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# Impact of L-citrulline supplementation on oxygen uptake kinetics during walking

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## Abstract

Supplementation with L-citrulline (Cit) has been shown to improve muscle oxygenation and oxygen uptake ($VO_2$) kinetics during moderate-to-high intensity cycling in young men. The aim of this study was to test the hypothesis whether Cit would improve $VO_2$ kinetics during walking in older and young adults. In a randomized, double-blind study, 26 (15 women, 11 men) adults between the ages of 20–35y (n = 15) and 64–86y (n = 11) completed 7 day periods of taking placebo and L-citrulline (6 g/day) in a crossover manner. Participants walked on a treadmill at 40%HRR while pulmonary $VO_2$ was measured using indirect calorimetry. Net oxygen cost, mean response time (MRT), and the oxygen deficit were calculated before and after each supplement period. There was no significant change ($P$>0.05) in net oxygen cost, MRT, or the oxygen deficit after Cit in older adults, while young adults showed a decrease ($P$=0.05) in the oxygen deficit after Cit that tended ($P$=0.053) to be different than the change after placebo. Sex-stratified analysis revealed that Cit decreased MRT ($P$=0.04, Cohen's d = 0.41) and the oxygen deficit ($P$<0.01, Cohen's d = 0.56) in men with the change after Cit being greater than the change after placebo (MRT: −4.5±2.1 vs. 3.4±2.1s, $P$=0.01; deficit: −0.15±0.05 vs. 0.01±0.05L, $P$=0.02). All $VO_2$ parameters were unchanged ($P$>0.05) following Cit and placebo in women. Citrulline does not alter the oxygen cost of moderate intensity walking in young or older adults, but Cit improved the rate of rise in $VO_2$ at exercise onset in men.

### Keywords

L-citrulline; $VO_2$ kinetics; walking; exercise; energy cost

## Introduction

Older adults have a slower adjustment in the utilization of oxygen at the onset of submaximal exercise as compared to young adults (Gurd et al. 2008; Scheuermann et al.

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2002). As a result, older adults must rely on anaerobic metabolism at the start of exercise while oxygen uptake ($VO_2$) increases to meet an elevated metabolic demand. This results in an early production of fatigue-developing metabolites (Idström et al. 1986) and associated decrements in functional performance (Alexander et al. 2003). The slower $VO_2$ response across the transition from rest to submaximal exercise in older adults is partly due to inadequate oxygen delivery to active muscle (Gurd et al. 2008). Interestingly, enhancing nitric oxide bioavailability has the potential to improve muscle oxygenation with implications for faster $VO_2$ kinetics (Bailey et al. 2009). For example, dietary nitrate taken for 3 days speeds the increase in $VO_2$ (i.e., mean response time) at the start of submaximal treadmill walking in older adults (Kelly et al. 2013). While dietary nitrate is effective at improving nitric oxide bioavailability through a one-electron reduction from nitrite, age-related deficits in nitric oxide synthase activity are not addressed. Aging is associated with elevated arginase activity (Santhanam et al. 2008), which competes with nitric oxide synthase for L-arginine thereby limiting nitric oxide production. Therefore, supplements that can improve the substrate pool for nitric oxide synthase may also be effective at improving $VO_2$ at the start of submaximal exercise in older adults.

Supplementation with the amino acid, L-citrulline (Cit), has been shown to increase plasma biomarkers of nitric oxide after 7 days of supplementation in young (Bailey et al. 2016) and older adults (Schwedhelm et al. 2008). L-citrulline has the potential to improve nitric oxide bioavailability by increasing systemic levels of L-arginine (Bailey et al. 2015; Kim et al. 2015; Schwedhelm et al. 2008), substrate needed by nitric oxide synthase to produce nitric oxide. Previous work has demonstrated that 7 days of Cit supplementation (6 g/day) increases the tissue oxygenation index of the vastus lateralis during moderate and high intensity cycling in young men suggesting improved muscle perfusion during exercise (Bailey et al. 2015; Bailey et al. 2016). Moreover, Cit is found to increase the rate of oxygen uptake (i.e., faster $VO_2$ mean response time) at the start of high intensity cycling exercise in young men (Bailey et al. 2015). While these studies in young men support faster oxygen delivery and utilization during submaximal exercise after Cit supplementation, whether Cit has the same effect on $VO_2$ kinetics in older adults has yet to be investigated.

