Short-Term Citrulline Supplementation Does Not Improve Functional Performance in Older Active Women

Original Research

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Abstract

Introduction: The aim of this study was to test the hypothesis that seven days of citrulline (CIT) supplementation would improve cardiovascular measures and functional performance in older active women.
Methods: Sixteen women (66.9±5.6yrs, 1.65±0.5m, 71.7±16.7kg) volunteered to participate in this randomized, double-blind, crossover-study. Participants underwent a series of functional fitness testing including a hand grip strength test, get-up and go, sit-to-stand, and a 6-minute walk test (6MWT). Heart rate and blood pressure (BP) were obtained at rest and following the 6MWT. Participants consumed 6g of citrulline or a placebo daily for seven days between pre- and post-testing in a counterbalanced fashion with a 14-day washout period between treatments. Data were analyzed via separate repeated measures analysis of variance.
Results: A significant time by treatment interaction was observed for resting diastolic BP ($F = 5.34$; $p = 0.028$) indicating lower resting diastolic BP values following seven days of CIT supplementation compared to placebo. No other differences in cardiovascular measures were observed. There were no significant ($p>0.05$) differences between CIT and placebo for any measure of functional performance following the interventions.
Conclusions: These results indicated that CIT did not influence functional performance, but CIT did improve resting diastolic blood pressure in older active female adults.

Key Words: L-Citrulline, Nitric Oxide, Fitness, Blood Pressure; Older Adult

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Introduction

Nitric oxide (NO) is a gaseous molecule which is responsible for numerous functions in the body and most notably for its role in cardiovascular health[1]. Improving NO bioavailability stimulates blood vessel dilation, improved blood flow [2], and the lowering of blood pressure (BP)[3], promoting cardiovascular health [4]. L-citrulline (CIT) is a non-essential amino acid which serves as a precursor to L-arginine and is found in high amounts in natural foods such as watermelon and cucumbers. Studies have shown that CIT administration effectively increases NO bioavailability in the body [5,6] subsequently improving muscle oxygenation during high-intensity exercise [7]. Furthermore, CIT supplementation has been demonstrated to decrease fatigue and muscle soreness [8], lower BP for those with hypertension [9], and a recent meta-analysis indicates a positive effect of CIT supplementation on muscular performance [10]. As such, CIT has become a popular dietary supplement for active populations to improve aerobic and anaerobic performance [7,10,11].

While an increased ability to sustain higher workloads is beneficial for athletes engaged in vigorous exercise, older adults may also reap benefits from CIT administration [12,13]. Aging is often associated with a progressive loss of muscle strength and exercise capacity which leads to decrements in functional performance [14-16]. Additionally, an age-related reduction in NO bioavailability is a central cause of endothelial dysfunction in older adults [3]. As such, nutritional aids which may increase exercise capacity and vascular function may improve the quality of life for older adults. Previous work has demonstrates CIT supplementation combined with high-intensity interval training (HIIT) may improve upper limb muscle strength and walking speed in dynapenic-obese older adults [15] and improve self-pace gait speed in healthy older adults [13]. Another study found that CIT supplementation improved the rate of rise in oxygen uptake at exercise onset in younger and older men [17]. While these data are promising, there are few data regarding the effects of supplementation in active older adults. Given the reported effects of CIT on muscle protein synthesis [18], improvement in exercise capacity[12], and NO mediated blood flow [2], it is likely that the salient benefits of CIT would extend to a more active older population.

Therefore, the purpose of this study was to determine the effect of seven days of CIT supplementation on measures of cardiovascular health and functional fitness in active older women. The hypothesis for this study is that short-term CIT supplementation will improve measures of physical performance in older active women and improve indices of cardiovascular health.

Methods

Participants

Sixteen older physically active women volunteered for this placebo controlled, randomized, double-blind cross-over study (Table 1). Participants were recruited from a senior fitness class at a local community center. To be included in this study participants were required to be over 55 years old and be physically active (engaged in moderate physical activity at least three times a week)[19]. Participants were excluded if they had any physical limitations which would not allow them to complete all testing procedures. All participants provided their written informed consent, completed a PARQ-2018+, and a medical history & activity form during their first visit to the lab. The study was approved by the Lipscomb's University Institutional Review Board prior to participant enrollment.

