The Effect of L-citrulline Supplementation on Gait Performance in Older Adults

by

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ABSTRACT

The purpose of this study was to determine if enhancing vascular function would lead to an improvement in gait performance in older adults. We hypothesized that L-citrulline would improve vascular function, specifically by reducing arterial stiffness and enhancing exercise blood flow as compared to placebo. We further hypothesized that the change in vascular function would associate positively with the change in gait performance. Twenty-four healthy older adults (60-79 years, 13 women, 11 men) were included in this study. The study was a placebo-controlled, double-blind, crossover study. Participants took a dietary supplement (L-citrulline, 6g/d) or placebo (maltodextrin) for 2 weeks followed by a washout period of 2 weeks before crossover. Participants performed walking tests and completed rhythmic calf exercise while vascular conductance (FVC) was measure in the superficial femoral artery using Doppler ultrasound. We found no significant change in arterial stiffness (pulse pressure, SFA β-stiffness index) following L-citrulline. However, the change in FVC during exercise was increased in men ($p<0.01$), but not women ($p=0.40$) following L-citrulline. Similarly, the change in gait speed reserve (fast minus usual pace) was elevated in men ($p<0.01$), but not in women ($p=0.11$) following L-citrulline as compared to placebo. The change in gait speed reserve was positively correlated with the change in FVC during exercise following L-citrulline when both sexes were combined ($r=0.66$, $p=0.01$). In conclusion, L-citrulline improved blood flow during exercise in older men. More research is needed to understand the possibility of L-citrulline improving physical function in other populations.

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CHAPTER 1

INTRODUCTION

Decreasing mobility in older adults typically leads to reduced physical activity and deconditioning which has a direct effect on physical capacity, health, and survival (Studenski et al., 2011). A valid indicator of mobility is gait speed. It is known that gait speed decreases with advancing age and contributes to reduced activities of daily living (Hardy et al., 2007; Ko et al., 2010; Schrack et al., 2010). Gait speed is also considered an indicator of vitality because it combines known and unrecognized disturbances in many organ systems (Studenski et al., 2011). For instance, gait speed is associated with clinical conditions, such as inflammatory status, and cardiovascular disease (Cesari 2011; Newman et al., 2006). In addition, gait speed has been shown to predict disability (Guralnik et al., 2000) and all-cause mortality (Newman et al., 2006).

Gait performance in older adults has multiple determining factors including perceived fatigue (Gonzales et al., 2015), neuromuscular function (Zierath & Hawley 2004), muscle strength (Delmonico et al., 2007; Jenkins et al., 2015), obesity (Lia et al., 2008) and decreased cognitive function (Rosso et al., 2013). Vascular function may also be an important determining factor of gait performance in older adults. Arterial stiffening of large central arteries (aorta, common carotid artery) occurs with advancing age (Hashimoto & Ito 2009). This arterial structural change, as assessed by measuring carotid-femoral pulse wave velocity or carotid artery distensibility, is associated with slower gait speed in healthy older adults (Gonzales 2012; Watson et al., 2011). Moreover, parameters of arterial function have also been shown to relate with gait performance. Sorond et al. (2010) looked at endothelial function and found that impaired cerebral vasoreactivity is associated with slower gait speed and falls in older adults. Gonzales et al. (2014) also showed that older adults that exhibited greater performance fatigue during walking at a fast pace had lower leg blood flow and vascular conductance responsiveness during rhythmic plantar-flexion exercise. These studies provide evidence that impaired vascular function may be associated with slow gait speed suggesting that vascular function may be an important determining factor of gait performance.

Purpose of the study

The purpose of this study was to determine if enhancing vascular function would lead to an improvement in gait performance in older adults. To improve vascular function, we used the dietary supplement L-citrulline, which has been shown to reduce arterial stiffness (Ochiai et al., 2010), blood pressure (Bailey et al., 2015; Cutrufelo et al., 2015), and speed oxygen uptake kinetics during exercise (Bailey et al., 2015). While research shows an inevitable slowing of gait speed in older adults, this study tested if L-citrulline will improve gait speed thereby increasing vitality in older adults. Our hypothesis was that arterial stiffness and blood flow during exercise will be improved in older adults following L-citrulline as compared to placebo. We further hypothesized that the positive change in arterial stiffness and exercise blood flow following L-citrulline will be associated with a positive change in gait performance.

Delimitations:

1. Older adults with manifest cardiovascular disease or diabetes were not able to participate in this study because of abnormal cardiovascular and metabolic systems as compared to healthy older adults.
2. Older adults taking medication for hypertension or hypercholosterolemia were unable to participate in this study because the medication could alter the effect of L-citrulline on vascular function. It would be unethical to have participants cease medication for the purpose of this study.

Assumptions:

1. Participants in this study were instructed to continue their normal daily lifestyle including dietary habits, exercise and physical activity, and not to begin any new activity (for example, a new exercise program or diet).
2. All supplements were taken as instructed during this study.
3. Participants followed instructions to refrain from food, drink and caffeine 12 hours prior to testing.
4. Participants gave full and consistent effort according to the instructions provided for each gait performance test.

CHAPTER II

REVIEW OF LITERATURE

Introduction

It is well known that mobility decreases with advancing age in older adults. One of the indicators of mobility is gait speed. Gait speed decreases with age in women and men (Ko et al., 2010), and contributes to reduced activities of daily living (Hardy et al., 2007; Shrack et al., 2010). In addition, gait speed is associated with limitations in motor-based activities such as dressing, shopping and using public transportation (Verghese et al., 2011). Thus, gait speed is an indicator of mobility in older adults and should be assessed not only to examine mobility, but also to identify early decline in overall physical function (Newman et al., 2006).

Multiple physiological factors affect gait performance in older adults. Some of these factors include neuromuscular function (Zierath & Hawley 2004), skeletal muscle energetics (Coen et al., 2013), and vascular structure (Watson et al., 2011) and function (Gonzales et al., 2014; Sorond et al., 2010), all of which if negatively affected, can have a detrimental impact on gait performance. Recent studies show that age associated changes in gait performance are more prominent throughout challenging walking conditions. Ko et al. (2010) had older men and women complete a 10 meter walk test at their usual-paced and fast-paced speed. They found fast-paced gait speed decreased at a steeper rate than usual-paced gait speed suggesting that fast-paced walking is more sensitive to age-related limitations than usual-paced walking (Ko et al., 2010).

Assessing gait performance can be done in multiple ways. There are short distance and long distance walking tests. Short distance tests generally range between 2.5 to 20 meters in length. However these tests are known to lack accuracy because they are less sensitive to capture the small differences in gait performance (Sayers et al., 2006) and do not assess aerobic fitness or endurance due to their short length (Newman et al., 2006). Long distance walking tests are 400 meters in length and involve walking 10 laps in a long corridor hallway, 40 meters each lap (Newman et al., 2006). The long distance walking test better assesses endurance as well as discriminates walking ability in highly functional older adults (Sayers et al., 2006; Simonsick et al., 2001). As a result, long distance walking tests can be used as a sensitive and early identifier of decline in physical function in older adults (Newman et al., 2006).