To date, studies that have reported a positive impact of Cit supplementation on $VO_2$ kinetics have been performed in young men (Bailey et al. 2009; Bailey et al. 2015). Animal studies have demonstrated that transport of L-arginine into the endothelium is influenced by age and sex (Schwartz et al. 2009a), with male rats showing higher aortic L-arginine transport velocities than female rats (Schwartz et al. 2009b). Our laboratory also recently demonstrated that Cit supplementation increases peripheral vasodilation (vascular conductance) during submaximal exercise in older men but not women (Gonzales et al. 2017), providing support for the notion that sex may alter the potential for augmented L-arginine to benefit nitric oxide bioavailability. Therefore, the purpose of this study was two-fold. Our primary aim was to determine whether Cit supplementation improves $VO_2$ kinetics during submaximal exercise in older adults. We hypothesized that Cit supplementation would speed $VO_2$ kinetics in older and young adults. A secondary aim of this study was to determine whether the impact of Cit supplementation is sex-dependent. We hypothesized, based on our past work (Gonzales et al. 2017), that men would show greater improvement in $VO_2$ kinetics than women after Cit supplementation.

## Methods

### Participants

Fifteen adults (8 women, 7 men) between the ages of 20–31 years (22 ± 2y) and 11 adults (7 women, 4 men) between the ages of 64–86 years (74 ± 7y) were recruited for this study. Participants were non-smokers and self-reported no history of physician-diagnosed cardiovascular, pulmonary, or metabolic disease. None of the participants were taking medications for blood pressure or drugs that alter heart rate. Participants were not obese (all had BMI <30 $kg/m^2$) and did not report or show visual signs of orthopedic limitations to walking. Lastly, participants were excluded if they reported >3 days per week of planned aerobic exercise in order to include participants with similar physical activity behavior.

### Study Procedures

The study procedures were approved by the Institutional Review Board at Texas Tech University (approval #2016-492). The subjects were required to attend a total of five visits. On the first visit, participants provided written informed consent and completed a medical history questionnaire. Participants were also familiarized with treadmill walking while wearing a respiratory mask. Study procedures were the same for visits two through five. On these visits, height and weight were measured using a standard scale followed by measurement of resting blood pressure using an automated device (Oscar2, Suntech Medical, Morrisville, NC) after the subject rested in a seated position for 10 min. Participants then performed a 5 min warm-up (<2.5 mph) wearing a respiratory mask to accustom the participant to the procedures and reduce anxiety prior to testing. Past work shows no effect of prior light exercise on $VO_2$ kinetics (Diamond et al. 1977). After 15 min of seated rest, participants returned to the treadmill for walking at 40% of their heart rate reserve (HRR). Age-predicted maximum heart rate was used to calculate HRR along with resting heart rate while standing on the treadmill prior to exercise.

The last four study visits were designed as a placebo-controlled, double-blind, crossover study. Participants were randomly assigned to placebo (maltodextrin, NOW supplements, Bloomingdale, IL) or L-citrulline (6g per day; Kyowa Hakko USA, New York, NY) capsules for 7 days, followed by a 2 week washout period, and crossed over to the other condition for another 7 days. The sequence of supplements was equally balanced within each age group. For each visit, participants were informed to arrive fasted, and no food or drink other than water for at least 4 hours prior to their appointment. Also, participants were asked to refrain from ingesting any vitamins, supplements, or caffeine ≥12 hours prior to their appointment.