Table 1. Participant Characteristics

Variable	n=16
Age (y)	66.9 ± 5.6
Height (cm)	165.3 ± 4.9
Weight (kg)	71.7 ± 16.7
Body Mass Index (BMI)	25.4 ± 4.2
Lean Body Mass (kg)	40.9 ± 5.5
Fat Mass (kg)	30.9 ± 13.2
Bone Mineral Content (kg)	2.4 ± 0.8

Data are presented as mean ± SD

Experimental Design

All participants were required to attend 5 separate testing visits. The first visit (T1) was to the University human performance & body composition labs where they completed body composition assessment and familiarization sessions with all experimental testing procedures. All subsequent testing visits (T2-T5) occurred at a local community fitness center and consisted of pre- and post-testing following seven days of supplementation with CIT or placebo (PL) and with participant reporting in a fasted state. Upon arrival for these visits, resting BP and heart rate (HR) was obtained with the participant in a seated position. Following HR & BP assessment, participants completed the following tests in order: maximal voluntary handgrip strength, timed get-up and go test (GUG), a 30-second sit-to-stand (STS) assessment, and a 6-minute walking test (6MWT) during which a measure of 5-meter gait time was obtained. Immediately following the 6MWT, post-exercise BP and HR was recorded. Between each experimental condition, a 14-day washout period was provided to minimize any carry-over effects from the prior condition. Participants then followed the same procedures as described above by completing pre-testing, 7-days of supplementation with the opposite condition (CIT or PL) and post-testing.

Anthropometric & Body Composition Assessment
Body mass, non-bone fat-free mass (FFM), and body fat percentage were determined using whole body–dual energy x-ray absorptiometry (DXA) scans (ProdigyTM; Lunar Corporation, Madison, WI). Total body estimates of percent fat and non-bone FFM (± 0.1 kg) were determined using the company's recommended procedures and supplied algorithms. Daily calibrations of quality assurance were completed prior to all DXA scans using the manufacturer supplied calibration block. All DXA assessments were completed using standardized subject positioning procedures by a single certified radiological technician. This has been previously reported as a valid and reliable method of estimating percent body fat, fat mass, and lean mass [20].

Heart Rate and Blood Pressure
During all experimental testing visits (T2-T4), participant HR and BP were recorded at rest before any experimental testing and immediately after the 6MWT. Before any performance testing, each participant was asked to rest seated in a chair for 5 min before their HR and BP was recorded. Immediately following the 6MWT, post-exercise HR and BP were recorded in the seated position. Measurements of HR and BP were obtained using an automatic BP monitor (Model MDS4001, Medline industries, Inc. Mundelein, IL). Each measurement of HR and BP were recorded twice and the average of the 2 measurements were used for analysis.

Hand grip strength
Maximal voluntary hand-grip strength was measured in kilograms (kg) as the maximal isometric force achieved on a hand grip dynamometer (Takei Scientific Instruments Co., Ltd, Tokyo, Japan). This test was administered with participants sitting square in an armless chair with their feet flat on the floor and the elbow bent at a 90° angle confirmed by a goniometer. The dynamometer was placed in the right hand and adjusted so the joints of the medial and distal phalanges were lined up. The grip size was adjusted so that the second metacarpals were flat with a 90° bend at the knuckles. Participants were asked to squeeze the dynamometer as hard as possible for 3 seconds. They were given 30 seconds of rest and were asked to repeat the hand grip assessment. The maximum value attained between the two trials was used in subsequent analysis.

Timed Get up and Go (GUG)
The GUG test measured the time taken in seconds for participants to stand from a seated position in an armless chair, walk 3 m, turn, return to the chair and sit down. During this test, participants were not allowed to use their hands for support while standing or sitting.

30 second Sit-to-Stand (STS)
The STS test was implemented as a measurement of lower-body muscular endurance. For this test, participants were instructed to sit in an armless chair with their arms folded across their chest and stand from a seated position and then seat back down, back to chair as many times as they could for 30 seconds. The number of repetitions were recorded as the STS score and was used for later analysis.

6-minute walk test (6MWT) & 5-meter Gait Time
For this assessment, participants were instructed to walk a 25 m straight-line course at a brisk pace, turn, return to the starting point as many times as they could in six minutes. A 25 m straight-line path was marked with cones and additional cones were placed at each 5 m mark to assist in tracking distance. Gait time was recorded as the time in seconds required to walk the first five meters of the 6MWT. Gait time was recorded when the participant moved one foot from the start point and stopped when one foot hit a 5-meter point. Participants were instructed to stop in place at the end of the 6 min wherever their weighted foot landed, and this location was used to calculate distance covered.

Supplementation Protocol
Participants were randomly assigned in a cross-over fashion to consume 6g of l-citrulline (Bulksupplements.com, Nevada, USA) or PL (Maltodextrin, Bulksupplements.com, Nevada, USA) once daily for seven days between pre-testing (T2 & T4) and post-testing (T3 & T5). Participants consumed their last dose or their respective supplement 60 minutes before completing each post-testing visit. Supplements were provided to participants in pre-labeled, coded

bags and both CIT and PL were flavored to be identical in taste and appearance. Only one researcher, who was not involved in experimental testing, knew which codes corresponded with each product. At each post-testing visit (T3 & T5), participants returned all used supplement bags to assess compliance.