Factors known to predict gait performance in healthy older adults

As humans age there is a physiological change in skeletal muscle and its function. Two well-known terms that describe this change are sarcopenia and dynapenia. Sarcopenia is the loss of muscle mass as age increases whereas dynapenia is the loss of muscle strength with age. Both of these phenomenon play an important role in muscle function as humans age and increase the risk of disability, functional impairment, and death (Baumgartner et al., 1998). The loss in muscle strength is related to the loss of type II muscle fibers (fast twitch fibers) (Jenkins et al., 2014) which are responsible for anaerobic capacity. Jenkins et al. (2014) found that decreases in peak torque and power in older adults is reflective of dynapenia due to the decreased type II (fast twitch) muscle fiber size and function. In relation to muscle strength, Misic et al. (2007) assessed muscle quality by the relationship between leg strength and leg lean mass. In this study, gait speed and time going up and down stairs were predicted by muscle quality in healthy older adults (Misic et al., 2007).

Muscle strength is not the only factor that affects gait performance. Declines in the efficiency and capacity of skeletal muscle energy production is also a factor associated with aging (Coen et al., 2013). As age increases, not only an increase in sarcopenia (loss of muscle mass) occurs, but also a decrease in whole body oxidative capacity and a decrease in muscular mitochondrial capacity and efficiency (Coen et al., 2012). In addition, older adults are reported to have mitochondrial dysfunction (Martins et al., 2012), leading to an increased energetic cost of walking (Coen et al., 2013). Coen et al. (2013) determined that the decrease in mitochondrial capacity and efficiency were directly related to decreased walking speed in older men and women. In relation to this finding, there is also a decline in overall energy reserve with increasing age (Schrack et al., 2010). More specifically, Schrack et al. (2010) studied energy availability (peak VO2 – resting VO2) in older adults and found that there was a total of 55-60% loss in available energy. It was concluded that the lower energy availability translates to a higher intensity of exercise (working closer to peak VO2) even during activities of daily living (Schrack et al., 2010).

Cognitive function has an effect on gait performance. Different diseases such as Alzheimers, vascular dementia, and stroke, all impair cognitive function and are known to negatively impact gait performance (Rosso et al., 2013). Other central nervous system abnormalities known to be a complex variety of “silent diseases” (Sonnen et al., 2011) are also common in older adults, and have been shown to affect gait and motor function (Verghese et al., 2012). Of the factors that directly affect cognitive function, neurovascular coupling, cerebral white matter hypertensies (Sorond et al., 2011), and cerebral blood flow (Sorond et al., 2010) are all independent predictors of gait speed in older adults. Sorond et al. (2010) found impaired cerebral vasoreactivity to physiological stimuli is associated with slower gait speed and increased risk for falls in older adults. In addition, Sorond et al. (2011) found individuals with decreased neurovascular coupling, a relationship between regional synaptic activity and cerebral blood flow, as well as those with higher white matter hypertensies, had slower gait speed. Adults who had both impairments were twice as likely to have reduced gait speed (Sorond et al., 2011).

Arterial stiffness and blood flow in relation to gait performance

Arterial structural changes occur with aging. Some of these changes include increased stiffness, increased thickness of the arterial wall and enlarged lumen diameter (Hashimoto & Ito 2009). As the arteries stiffen, aortic pulse wave velocity and pulse pressure increases (Mitchell 2008). Watson et al. (2011) assessed pulse wave velocity and gait speed in participants over a 7 year period and determined that participants with previous peripheral arterial disease, but not healthy patients, were reported to have higher pulse wave velocity along with a slower gait speed. Gonzales (2012) assessed arterial stiffness in central and peripheral arteries in presumably healthy older adults. He found that higher central arterial stiffness was related to decreased distance traveled during a 2-minute walk test, as well as slower gait speed. In contrast, Gonzales (2012) did not find peripheral arterial stiffness and resting leg blood flow to relate to gait performance. More recently, Gonzales et al. (2014) showed that older adults who displayed a decrease in walking speed during a fast-pace 400 meter walk test (i.e., performance fatigue) had lower responses in leg blood flow and leg vascular conductance to rhythmic plantar-flexion exercise. These studies support the concept that central arterial stiffness and diminished peripheral vascular function during exercise is associated with gait performance.

Pulse pressure is also a known predictor of gait speed. In the assessment of a usual-paced 400 meter walk test, older adults with higher pulse pressure has slower gait speed (Heffernan et al., 2012). Hajjar et al. (2009) also found patients with poor cognitive function, slower gait speed, and symptoms of depression had increased pulse pressure (vascular load). The elevated pulse pressure was associated with cardiovascular disease and lower daily living activities. More specifically, Hajjar et al. (2009) found that systolic blood pressure, more than diastolic blood pressure, was more strongly associated with the impaired functional performance. Together these studies support the idea that pulse pressure can be used as a predictor of gait speed and physical function.

Other vascular parameters are shown to be related to gait performance. Mitchell et al. (2011) assessed the relationship between arterial structure and function and the brain in older adults. Their data showed higher pulse pressure and carotid-femoral pulse wave velocity were associated with higher brain lesions, greater white matter hypertensie volume, and impaired cognitive function (Mitchell et al., 2011). The authors speculated that, increased arterial stiffness leads to increased pressure in the carotid arteries which are transmitted to brain tissue leading to cerebral vessel damage and impaired motor control (Mitchell et al., 2011). Sorond et al. (2010) also looked more specifically at endothelial function of cerebral arteries and found that elderly persons with lower cerebral vasoreactivity had slower gait speed and higher rates of falls. Furthermore, impaired neurovascular coupling is also related to decreased gait speed in elderly with white matter hypertensies (Sorond et al., 2011). Sorond et al. (2014) discussed the importance that the central nervous system plays in relation to aging and mobility in older adults and concluded that there is a strong relationship between vascular dysfunction and structural brain changes which can impair mobility in older adults.

While change in arterial structure and function occurs naturally with age, they are associated with decreased vascular function and lower physical function with aging. Previous research established that arterial stiffness and gait performance suggests that if arterial stiffness was lowered it may improve gait performance and overall physical function in older adults. Recent research has started to study different supplements that could potentially improve vascular function and physical performance in older adults (Bailey et al., 2015; Cutrufello et al., 2015, Figueroa et al., 2011; Ochiai et al., 2010; Schwedhelm et al., 2007); one of which is L-citrulline.

L-citrulline and Vascular Function

L-citrulline is a nonessential amino acid involved in the urea cycle. At the endothelial cell level, L-citrulline is involved in L-arginine biosynthesis (Figure #1). L-citrulline is a byproduct of L-arginine once it is hydroxylated and forms N-hydroxyl-L-arg. Nitric oxide (NO) is produced in the endothelium from the amino acid L-arginine by nitric oxide synthase (Schmidt et al., 1988). Nitric oxide increases activity of guanylyl cylase (sGC) in arterial smooth muscle cells which leads to increased intracellular levels of cyclic guanosine monophosphate (cGMP). cGMP causes relaxation of the arterial smooth muscle and leads to vasodilation (Fiscus et al., 1988). Meanwhile, L-citrulline is being recycled back into the L-Arginine pathway by being converted to L-arginosuccinate (Hecker et al., 1990), making it an important precursor for L-arginine and playing a significant role in metabolism and regulation of endothelial nitric oxide production. Recent studies have found that dietary supplementation with L-citrulline leads to an increase in plasma concentrations of L-arginine which in turn leads to more production of nitric oxide (Ochiai et al., 2010, Schwedhelm et al., 2007).