### Pulmonary Gas Exchange

Oxygen uptake was measured using indirect calorimetry (Ultima Cardio$O_2$, MedGraphics, St. Paul, MN) and a respiratory mask. Participants stood for 5 min to achieve a resting steady-state $VO_2$. The speed of the treadmill was increased to a level previously recorded during the first visit to achieve 40%HRR then adjusted, if necessary, to meet the set intensity. The participants walked for 5 min until a steady-state $VO_2$ was reached. If steady-state was not reached, an additional minute was added until participants achieved steady-state. To ensure participants were maintaining an aerobic intensity, respiratory exchange

ratio was monitored for a value ≤ 0.9. Heart rate was monitored during exercise using a Polar chest strap and watch (model FS1, Polar Electro, Bethpage, NY).

The last two minutes of $VO_2$ during standing rest and exercise steady-state was averaged to determine resting and exercise $VO_2$, respectively. The net energy cost of walking was determined by subtracting resting from exercise $VO_2$. To calculate the rate constant (k), the breath-by-breath $VO_2$ response to exercise was subtracted from resting $VO_2$ and entered into SigmaPlot software (version 13.0, Systat Software, San Jose, CA) for monoexponential modeling. The oxygen ($O_2$) deficit was calculated by dividing net $VO_2$ from the rate constant ($\Delta VO_2/k$) as described previously (Whipp 1971). Mean response time (MRT) was calculated by the inverse of the rate constant (1/k) (Lamarra 1990). Lastly, the 95% confidence interval for the rate constant ($CI_{95}$) was also reported as recommended for studies reporting $VO_2$ kinetics from a single on-transient response (Lamarra et al. 1987).

### Treadmill Stepping Cadence

Stepping cadence was assessed during treadmill walking using an accelerometer based system (APDM Mobility Lab, Dynavision, West Chester, OH). Three sensors were secured to participants using elastic straps. One small plastic sensor was placed on top of each shoe and another at the lumbar spine on the participants' backside. The sensors transmitted motion data in real-time during walking allowing for the measurement of stepping cadence. Cadence was recorded in order to determine if the number of steps taken per minute on the treadmill varied between visits.

### Statistical Analysis

Net $VO_2$, MRT, $CI_{95}$, and the oxygen deficit were normally distributed in the group analyses based on Kolmogorov-Smirnov tests of normality. For the main analysis, primary outcome variables examined were the pre-to-post change scores for the $VO_2$ kinetic parameters (i.e., net $VO_2$, MRT, and the $O_2$ deficit) after each treatment condition (placebo and Cit). The analysis of crossover data was conducted based on a linear mixed modeling approach proposed by Senn (2002) in order to test and adjust for possible nuisance effects related to order (sequence supplement was taken) and period (first week versus second week). Non-significant interaction terms ($P>0.05$) between age, sex, and treatment conditions were removed from the final model. Primary parameters of interest examined from the model included 1) significance of pre-to-post change scores of $VO_2$ kinetic parameters after treatment; 2) between-condition differences in pre-post change scores of $VO_2$ kinetic parameters; and 3) between-group (age or sex) differences in between-condition differences in pre-post change scores of $VO_2$ kinetic parameters. Effect sizes (Cohen's d) were calculated and interpreted as weak (<0.20), small (0.20–0.49), medium (0.50–0.79), and large (≥0.80) (Cohen 1988). Paired t-test was used to test for differences in demographic variables before and after supplementation within each treatment condition. SAS v.9.4 (SAS Institute, Cary, NC) was used for data analysis and statistical significance was considered $P \leq 0.05$.

## Results

Table 1 presents participant characteristics before and after Cit and placebo when participants are categorized based on age. Resting diastolic blood pressure was higher ($P<0.01$) after placebo in young adults, but was not different ($P>0.05$) after Cit or in older adults after either condition. Heart rate during exercise was not different ($P>0.05$) after Cit or placebo in young adults, but was lower ($P=0.03$) after Cit in older adults.

