-3) and adenosine nucleotide translator expression (25), but enhanced excitation-contraction efficiency through increased expression of calcium-handling proteins in muscle (calsequestrin 1 and dihydropyridine receptor) may be an alternative mechanistic explanation (19). As both potential mechanisms are influenced by changes in protein expression, changes are more likely to be observed with longer-term (multiday) supplementation strategies (19). However, it should be noted that Whitfield et al. (40) found no change in calcium-handling proteins after 1 week of BEET supplementation, despite improvements in muscle force production. There is also evidence to indicate that acute nitrate supplementation enhances contraction efficiency through nitrosylation of ryanodine receptors and activation of guanylyl cyclase (10), but these effects have largely been observed with nitrate doses of 8.8 mmol and above. As such, dosing strategies using longer durations of supplementation or larger acute doses seem to be warranted.

Blood flow-related outcomes in the current study (aDIAM, aBF, CSA, and EI) suggest no effect of either supplement on blood flow. The current study was performed in a young, healthy, and relatively active sample, which may explain the absence of effect. Nitric oxide production and bioavailability decrease with age and are increased by regular physical activity. As such, Casey et al. (6) demonstrated that BEET

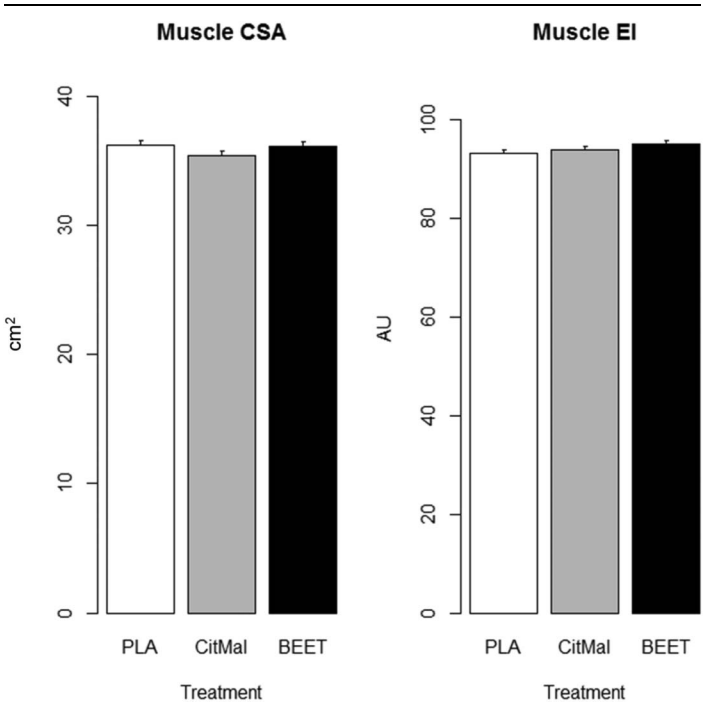

Figure 4. Effects of treatment on postexercise muscle cross-sectional area (CSA) and fat-corrected echo intensity (EI). Data are presented as adjusted least squares mean + *SE*. AU = arbitrary units.

supplementation increases the compensatory vasodilator response to hypoxic handgrip exercise in older adults (age: 64 ± 2 years), but not in younger adults (age: 25 ± 1 year). Aside from conduit artery measurements, local ultrasound assessments of muscle CSA and EI can be used to indirectly assess the hyperemic response to exercise. Recent research has documented acute increases in CSA and EI values immediately after fatiguing resistance exercise of the elbow flexors (20), presumably due to fluid accumulation from exercise hyperemia. In the current study, neither CitMal nor BEET influenced CSA or fat-corrected EI values in comparison with PLA. Similarly, Gonzalez et al. (17) assessed the effect of CitMal on muscle thickness, measured through ultrasound, after acute resistance exercise. In line with the current findings, CitMal had no influence on the exercise-induced increase in muscle thickness (17). Taken together, the current results do not suggest that CitMal or BEET increases indices of blood flow or muscle fluid accumulation in response to resistance exercise, presumably due to the use of a young, healthy, physically active sample.

The primary limitation of this study is the use of single-leg, isokinetic leg extension as the exercise task. Full-body, dynamic constant external resistance exercise tests loading both eccentric and concentric muscle actions would impose a greater metabolic demand, and thereby more pronounced changes in the physiological measurements obtained, such as plasma lactate. Similarly, isokinetic testing constrains the velocity of movement, which could potentially mask supplement effects related to the rate of force production or shortening velocity of muscle actions which may be seen at higher isokinetic velocities. Finally, the current study implemented a 2-hour wait period after a single supplement dose. Pharmacokinetic data suggest that such a compromise is appropriate, but studies documenting the efficacy of CitMal and BEET often implement waiting periods of 1 and 2.5–3 hours, respectively, and the effects of BEET seem to be more pronounced when using larger doses or multiday dosing protocols.

Practical Applications

Recent studies have reported that both CitMal and BEET increase RTF during dynamic constant external resistance exercise tests (30,38,39), but the effects of these NO precursor supplements on resistance exercise performance have not been directly compared to date. In addition, the mechanisms underlying this effect are not conclusively known, but it has been speculated that alterations in NO production/bioavailability, blood flow, energy efficiency, substrate utilization, lactate clearance, or ammonia clearance may contribute. Results of the current study suggest that neither 8-g CitMal nor 70-ml BEET enhances isokinetic leg extension performance in recreationally active men. Similarly, neither treatment improved indices of blood flow, metabolic efficiency, or the hormonal response to exercise to a meaningful degree. It is possible that higher or more sustained dosing strategies, or exercise tests involving more rapid muscle actions and activation of more total musculature, may be required to observe ergogenic effects of CitMal and BEET. Null effects on blood flow, metabolic efficiency, lactate clearance, and plasma BUN suggest that additional focus on the direct effects of NO on muscle contractile properties (10) is warranted in future mechanistic research on acute NO precursor supplementation. Although further research is needed to investigate optimal dosing protocols and the various physiological mechanisms mediating the effects of acute NO precursor supplementation, the current data do not suggest that acute CitMal or BEET supplementation enhances isokinetic leg extension performance.

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