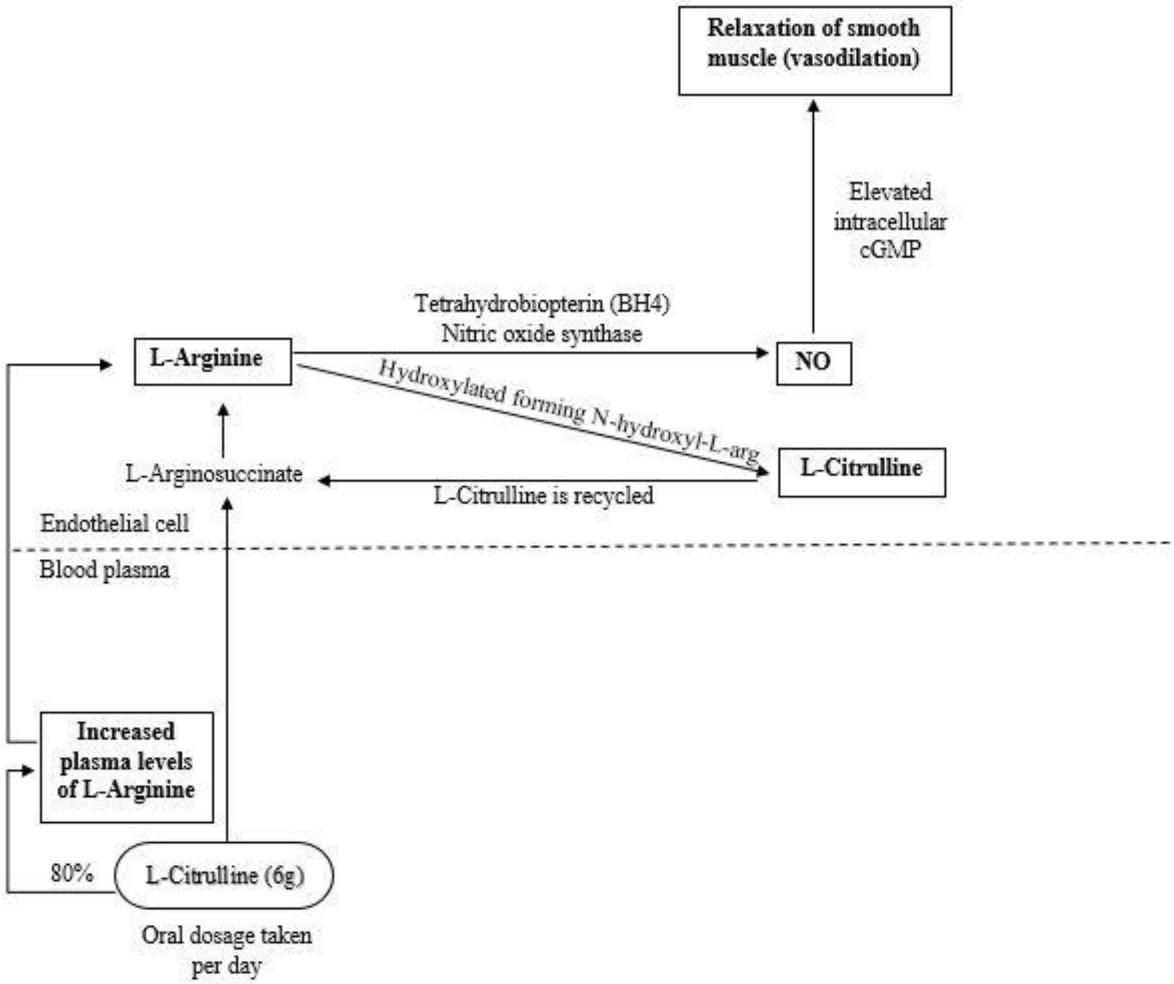

Figure 1. Role of L-citrulline supplementation on Nitric Oxide (NO) biosynthesis.

L-citrulline can be taken as an oral supplement in a variety of doses (e.g., 6g). It is found naturally in watermelon. When L-citrulline supplementation is taken up and released into circulation, it bypasses metabolism and is transported to the kidneys where 80% is catabolized to L-arginine. This factor leads to an increase in plasma levels of L-arginine which then increases the substrate for nitric oxide (NO) (Sureda et al., 2013). It has been suggested that with the supplementation of L-citrulline, L-arginine may act as a precursor for nitric oxide production (Sureda et al., 2013) because previous studies have shown that L-arginine supplements increase nitric oxide biomarkers, reduce blood pressure and improve exercise economy and efficiency (Bailey et al., 2015). Ochiai et al. (2010) looked at the effect of 5.6g of L-citrulline supplementation per day for 7 days on men and found a significant reduction in brachial-ankle pulse wave velocity (arterial stiffness), but did not find a difference in blood pressure. Bailey et al. (2015) studied the effect of 6g/day of L-citrulline supplementation on plasma and NO2 concentration, blood pressure and exercise performance. The results showed short term supplementation of L-citrulline significantly reduced resting blood pressure in healthy adults. Bailey et al. (2015) also showed a speeding of oxygen uptake kinetics due to the improvement in oxygen delivery and muscle oxidative metabolism which lead to longer exercise duration and work performed during cycling.

Several studies have found 6g/day of L-citrulline to be effective in improving exercise performance, however Cutrufello et al. (2015) found that 6g of oral L-citrulline given 1-2 hours before measurements showed no significant improvement in exercise performance in college-aged intercollegiate athletes. This lack of significant effect may be due to timing suggesting that, in relation to the studies mentioned above, the supplement may have the most effect after 7 days or more of supplementation. It may also be possible that L-citrulline has less of a positive effect on exercise performance in young healthy adults with assumedly healthy vasculature. The study of L-citrulline on humans and performance is a fairly new area and many of the studies contradict with each other in timing of supplementation, dosage, and tests performed. More research needs to be done in order to verify the effect the L-citrulline truly has on exercise performance.

Future Directions

While research shows an inevitable decline in physical function as evidenced by slowing of gait speed in older adults, recent studies suggest that there are possible ways to lessen this decline. A future direction of interest is to study the effect of L-citrulline on gait performance. We hoped to find that the supplement L-citrulline would have a positive effect on gait speed with implications on improved vitality in older adults. Our hypothesis was that arterial stiffness and blood flow during exercise will be improved in older adults following L-citrulline as compared to placebo. We further hypothesized that the positive change in arterial stiffness and exercise blood flow following L-citrulline will be associated with a positive change in gait performance.