Figure 1 shows $VO_2$ kinetic parameters measured during walking before and after Cit and placebo in young and older adults, and the change in variables compared between conditions is shown in Table 3. Net $VO_2$ or $CI_{95}$ were not different ($P>0.05$) after Cit or placebo in young adults. Mean response time was slower ($P=0.05$) after placebo in young adults (by 7%), but was not different ($P>0.05$) after Cit. The $O_2$ deficit was lower ($P=0.05$, Cohen's d = 0.48) after Cit in young adults (by 11%), but the change in the $O_2$ deficit following Cit tended (p=0.053) to be different as compared to the change after placebo. Net $VO_2$, MRT, $CI_{95}$, and the $O_2$ deficit were not different ($P>0.05$) after Cit or placebo in older adults.

Sex-stratified analysis of participant characteristics before and after Cit and placebo is shown in Table 2. Resting diastolic blood pressure was higher ($P<0.01$) after placebo in women, but was not different ($P>0.05$) after Cit. Resting systolic blood pressure was reduced ($P=0.03$, Cohen's d = 0.46) after Cit in men with no difference ($P>0.05$) after placebo. Figure 2 shows $VO_2$ kinetic parameters measured during walking before and after Cit and placebo in women and men, and the change in variables compared between conditions is shown in Table 3. Net $VO_2$, MRT, $CI_{95}$, and the $O_2$ deficit were not different ($P>0.05$) after Cit or placebo in women. Similarly, net $VO_2$ and $CI_{95}$ were not different ($P>0.05$) after Cit or placebo in men. However, the change in MRT and the $O_2$ deficit in men was significantly different ($P<0.05$) after Cit when compared to the change after placebo. Mean response time significantly decreased by 10% ($P=0.04$, Cohen's d = 0.41) and the $O_2$ deficit was reduced by 17% ($P<0.01$, Cohen's d = 0.56) after Cit in men, whereas no significant differences ($P>0.05$) were observed after placebo. The reduction in MRT and $O_2$ deficit after Cit supplementation was observed in young men (pre vs. post Cit: 48.66±2.77 vs. 43.55±3.61s, $P<0.01$; 1.01±0.06 vs. 0.84±0.06L, $P<0.01$), but not older men (pre vs. post Cit: 50.05±5.00 vs. 45.55±2.02s, $P=0.33$; 0.69±0.10 vs. 0.59±0.07L, $P=0.15$) when examined separately.

## Discussion

This study aimed to determine if Cit supplementation would alter $VO_2$ kinetics during submaximal walking. L-citrulline, when taken orally, is absorbed by the small intestine, enters the circulation, and after renal uptake is converted to L-arginine through an argininosuccinate pathway (Figueroa et al. 2017). Plasma levels of L-arginine increase in young (Bailey et al. 2015; Bailey et al. 2016) and older adults (Schwedhelm et al. 2008) following oral intake of Cit. The endothelium is a tissue that can uptake L-arginine from the blood through a CAT-1 transporter (Bentur et al. 2015). In the endothelial cell, L-arginine can be oxidized to nitric oxide by nitric oxide synthase. We therefore postulated that Cit supplementation would enhance nitric oxide bioavailability that would result in faster $VO_2$

kinetics due to improved oxygen delivery and/or distribution (Bailey et al. 2016; Gonzales et al. 2017), although other mechanisms such as reducing the energy cost of contraction (Bailey et al. 2010) or increasing the maximal rate of oxidative ATP production (Larsen et al. 2011) may also alter $VO_2$ kinetics. Bailey et al. (2015) found 7 days of Cit supplementation increases skeletal muscle oxygenation and speeds $VO_2$ kinetics (MRT) by 10% during high intensity cycling in young men. Similarly, the present study finds faster uptake of oxygen at the start of moderate intensity walking (i.e., MRT) in men (by 11% in young men) after Cit supplementation. Considering we did not observe lower net $VO_2$ during walking it is unlikely that the improved $VO_2$ kinetics after Cit supplementation in men was due to a lower energy cost of walking.