Statistical Analysis

Prior to analysis, all data was assessed to ensure normal distribution, homogeneity of variance and sphericity. If assumption of sphericity was violated, a Greenhouse Geisser correction was applied. Separate repeated measures analyses of variance (ANOVA) were performed to analyze group by time interactions for all variables measured. Statistical software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) was used to perform all statistical analyses. When appropriate, follow-up paired t-tests were utilized for post hoc comparisons. An alpha level of $p < 0.05$ was considered statistically significant for all comparisons. All data are reported as mean ± standard deviation SD.

Results

All 16 participants completed each experimental test with 89.7% compliance as measured by returned empty supplement bags. However, while not all supplement bags were returned, all 16 participants verbally confirmed that all supplements issued were ingested.

Functional Performance

Functional performance results are presented in Table 2. Briefly, there were no significant differences ($p > 0.05$) at PRE for any variable in the functional performance testing battery between treatment conditions. However, there was a significant main effect for time for handgrip strength ($p = 0.045$; $\eta^2 = 0.128$), STS time ($p = 0.002$, $\eta^2 = 0.275$), and the 6MWT ($p < 0.001$, $\eta^2 = 0.382$).

Table 2. Performance Values

Variable	Group	Pre	Post	Time	Time x Group
Handgrip (kg)	Placebo	21.4 ± 5.3	22.1 ±4.4	p = 0.045	$p = 0.593$; $\eta^2 = 0.010$
	Citrulline	20.7 ± 5.1	21.9 ± 4.7		
Sit-to-Stand (repetitions)	Placebo	14.6 ± 0.9	15.2 ± 1.0	p = 0.002	$p = 0.860$; $\eta^2 = 0.001$
	Citrulline	14.2 ± 1.2	14.8 ± 1.2		
Get-Up and Go (s)	Placebo	6.7 ± 0.9	6.6 ± 0.6	p = 0.108	$p = 0.877$; $\eta^2 = 0.001$
	Citrulline	6.8 ± 0.7	6.7 ± 0.7		
6 Min Walk Test (s)	Placebo	548.3 ± 68.9	577.7 ± 64.5	p < 0.001	$p = 0.563$; $\eta^2 = 0.011$
	Citrulline	561.3 ± 68.9	583.6 ± 61.2		
5m Gait (s)	Placebo	3.2 ± 0.6	3.0 ± 0.4	p = 0.500	$p = 0.088$; $\eta^2 = 0.094$
	Citrulline	3.2 ± 0.4	3.3 ± 0.6		

Data presented as mean ± SD

Cardiovascular Measures

The results for all cardiovascular measures are presented in Table 3. Briefly, there was no significant time by treatment interaction nor main effect of time found for resting or post-exercise heart rate ($p > 0.05$). There was a significant time by treatment interaction observed for resting diastolic BP ($p = 0.028$, $\eta^2 = 0.151$) indicating lower resting diastolic BP values following seven days of CIT supplementation compared to placebo. Additionally, no main effect for time was found ($p = 0.820$, $\eta^2 = 0.002$). No significant time by treatment interaction was observed for post exercise systolic BP ($p = 0.702$, $\eta^2 = 0.005$). There were no significant time by treatment interactions or main effects for time seen for any other BP measure.

Discussion

The purpose of this study was to determine the effect of short-term CIT supplementation on measures of health and functional performance in older active women. The results demonstrated CIT provided no beneficial effects on handgrip strength, STS, GUG, 5m gait time, or 6MWT performance. Interestingly, resting diastolic BP was significantly lower following seven days of CIT supplementation compared to PL with no other differences found in any other cardiovascular measure.

Table 3. Cardiovascular Measures

Variable	Group	Pre	Post	Time	Time x Group
Resting Heart Rate (bpm)	Placebo	73.1 ± 10.4	73.0 ± 8.6	p = 0.868	p = 0.793; η^2 = 0.002
	Citrulline	73.3 ± 7.9	73.9 ± 13.4		
Post-Exercise Heart Rate (bpm)	Placebo	94.9 ± 6.2	96.3 ± 11.1	p = 0.619	p = 0.971; η^2 = 0.000
	Citrulline	96.3 ± 16.0	97.4 ± 12.0		
Resting Systolic BP (mmHg)	Placebo	131.6 ± 17.6	133.4 ± 19.8	p = 0.898	p = 0.487; η^2 = 0.016
	Citrulline	131.1 ± 18.3	129.9 ± 19.9		
Resting Diastolic BP (mmHg)	Placebo	73.6 ± 16.3	77.9 ± 15.7	p = 0.820	p = 0.028*; η^2 = 0.151
	Citrulline	78.8 ± 12.9	73.6 ± 13.1*		
Post-Exercise Systolic BP (mmHg)	Placebo	146.9 ± 21.0	148.5 ± 30.5	p = 0.942	p = 0.702; η^2 = 0.005
	Citrulline	148.2 ± 20.3	147.1 ± 20.4		
Post-Exercise Diastolic BP (mmHg)	Placebo	83.4 ± 15.4	78.1 ± 15.0	p = 0.108	p = 0.874; η^2 = 0.001
	Citrulline	84.8 ± 19.5	80.4 ± 13.9		