Conclusion

The mobility decline observed in older adults as well as reduced physical activity and deconditioning has been shown to have a direct effect on physical capacity, health, and survival (Studenski et al., 2011). This is partly due to decreases in physical activity and strength as well as physiological factors such as stiffening of central arteries, cognitive decline, and changes in blood flow. Gait speed is an important factor to assess when evaluating function in older adults and can also be used to predict and possibly prevent future disabilities. L-citrulline has been shown to reduce blood pressure (Bailey et al., 2015; Cutrufelo et al., 2015), speed oxygen uptake kinetics (Bailey et al., 2011), and enhance aerobic exercise performance (Bailey et al., 2011). Our aim is to use 6g/day of L-citrulline supplementation for 2 weeks to improve vasculature by decreasing arterial stiffness and improving peripheral vascular factors which will, in theory, improve gait performance in older adults.

CHAPTER III

METHODS

Participants

Eleven men and 13 women between the ages of 60 and 79 years old were recruited for this study. Participants were non-smokers with no physician diagnosed cardiovascular, lung, or metabolic disease. Participants were not obese and had a BMI of less than 30 kg/m^2 or had any skeletal or muscle issues that kept them from completing the walking tests. Also, participants were not taking hormone replacement therapy or medications that affect cardiovascular function. Lastly, participants were not allowed to participate if they had any metal in their body due to MRI scanning (not part of this thesis).

Study Design

This study took place at Texas Tech University in the Kinesiology and Sport Management building. There were a total of five visits in this study. The first visit primarily served as a screening and familiarization visit. Participants reviewed and filled out a study consent form, medical history questionnaire, and had their height, weight, blood pressure and fasting blood glucose measured. Participants were also familiarized with the walking tests.

The last four visits were about 3 hours in duration. Participants were informed to come in to the Vascular Assessment Lab in a fasted state, that is, no consumption of food, drink other than water, vitamins, supplements, or caffeine within 8 hours of their appointment. The last four visits were designed as a placebo-controlled, double-blind, crossover study. Double-blind meaning that neither the researchers nor the participants knew which supplement was being taken, it was controlled by a third-party. Briefly, participants were asked to take a dietary supplement (L-citrulline, 6g/d) or placebo (maltodextrin) for 2 weeks followed by a washout period of 2 weeks. After the washout period, participants were asked to take either the supplement or placebo for 2 weeks in a crossover manner. This study design allowed for each participant to serve as their own control for comparison purposes.

Vascular Measurements

The participant sat in an angled weight-lifting bench with their right leg elevated and in a horizontal position parallel to the ground. The right foot was attached to a pulley system that allowed for adjustment of workload (i.e, weight lifted). Participants were instructed to move their right foot up (dorsi flexion) and down (plantar flexion) at full range of motion focusing only on using their calf muscle for the movement. A metronome was set at 40 bpm so that the participant could move their foot up and down with the beat of the metronome. The participant was asked to complete 3 minutes of exercise with a 1.5 kg weight. Blood pressure was taken at rest and every minute throughout the exercise using an automated device (Omron Healthcare, HEM-907XL) with the left arm relaxed on a table at heart level. Pulse pressure was calculated by systolic blood pressure (SBP) minus diastolic blood pressure (DBP). Mean blood velocity and diameter was measured during exercise at the right superficial femoral artery using Doppler ultrasound (GE Healthcare, Vivid 7). Arterial stiffness was measured using the β-stiffness index which was calculated using the equation, ln(SBP/DBP) x (maximum minus minimum diameter)/mean diameter. Blood flow was calculated using the equation, mean blood velocity x cross-sectional area of artery x 60. Femoral artery vascular conductance (FVC) was calculated using the equation, mean blood flow / mean blood pressure.

Walking Performance Tests

Participants completed several walking tests. First they completed a 5 minute warm-up on the treadmill at 1.5 miles per hour. After the warm-up, participants were asked to walk 7 meters under four different conditions: usual-pace walking, fast-pace walking, usual-pace walking while pausing to pick up an object (plastic spoon or fork), and fast-pace walking while stepping over two obstacles placed 2 meters and 4 meters from the start point. The first and second obstacles were 6 cm and 30 cm in height, respectively. The order if the walking tests was randomized between visits within each subject. Participants were timed during each test so walking speed in meters per second could be calculated. At least four trials were completed of each test and the slowest trial from all trial were used for each task. The fast-pace gait speed was subtracted from the usual-pace gait speed to calculate a measure of reserve capacity that we will refer to as gait speed reserve (Middelton et al., 2016). Lastly, participants completed a 400 meter walk test. Participants walked a long corridor, 20 meters each way, until they reached 400 meters (i.e., 10 laps). The instructions given to participants were to complete the test “as quickly as possible". Main variables derived from this test were time to complete 400 meters and 2 minute walk distance.

Statistical Analysis

We used a paired t-test to determine if differences in gait and blood flow parameters were present in the pre to post change for each variable within each sex between L-citrulline and placebo. A separate paired t-test was used to compare pre to post values for each gait and blood flow parameter within each condition. A Pearson correlation was used to determine if there was a correlation between the change in gait performance and the change in blood flow. Statistical significance was considered $p \leq 0.05$. Values are presented as mean ± standard deviation.

CHAPTER IV

RESULTS

Table 1 shows the comparison of demographic information between women and men. As expected, on average men weighed more ($p<0.01$) and were taller ($p<0.01$) than women. Similarly, men also had a higher body mass index ($p<0.01$) than women consistent with their greater height and weight. Men also had significantly higher femoral vascular conductance during exercise than women as expressed in absolute units ($p=0.02$) or as a change from rest ($p<0.01$).

Table 1. Comparison of Baseline Characteristics between Women and Men

	Women(*n*=13)	Men(*n*=11)	p-value
Age (years)	72.46 ± 5.25	68.63 ± 5.16	0.08
Height (cm)	163.90 ± 5.17	176.74 ± 5.24	<0.01
Weight (kg)	59.15 ± 8.57	78.43 ± 9.01	<0.01
Body Mass Index (m/kg^2)	21.98 ± 2.8	25.04 ± 2.00	<0.01
Usual Pace (m/s)	1.30 ± 0.16	1.40 ± 0.13	0.13
Fast Pace (m/s)	1.88 ± 0.21	1.91 ± 0.21	0.81
Fast-Usual Reserve (m/s)	0.58 ± 0.22	0.50 ± 0.19	0.38
Pick up object (m/s)	1.09 ± 0.12	1.11 ± 0.12	0.78
Over Obstacle (m/s)	1.56 ± 0.17	1.69 ± 0.19	0.11
400 meter (s)	247.96 ± 25.88	242.27 ± 25.12	0.59
2 minute distance (ft)	641.92 ± 65.36	657.18 ± 64.14	0.57
β-Stiffness Index (U)	18.71 ± 5.06	21.09 ± 4.74	0.25
Pulse Pressure	55.03 ± 10.28	55.04 ± 4.61	0.99
Rest FVC (ml/min/mmHg)	0.94 ± 0.27	0.90 ± 0.45	0.75
Exercise FVC (ml/min/mmHg)	4.53 ± 0.94	5.54 ± 1.16	0.02
Change in FVC (ml/min/mmHg)	3.58 ± 0.76	4.64 ± 0.81	<0.01

VO2, maximal oxygen uptake; FVC, femoral vascular conductance. Values presented as mean ± standard deviation. Comparisons made using an independent sample t-test.