Interestingly, when data were separated according to sex, men showed a significant improvement following Cit supplementation in MRT and the $O_2$ deficit during walking that was largely attributed to young men. We acknowledge that this study has a small sample size and is underpowered to claim that there is a sex difference, but evidence does exist in the literature for differential regulation of L-arginine transport between sexes. While acute infusion of L-arginine (Forte et al. 1998) or Cit ingestion (Kim et al. 2015) increases whole-body nitric oxide synthesis in women and men, animal studies show higher aortic L-arginine transport velocities in young male rats as compared to young female rats (Schwartz et al. 2009b). In addition, although both older male and female rats have elevated levels of PKCα (Schwartz et al. 2009a), an inhibitor of L-arginine transporter CAT-1, only male rats show increased L-arginine transport when PKCα is blocked with α-tocopherol (Schwartz et al. 2009b). The level of PKCα is higher in older male as compared to young male rats (Schwartz et al. 2009b; Schwartz et al. 2009a), which may partly explain the greater effect of Cit on $VO_2$ kinetics in young men presented here. Although, our findings for older men should be interpreted with caution due to their low sample size relative to the other groups.

The ineffectiveness of Cit to alter net $VO_2$ or the oxygen cost of walking is consistent with other studies that observe no effect of short-term Cit supplementation on absolute $VO_2$ during submaximal cycling exercise (Bailey et al. 2015; Suzuki et al. 2016). However, it is in contrast to a study by Lansley et al. (2011) that showed 6 days of nitrate supplementation via beetroot juice to lower steady-state $VO_2$ by 12% in young men during slow walking on a treadmill at a set speed (~1.11 m/s). The gait speed in the present study was faster (all: 1.49 ± 0.25 m/s), but Lansely et al. (2011) also found lower exercise $VO_2$ during moderate intensity running so the differences between studies is not likely due to exercise intensity. Examining young men separately from older men or young women also found no effect of Cit on net $VO_2$ during walking (before: 15.7 ± 0.7 vs. after: 15.1 ± 1.3 mL/kg/min, p=0.31), so the discrepancy between studies is not due to variance explained by age or sex. Considering that dietary nitrate increases nitric oxide bioavailability dependent on factors affected by metabolism (changes in pH, oxygen tension, and redox status), it is possible that nitrate supplementation is more effective than Cit supplementation at improving muscle efficiency (i.e., lower oxygen cost) during exercise. However, it should be noted that dietary nitrate supplementation with beetroot juice for 3 to 7 days in older adults results in no change in $VO_2$ during moderate intensity walking (Kelly et al. 2013), similar to the present results. Therefore, further research is needed to clarify if the oxygen cost of walking can be improved by dietary supplements aimed at increasing nitric oxide production.

Limitations to this study include no measures of nitric oxide bioavailability to support the mechanistic pathway thought to be responsible for changes in $VO_2$ kinetics with Cit supplementation. Past work giving Cit at 6 g/day for 7 days have reported a tendency for increased plasma [nitrite] in young men (Bailey et al. 2015), and significantly increased plasma [nitrite+nitrate] and urinary [nitrate] in middle-aged to older adults (Ochiai et al. 2012; Schwedhelm et al. 2008). The present study used the same dose and duration of Cit as these past studies so we assume a similar improvement in nitric oxide bioavailability occurred. Support for a change in vascular function was observed in men through a 4% reduction in resting systolic blood pressure after Cit supplementation. This finding is consistent with past work that finds 4% to 9% reductions in systolic blood pressure after Cit supplementation in young men (Bailey et al. 2015; Sanchez-Gonzalez et al. 2013). Another limitation to this study is the small sample size. Despite our low power to test for differences, the small to medium effect sizes for the difference in MRT and the $O_2$ deficit after Cit in men indicates a considerable change after Cit. Lastly, we recruited participants with similar physical activity levels, and set walking speed to a similar relative intensity across all participants based on HRR using age-predicted maximum heart rate, but we did not assess for cardiorespiratory fitness level. Peak $VO_2$ is associated with faster $VO_2$ kinetics during submaximal exercise in young and older adults (Chilibeck et al. 1996), and may have limited the capacity for Cit to improve $VO_2$ kinetics in individuals with higher cardiorespiratory fitness due to a possible ceiling effect.