Data presented as mean ± SD. * Citrulline significantly different than placebo

Our data revealed that CIT supplementation produced no beneficial effects on any measure of functional performance in older active women compared to placebo, which is contrary to recent literature showing improvement in exercise performance after CIT supplementation in younger adults [7,11,21]. In older dynapenic obese adults, timed GUG (Δ -16.1 vs. -11.8sec) and upper-limb muscle strength (Δ +9.3 vs. 3.3kg) improved more after 12 weeks of HIIT and CIT supplementation compared to HIIT and placebo [15]. In a follow-up study, Buckinx and colleagues [13] reported that CIT supplementation improved BMI, android fat mass, and self-paced gait time in sedentary healthy older adults engaging in HIIT training. In partial support of the present findings, Ashley et al. [17] measured oxygen uptake kinetics during a 15 min of treadmill walking at 40% of the participant's HRR. This investigation found no significant change in net oxygen cost of moderate intensity walking in young or older adults as a result of CIT supplementation, suggesting no performance benefits of CIT consumption. As the participants in the present study were active older women who participated in moderate intensity strength training 2-3 times per week, they may have been less responsive to the CIT treatment as compared to previous work utilizing inactive or obese older adults [13,15]. Another reason for the difference in results are that one of the studies [15] supplemented for 12 weeks while the supplementation intervention in this study was for only seven days. This same study recruited obese dynapenic older adults while the present study recruited active older adult women. It is likely that sedentary older adults benefit from NO precursors to a greater extent than active individuals due to their lower NO bioavailability [22].

Our data indicated that resting DBP was significantly lower (5.5%) following one week of CIT supplementation which is consistent with other work reporting reduced diastolic BP following CIT administration [2,17]. One study showed that while two weeks of CIT supplementation significantly lowered (5%) diastolic BP for older men, there was no significant difference in older women [17]. Furthermore, mean BP, blood flow, and vascular conductance was not different in women at rest or during exercise [2]. The authors noted that the older men had higher diastolic BP at baseline (~13%) than the older women participants. Thus, it is plausible that CIT may be most beneficial for those who have higher initial BP readings indicating endothelial dysfunction. Therefore, while improvements in DBP were detected, a larger effect may have been observed with less-active participants. With resting diastolic BP improved following the CIT intervention, it may suggest that muscle blood flow was improved as well. However, as blood flow was not directly measured in this study, this assumption cannot be confirmed and more work in needed in this area.

While the present study showed improvement in resting diastolic BP after seven days of supplementation, there were no changes observed in systolic BP in either treatment condition. Two investigations specifically report that after CIT supplementation, systolic BP was significantly lowered, while no differences in diastolic BP were found for either group at any time point in these studies [7,23]. In addition, some studies found that systolic BP lowered for older men along with diastolic BP, without any effect found in older women [2,17]. Taken together with the results of this study, there may be a differential responses between sexes which has been reported following consumption of other NO-precursors [24].

The results of this study are not without limitations. While most studies to date have utilized acute or short-term periods (seven days) of CIT supplementation, longer supplementation protocols may be more beneficial to detect any favorable effects of CIT consumption similar to the two longer duration HIIT training studies in the literature[13,15]. Additionally, this study sought to determine the effects of CIT on functional performance, the addition of more sensitive testing measures (e.g., electromyography, tensiomyography, force place data) in addition to functional testing may have been able detect subtle changes in muscular function and performance. Furthermore, measurement of plasma arginine and NO metabolites or a measurement of muscle oxygenation (e.g., near-infrared spectroscopy) would have been useful to interpret individual responses to CIT supplementation and the functional outcomes in the current study.

The present study is one of few to investigate the effects of functional movement in older active women after CIT supplementation. These results indicate that seven days of CIT supplementation does not improve functional in older active women. However, this study provides evidence that CIT may improve resting DBP after seven days of supplementation. By lowering DBP, CIT may improve cardiovascular health in older active women.

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