Table 2 compares the pre to post change in gait variables between treatments in women. The change in distance walked during the first 2 minutes of the 400 meter walk test was significantly different between placebo and L-citrulline (p=0.02). Distance covered increased by 2.3% following placebo (p<0.01) as compared to 0.5% following L-citrulline (p>0.05).

Table 2. Comparison of Pre to Post Change between Treatments in Women

Outcome	Placebo	L-citrulline	p-value
Usual Pace (m/s)			
Before	1.31 ± 0.17	1.29 ± 0.18	
After	1.28 ± 5.17	1.30 ± 0.15	
Change	-0.02 ± 0.11	0.01 ± 0.09	0.44
Fast Pace (m/s)			
Before	1.88 ± 0.22	1.88 ± 0.21	
After	1.93 ± 0.26	1.84 ±0.21	
Change	0.04 ± 0.13	-0.03 ± 0.12	0.13
Fast-Usual Reserve (m/s)			
Before	0.57 ± 0.22	0.59 ± 0.25	
After	0.64 ± 0.27	0.55 ± 0.26	
Change	0.05 ± 0.17	-0.04 ± 0.16	0.11
Pick-Up Object (m/s)			
Before	1.10 ± 0.15	1.08 ± 0.11	
After	1.12 ± 0.14	1.08 ± 0.11	
Change	0.01 ± 0.16	-0.01 ± 0.07	0.63
Over Obstacle (m/s)			
Before	1.56 ± 0.18	1.56 ± 0.17	
After	1.60 ± 0.19	1.56 ± 0.20	
Change	0.01 ± 0.08	-0.01 ± 0.11	0.72
400 meter (s)			
Before	250 ± 29.21	246 ± 23.60	
After	245 ± 27.55*	246 ± 25.43	
Change	-4.76 ± 6.12	-0.23 ± 4.912	0.06
2 minute distance (ft)			
Before	637 ± 72.46	647 ± 60.60	
After	652 ± 72.70*	650 ± 64.00	
Change	14.53 ± 15.42	2.92 ± 14.17	0.02

Comparisons made using a paired t-test. Values presented as mean ± standard deviation.
*, significant difference between before and after value within condition (p<0.05).

Table 3 compares the pre to post change in gait variables between treatments in men. The change in usual-pace gait speed was significantly different ($p=0.02$) between placebo and L-citrulline. Usual-pace gait speed was slowed by 6.8% following L-citrulline ($p<0.5$) as compared to an increase of 5.9% following placebo ($p>0.5$). The change in gait speed reserve was also significantly different between treatments ($p<0.01$). Gait speed reserve was enhanced by 40.9% following L-citrulline ($p=0.01$) as compared to -10.7% following placebo ($p>0.05$).

Table 3. Comparison of Pre to Post Change between Treatments in Men

Outcome	Placebo	L-citrulline	p-value
Usual Pace (m/s)			
Before	1.34 ± 0.18	1.46 ± 0.14	
After	1.42 ± 0.11	1.36 ± 0.14*	
Change	0.07 ± 0.16	-0.09 ± 0.14	0.02
Fast Pace (m/s)			
Before	1.91 ± 0.12	1.90 ± 0.20	
After	1.93 ± 0.26	1.98 ± 0.25†	
Change	0.02 ± 0.14	0.09 ± 0.14	0.19
Fast-Usual Reserve (m/s)			
Before	0.56 ± 0.19	0.44 ± 0.25	
After	0.50 ± 0.27	0.62 ± 0.21*	
Change	-0.03 ± 0.20	0.18 ± 0.19	<0.01
Pick-Up Object (m/s)			
Before	1.09 ± 0.12	1.12 ± 0.15	
After	1.11 ± 0.14	1.13 ± 0.11	
Change	0.01 ± 0.07	0.01 ± 0.08	0.82
Over Obstacle (m/s)			
Before	1.69 ± 0.23	1.69 ± 0.16	
After	1.70 ± 0.15	1.73 ± 0.19	
Change	0.02 ± 0.09	0.03 ± 0.09	0.78
400 meter (s)			
Before	241 ± 26.89	243 ± 24.75	
After	238 ± 28.54*	238 ± 23.62*	
Change	-3.00 ± 2.93	-5.27 ± 6.95	0.36
2 minute distance (ft)			
Before	663 ± 66.91	652 ± 66.06	
After	669 ± 76.21	669 ± 61.37*	
Change	6.18 ± 12.68	17.36 ± 21.31	0.14

Comparisons made using a paired t-test. Values presented as mean ± standard deviation. *, significant difference between before and after value within condition ($p<0.05$); †, difference between before and after value with condition ($p=0.06$).

Table 4 shows the comparison of pre to post change between treatments for FVC in women and Table 5 shows the comparison of pre to post change between treatments for FVC in men. Neither women nor men showed significant differences in the change in arterial stiffness or pulse pressure between placebo and L-citrulline. Similarly, women did not show significant differences in FVC at rest or during exercise between treatments. In contrast, men showed a significant difference ($p<0.01$) in FVC during exercise between treatments. The change in FVC during exercise increased by 15.3% following L-citrulline ($p<0.01$) as compared to a decline of 2.5% following placebo ($p>0.05$). There was also a significant increase in the FVC response to exercise (exercise minus rest) in men with L-citrulline ($p=0.04$) as compared to the placebo ($p>0.05$).

Table 4. Comparison of Pre to Post Change between Treatments for Femoral Vascular Conductance (FVC) in Women

Outcome	Placebo	L-citrulline	p-value
β-Stiffness Index (U)			
Before	18.08 ± 4.25	19.35 ± 9.15	
After	19.68 ± 6.48	16.90 ± 5.19	
Change	1.60 ± 7.79	-2.44 ± 7.04	0.21
Pulse Pressure			
Before	56.53 ± 13.92	53.53 ± 10.42	
After	56.15 ± 9.09	53.00 ± 10.66	
Change	-0.38 ± 8.60	-0.53 ± 7.9	0.34
Rest FVC			
Before	0.92 ± 0.32	0.96 ± 0.34	
After	0.88 ± 0.48	0.85 ± 0.39	
Change	-0.03 ± 0.49	-0.11 ± 0.31	0.59
Exercise FVC			
Before	4.56 ± 1.26	4.50 ± 0.95	
After	4.66 ± 0.94	4.32 ± 1.02	
Change	0.10 ± 0.85	-0.17 ± 0.69	0.40
Change in FVC			
Before	3.63 ± 1.17	3.53 ± 0.87	
After	3.77 ± 0.81	3.46 ± 0.94	
Change	0.14 ± 1.11	-0.06 ± 0.67	0.59

Comparisons made using a paired t-test. Values presented as mean ± standard deviation.