In conclusion, seven days of L-citrulline supplementation does not improve the oxygen cost of moderate intensity walking in young or older adults. Rather, supplementation with L-citrulline improved oxygen uptake kinetics during walking in men, but not women. Further research is needed to confirm if women experience different outcomes after L-citrulline supplementation as compared to men, and if confirmed, to reveal the mechanisms that may attribute to the sex difference.

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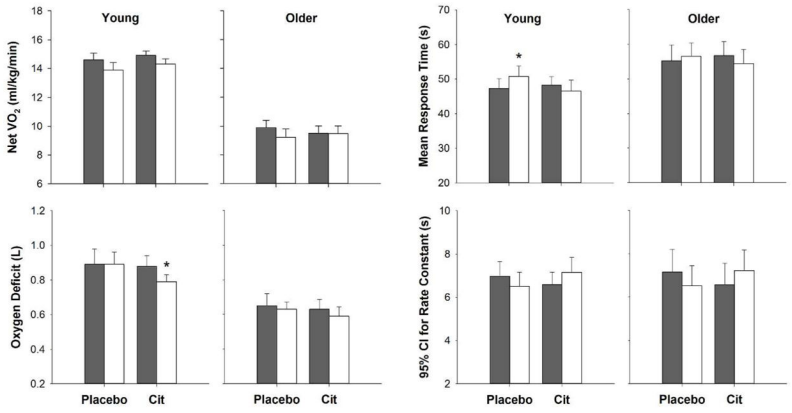


**Figure 1.**

Comparison of net oxygen uptake (VO2), the oxygen deficit, mean response time (MRT), and the 95% confidence interval (CI) for the rate constant before (shaded bars) and after (white bars) placebo and supplementation with L-citrulline (Cit) in young and older adults. *, significant difference compared to before supplementation after adjusting for sex, period, and sequence ($p \leq 0.05$).

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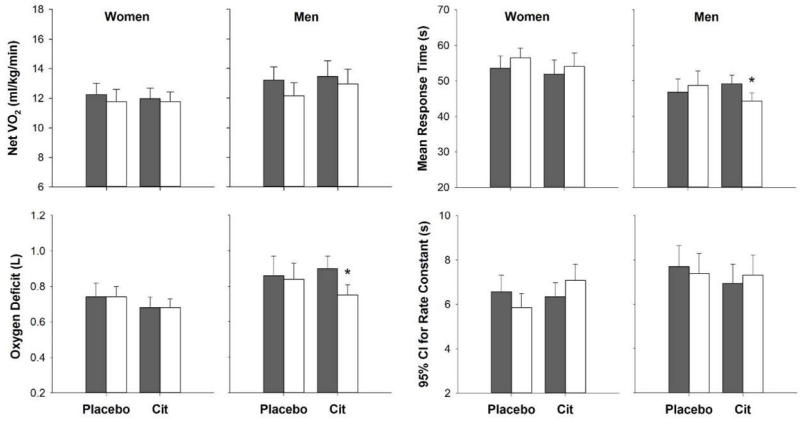


**Figure 2.**

Comparison of net oxygen uptake (VO2), the oxygen deficit, mean response time (MRT), and the 95% confidence interval (CI) for the rate constant before (shaded bars) and after (white bars) placebo and supplementation with L-citrulline (Cit) in women and men. *, significant difference compared to before supplementation after adjusting for age, period, and sequence ($p \leq 0.05$).