Table 5. Comparison of Pre to Post Change between Treatments for Femoral Vascular Conductance (FVC) in Men

Outcome	Placebo	L-citrulline	p-value
β-Stiffness Index (U)			
Before	20.51 ± 7.61	21.66 ± 5.97	
After	18.70 ± 4.79	19.46 ± 5.60	
Change	-1.80 ± 7.41	-2.21 ± 5.86	0.91
Pulse Pressure			
Before	54.63 ± 8.24	55.45 ± 9.60	
After	54.18 ± 7.01	55.09 ± 7.48	
Change	-0.45 ± 6.26	-0.36 ± 6.37	0.97
Rest FVC			
Before	1.02 ± 0.64	0.77 ± 0.49	
After	0.93 ± 0.62	0.79 ± 0.46	
Change	-0.09 ± 0.69	0.02 ± 0.44	0.65
Exercise FVC			
Before	5.80 ± 1.11	5.29 ± 1.41	
After	5.65 ± 1.14	6.10 ± 1.63*	
Change	-0.15 ± 0.82	0.80 ± 0.96	<0.01
Change in FVC			
Before	4.77 ± 1.04	4.52 ± 1.14	
After	4.71 ± 1.04	5.30 ± 1.46*	
Change	-0.05 ± 1.22	0.78 ± 0.73	0.04

Comparisons made using a paired t-test. Values presented as mean ± standard deviation.
*, significant difference between before and after value within condition ($p<0.05$).

The change in usual-pace gait speed was not correlated with the change in exercise FVC after L-citrulline for women (r=-0.11, p=0.72; Figure 2) and men (r=-0.07, p=0.81; Figure 2). In contrast, the change in fast-pace gait speed was correlated with the change in exercise FVC after L-citrulline for women (r=0.77, p<0.01; Figure 3), but not in men (r=0.35, p=0.28; Figure 3). Similarly, the change in gait speed reserve was correlated with the change in exercise FVC after L-citrulline for women (r=0.66, p=0.01; Figure 4), but not in men (r=0.31, p=0.34; Figure 4). When both men and women were combined, the change in exercise FVC was not correlated with the change in usual-pace gait speed (r=-0.30, p=0.14), but was positively correlated with fast-pace gait speed (r=0.63, p<0.01) and gait speed reserve (r=0.61, p<0.01).

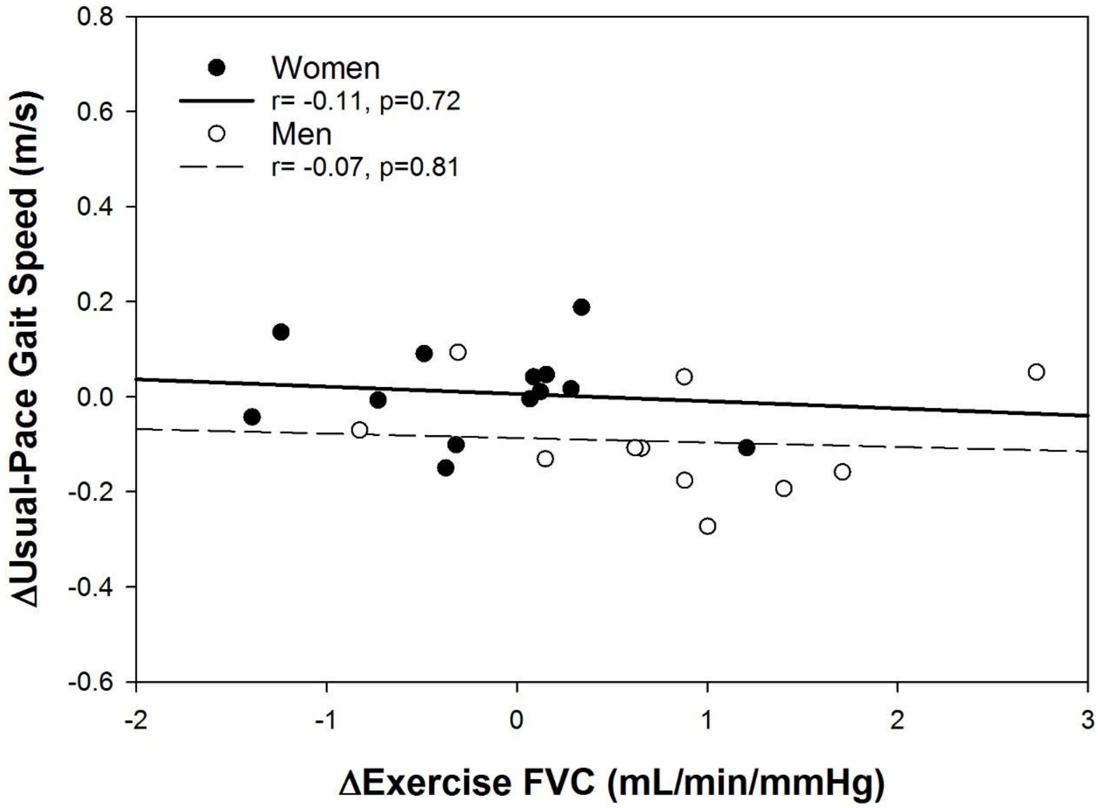

Figure 2. Relationship between the change in exercise femoral vascular conductance (FVC) and the change in usual-pace gait speed following L-citrulline supplementation.