## Table 1

Participant characteristics before and after supplementation in young and older adults

| | *Placebo* | | *L-citrulline* | |
|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** |
| *Young (n = 15; 8 women, 7 men)* | | | | |
| Weight (kg) | 66 ± 3 | 66 ± 3 | 66 ± 3 | 66 ± 3 |
| Body Mass Index (kg/m$^2$) | 23.4 ± 0.6 | 23.3 ± 0.6 | 23.3 ± 0.6 | 23.2 ± 0.6 |
| Seated SBP (mmHg) | 120 ± 2 | 120 ± 3 | 120 ± 2 | 119 ± 2 |
| Seated DBP (mmHg) | 67 ± 2 | 73 ± 2* | 70 ± 2 | 72 ± 3 |
| Resting VO$^2$ (mL/kg/min) | 3.36 ± 0.09 | 3.41 ± 0.14 | 3.55 ± 0.12 | 3.63 ± 0.18 |
| Exercise HR (bpm) | 125 ± 2 | 126 ± 2 | 126.1 ± 2 | 127 ± 2 |
| Cadence (steps per min) | 129 ± 1 | 129 ± 1 | 129 ± 1 | 129 ± 1 |
| *Older (n = 11; 7 women, 4 men)* | | | | |
| Weight (kg) | 67 ± 3 | 66 ± 3 | 67 ± 3 | 67 ± 3 |
| Body Mass Index (kg/m$^2$) | 23.8 ± 0.9 | 23.6 ± 0.9 | 23.7 ± 0.9 | 23.7 ± 0.9 |
| Seated SBP (mmHg) | 129 ± 5 | 127 ± 3 | 130 ± 3 | 129 ± 3 |
| Seated DBP (mmHg) | 73 ± 3 | 74 ± 2 | 75 ± 2 | 77 ± 2 |
| Resting VO$^2$ (mL/kg/min) | 3.04 ± 0.18 | 2.88 ± 0.11 | 3.00 ± 0.13 | 2.90 ± 0.10 |
| Exercise HR (bpm) | 102 ± 1 | 102 ± 1 | 102 ± 1 | 100 ± 1* |
| Cadence (steps per min) | 118 ± 4 | 119 ± 4 | 118 ± 4 | 118 ± 3 |

Values are mean ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate;

* significant difference ($P \leq 0.05$) compared to before within condition.

## Table 2

Participant characteristics before and after supplementation in women and men

| | *Placebo* | | *L-citrulline* | |
|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** |
| *Women (n = 15; 8 young, 7 older)* | | | | |
| Weight (kg) | 60 ± 2 | 60 ± 2 | 60 ± 2 | 60 ± 2 |
| Body Mass Index (kg/m$^2$) | 22.8 ± 0.5 | 23.7 ± 0.5 | 22.6 ± 0.6 | 22.6 ± 0.5 |
| Seated SBP (mmHg) | 121 ± 3 | 120 ± 3 | 121 ± 3 | 123 ± 3 |
| Seated DBP (mmHg) | 67 ± 2 | 71 ± 1* | 70 ± 1 | 71 ± 2 |
| Resting VO$^2$ (mL/kg/min) | 3.20± 0.14 | 3.23 ± 0.14 | 3.22 ± 0.13 | 3.24 ± 0.12 |
| Exercise HR (bpm) | 114 ± 3 | 115 ± 3 | 114 ± 3 | 113 ± 3 |
| Cadence (steps per min) | 126 ± 2 | 126 ± 2 | 126 ± 2 | 126 ± 2 |
| *Men (n = 11; 7 young, 4 older)* | | | | |
| Weight (kg) | 75 ± 3 | 74 ± 3 | 75 ± 3 | 74 ± 3 |
| Body Mass Index (kg/m$^2$) | 24.7 ± 0.9 | 24.4 ± 0.9 | 24.7 ± 0.9 | 24.6 ± 0.9 |
| Seated SBP (mmHg) | 128 ± 4 | 127 ± 3 | 130 ± 3 | 125 ± 3* |
| Seated DBP (mmHg) | 73 ± 3 | 77 ± 2 | 76 ± 2 | 79 ± 3 |
| Resting VO$^2$ (mL/kg/min) | 3.25 ± 0.14 | 3.12 ± 0.18 | 3.44 ± 0.16 | 3.43 ± 0.27 |
| Exercise HR (bpm) | 117 ± 3 | 117 ± 4 | 118 ± 4 | 119 ± 4 |
| Cadence (steps per min) | 122 ± 3 | 122 ± 3 | 123 ± 3 | 122 ± 3 |