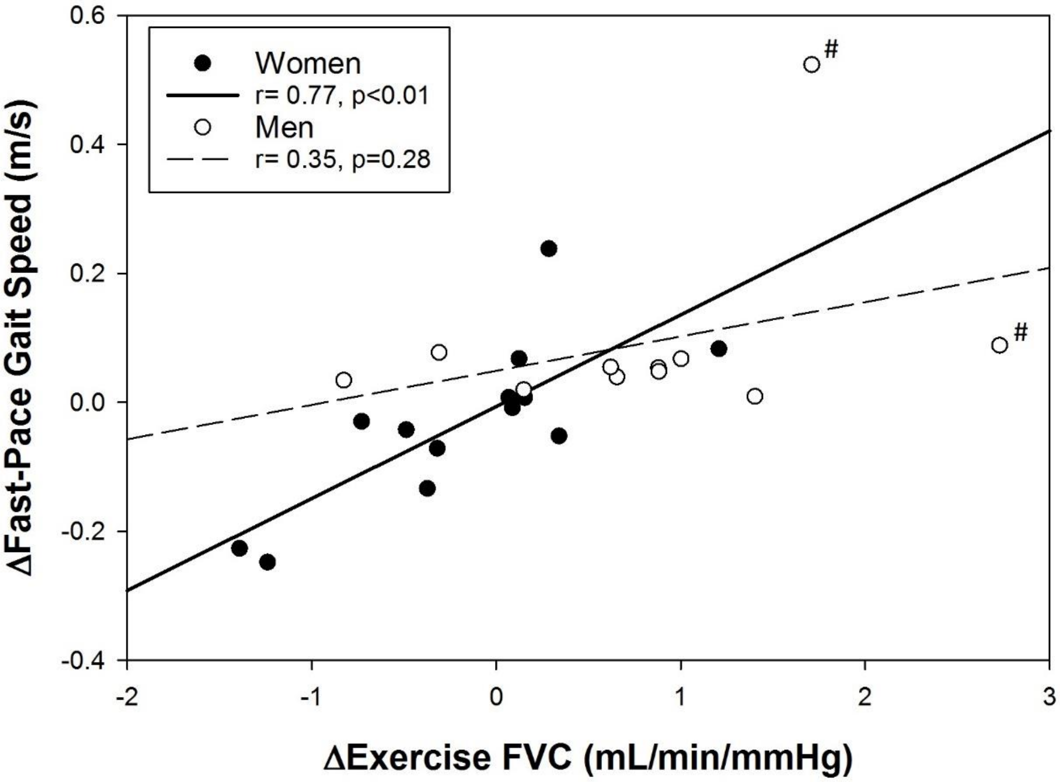

Figure 3. Relationship between the change in exercise femoral vascular conductance (FVC) and the change in fast-pace gait speed following L-citrulline supplementation. The two men denoted by # symbol may be considered outliers. Performing the correlation analysis for the men without the two outliers resulted in a similar result (r= -0.15, p=0.65).

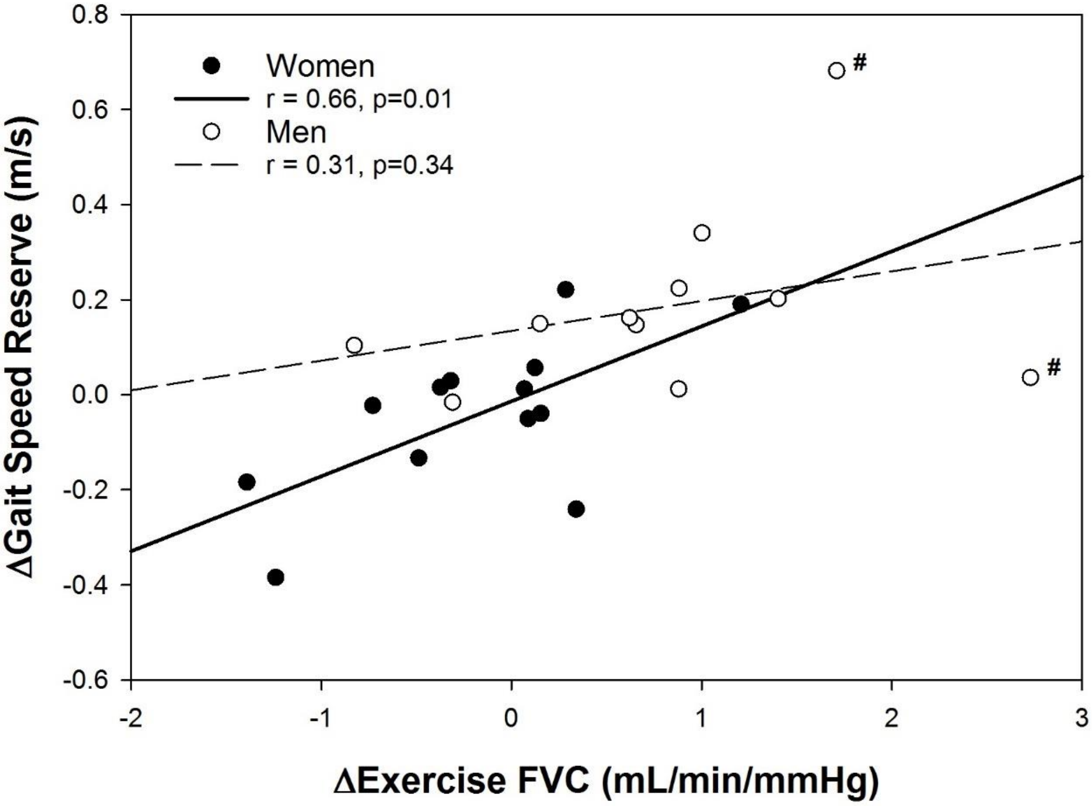

Figure 4. Relationship between the change in exercise femoral vascular conductance (FVC) and the change in gait speed reserve following L-citrulline supplementation. The two men denoted by # symbol may be considered outliers. Performing the correlation analysis for the men without the two outliers resulted in a similar result (r=0.52, p=0.15).

CHAPTER V

DISCUSSION AND CONCLUSION

Significant differences in blood flow and gait performance in older adults were found after 14 days of L-citrulline supplementation as compared to placebo. We hypothesized that L-citrulline would have a positive effect on the change in arterial stiffness and the change in exercise blood flow as compared to the placebo. In support of this hypothesis, we found exercise blood flow was increased following L-citrulline supplementation in men, but without a change in arterial stiffness. We also hypothesized that the change in gait performance would be improved following L-citrulline as compared to the placebo. Interestingly, usual-pace gait speed was slowed and fast-pace gait speed tended to increase after L-citrulline in men, which significantly enhanced their gait speed reserve. Importantly, we found some evidence that the change in exercise blood flow was correlated with the change in gait speed reserve when increasing the range of values by including all participants. These findings suggest that L-citrulline supplementation may improve vascular function during exercise in older adults which has implications for gait performance, although the impact may be specific to sex as no differences were found in women following L-citrulline.

Walking Performance

A significant improvement in gait speed reserve was found after L-citrulline. This metric involves subtracting fast-pace gait speed from usual-pace gait speed. The derived value is the subject’s walking speed reserve beyond their normal pace. Middleton et al.

(2016) has shown that gait speed reserve is an indicator of adaptability of walking in older adults, with lower reserves being predictive of fall risk in older adults. In the present study, men increased their reserve by a combination of increasing fast-pace gait speed and significantly lowering their usual-pace gait speed after L-citrulline supplementation.

Our finding that usual-pace gait speed slowed after L-citrulline was opposite of what was expected as faster walking indicates greater mobility, particularly in older adults. Aging is associated with slower gait speed which is linked to decreased activities of daily living (Hardy et al., 2007; Ko et al., 2010; Schrack et al., 2010). In addition, Studenski et al. (2001) found that faster gait speeds in older adults lead to a higher life expectancy. Hardy et al. (2007) similarly found that older adults who increased their gait speed had a significant reduction in relative risk of absolute death. However, it should be emphasized that the slower usual-pace gait speed following L-citrulline in men (1.36 m/s) was still above the speed considered to be mobility-limited (0.80 m/s) (Abellan van Kan et al., 2009; Studenski et al., 2011), thus not at a level to negatively impact function.