Values are mean ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate;

* significant difference ($P \leq 0.05$) compared to before within condition.

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**Table 3**

Change in oxygen uptake responses to walking after supplementation

| | ***Placebo*** | | ***L-citrulline*** | | **Placebo vs. Cit *P* value‡** |
|---|---|---|---|---|---|
| | **Change** | ***P* value†** | **Change** | ***P* value†** | |
| *Young* | | | | | |
| Net $VO_2$ (mL/kg/min) | −0.73 ± 0.39 | NS | −0.73 ± 0.39 | 0.07 | NS |
| MRT (s) | 3.80 ± 1.83 | 0.05 | −1.79 ± 1.83 | NS | 0.03 |
| $CI_{95}$ (s) | −0.46 ± 0.66 | NS | 0.54 ± 0.66 | NS | NS |
| $O_2$ deficit (L) | 0.01 ± 0.04 | NS | −0.10 ± 0.04 | 0.01 | 0.053 |
| *Older* | | | | | |
| Net $VO_2$ (mL/kg/min) | −0.57 ± 0.45 | NS | −0.01 ± 0.45 | NS | NS |
| MRT (s) | 2.59 ± 2.15 | NS | −0.50 ± 2.15 | NS | NS |
| $CI_{95}$ (s) | −0.60 ± 0.78 | NS | 0.59 ± 0.78 | NS | NS |
| $O_2$ deficit (L) | −0.01 ± 0.05 | NS | −0.05 ± 0.05 | NS | NS |
| *Women* | | | | | |
| Net $VO_2$ (mL/kg/min) | −0.41 ± 0.38 | NS | −0.16 ± 0.38 | NS | NS |
| MRT (s) | 2.93 ± 1.81 | NS | 2.26 ± 1.81 | NS | NS |
| $CI_{95}$ (s) | −0.74 ± 0.66 | NS | 0.76 ± 0.66 | NS | NS |
| $O_2$ deficit (L) | 0.01 ± 0.04 | NS | 0.01 ± 0.04 | NS | NS |
| *Men* | | | | | |
| Net $VO_2$ (mL/kg/min) | −0.89 ± 0.46 | NS | −0.58 ± 0.46 | NS | NS |
| MRT (s) | 3.46 ± 2.17 | NS | −4.54 ± 2.17 | 0.04 | 0.01 |
| $CI_{95}$ (s) | −0.32 ± 0.79 | NS | 0.38 ± 0.79 | NS | NS |
| $O_2$ deficit (L) | 0.01 ± 0.05 | NS | −0.15 ± 0.05 | <0.01 | 0.02 |

Values are least square adjusted mean ± SE estimated from a single linear mixed model controlling for sequence and period effects of crossover design in addition to age and sex.

MRT, mean response time; $CI_{95}$, 95% confidence interval for rate constant; NS, non-significant ($P > 0.05$)

† statistical significance of change scores before and after supplementation

‡ differences in change scores between placebo and L-citrulline conditions