Contrary to previous studies, we believe the decrease in usual-pace gait speed may not be an adverse finding. A slower speed at a usual-pace may reflect a more efficient walking pattern in an effort to use less energy to walk. In order to support this speculation, we estimated oxygen uptake (VO2) for the usual-pace and fast-pace gait speeds using ACSM's metabolic equation for walking. Assuming that fast-pace gait speed reflects peak VO2, this additional analysis revealed that after L-citrulline men used less of their peak VO2 during usual-pace walking (75% compared to 82% before supplementation) and were able to achieve a higher VO2 during the fast-pace walking (15.38 compared to 14.90 ml/kg/min). The data from this analysis is consistent with Schrack et al. (2010) who showed lower peak energy availability translates to a higher intensity of exercise during usual pace walking (i.e., working closer to peak VO2). In the present study, men walked further from their peak VO2 during usual-pace walking after L-citrulline, possibly in order to achieve a level of low perceived effort relative to their reserve capacity. Because L-citrulline supplementation increased gait speed reserve and decreased the percentage of VO2 used to maintain usual-pace gait speed, it can be suggested that older adults could see benefit in activities of daily living because they are using their energy more efficiently (Schrack et al., 2010).

In the present study, fast-paced gait speed increased in men non-significantly ($p=0.06$) by 4.2% after L-citrulline. Other studies have also reported improved lower-limb exercise performance in men following L-citrulline. Bailey et al. (2015) found that short-term (7 days) L-citrulline supplementation improved the performance of 19 year old male cyclists during vigorous intensity exercise by leading to longer time to exhaustion and total-work performed. Suzuki et al. (2016) has also shown 7 days of L-citrulline supplementation improves time to finish a cycling exercise trial in healthy young men as well as reduced perceived exertion during exercise. Cutrufello et al. (2014) tested the short term effect of L-citrulline and watermelon juice, a food that has high concentrations of L-citrulline, on exercise performance given 1 or 2 hours before exercise in young men and women. This study did not see an improvement in duration during a graded exercise test suggesting that short term supplementation is not as effective as 7 days or more. Together, these findings suggest that long-term L-citrulline supplementation, but not short-term, has the potential to enhance exercise performance as suggested by our results in older men along with previous studies findings in younger adults.

Our observation that gait performance did not change in women requires further investigation, as the studies cited above examined only men, thus little research is currently available on the effect of L-citrulline on exercise performance in women. The present finding of improved gait speed reserve, and possibly fast-pace gait speed, in men but no differences in women indicate a sex difference in L-citrulline's ability to provide an ergogenic effect.

Blood Flow

Studies have examined the effect of L-citrulline on muscle oxygenation during exercise. Bailey et al. (2015) found 7 days of L-citrulline to improve oxygen uptake kinetics during high-intensity cycling in young men. In a separate study, Bailey et al. (2016) also observed greater muscle oxygenation during moderate intensity cycling in young men following 16 days of watermelon supplementation. Similar to these findings, we observed FVC during calf exercise to increase by 15% following 14 days of L-citrulline supplementation. Femoral vascular conductance is a measure of how well muscle vasculature dilates to increase blood flow. Therefore, our finding that FVC was improved following L-citrulline indicates greater muscle perfusion (blood flow) during exercise. With a greater FVC response to exercise, older men had a better delivery of oxygen to active skeletal muscle in order to meet metabolic demand. It is possible that the greater FVC response to exercise may have influenced men's ability to walk faster and/or allowed greater distribution of blood flow to skeletal muscle providing a more efficient energy expenditure (i.e., possibly slowing usual-pace speed). Indeed, we found some evidence that the change in FVC during exercise following L-citrulline was related to the change in gait speed reserve when all subjects were included thereby expanding the range of values to include those men and women that showed little change to those that had the greatest change in exercise FVC following L-citrulline.

L-citrulline has the potential to increase nitric oxide, a vasodilator important for exercise blood flow. The increase in nitric oxide is not directly from L-citrulline, rather L-citrulline is transported to the kidneys where 80% is catabolized to L-arginine causing an increase in plasma levels of L-arginine and an increase in substrate for nitric oxide production (Sureda et al., 2013). Nitric oxide increases activity of guanylyl cyclase in arterial smooth muscle cells which leads to increased intracellular levels of cyclic guanosine monophosphate which causes relaxation of the arterial smooth muscle and leads to vasodilation (Fiscus et al., 1988). We believe these physiological mechanisms are what caused the increase in exercise FVC with L-citrulline supplementation. Indeed, past studies examining the effect of L-citrulline supplementation have reported elevated plasma levels of nitrate, a biomarker for nitric oxide supplementation (Bailey et al., 2015).

Interestingly, we did not observe FVC during exercise to increase significantly in women following L-citrulline supplementation as compared to placebo. This was surprising considering that older women have lower FVC responses to exercise than older men (Parker et al., 2008), thus greater potential for improvement with a supplement that may enhance blood flow. Additionally, past studies in older women found L-citrulline to lower blood pressure indicating greater dilation of arterioles that regulate blood pressure (Figueroa et al., 2013). It is possible that the women included in this study were too healthy in comparison to women studied by others. Indeed, the older women in the study by Figueroa et al. (2013) were obese and hypertensive while all the women in the present study had a normal body mass index (average BMI = 21.98 ± 2.8 kg/m^2) and normotensive. A recent review on the effect of L-citrulline on vascular function reports little-to-no effect of L-citrulline on blood pressure in adults with normal blood pressure but a reducing effect in adults with hypertension (Figueroa et al., 2016). Therefore, more research is needed to clarify if the effect of L-citrulline on vascular function in women is determined by their state of health.

Limitations

There are several limitations to this study. Our sample size is small and consisted of only healthy, non-hypertensive older adults. As stated above, due to the healthiness of the individuals, L-citrulline supplementation may not have had as much of an effect as it may have on unhealthy older adults as previous studies report. While all of these adults were presumably heathy, some were more physically active than others. Those who are active may have had less change in FVC with L-citrulline because their endothelium is already healthy and promoting good blood flow, opposed to those who are sedentary and at risk for vascular disease such as atherosclerosis. More research is required to know if there is a difference in L-citrulline supplementation between sedentary and active healthy older adults. In addition, during the walking performance tests, the participants pace was based on their perception of their usual-pace and fast-paces. Some participants struggled with choosing a consistent pace between visits, which may have had an effect on our findings for changes in gait speed. However in order to account for this possibility we measured multiple walking trials until at least four timed attempts were within 10 milliseconds of each other. Moreover, we calculated gait speed from the average, median, minimum and maximum. While the coefficient of variations varied between trials (e.g., usual- vs. fast-pace) between 4% and 8%, the coefficients were similar between calculations. Thus, we selected the slowest timed attempt as a conservative approach to calculate gait speed. Lastly, there were no significant differences ($p>0.05$; data not shown) in gait speeds between the pre-visits (i.e., baseline before supplementation) for each treatment indicating low variability between visits.

Conclusion

In conclusion, 14 days of L-citrulline supplementation had a positive effect on exercise blood flow and gait performance in men but not women. These findings support the potential for L-citrulline to improve overall daily activities of living in healthy older adults possibly by enhancing gait speed reserve capacity. However, more research is needed to study our observed sex difference as well as differences between healthy and unhealthy older adults in terms of the effect of L-citrulline supplementation in these parameters.

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