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THE EFFECTS OF SHORT-TERM L-CITRULLINE SUPPLEMENTATION ON ARTERIAL STIFFNESS IN PREDIABETIC AND HEALTHY ADULTS

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THE EFFECTS OF SHORT-TERM L-CITRULLINE SUPPLEMENTATION ON ARTERIAL STIFFNESS IN PREDIABETIC AND HEALTHY ADULTS

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ABSTRACT

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Background: Prediabetes is marked by elevated levels of plasma glucose. Chronic hyperglycemia may induce thickening of the arteries, oxidative stress, and endothelial dysfunction, a primary cause for cardiovascular disease. In cold temperatures, exaggerated vascular responses augments the risk for cardiovascular events. L-citrulline may attenuate the exaggerated hemodynamic responses to cold exposure. The purpose of this study was to measure the effects of a 2-week L-citrulline supplementation on hemodynamics at rest and during a cold pressor test (CPT).

Methods: Blood pressures, indices of wave reflection (augmented pressure (AP) and augmentation index (AIx)), and arterial stiffness (pulse wave velocity (PWV)) were measured at rest and during a CPT in 16 normal and prediabetic older adults (60-85 yrs). Subjects were randomly assigned to 2 weeks of L-citrulline (6g/day) or placebo supplementation in a double-blind crossover design.

Results: No significant changes with either intervention at rest. Central pulse pressure (*P* =.031) and AP (*P* =.046) in non-prediabetics and AP (*P* =.027) and Alx (*P* =.028) in prediabetics were significantly decreased following L-citrulline supplementation compared with placebo.

Conclusions: Although L-citrulline had no effect on resting hemodynamics, supplementation attenuates exaggerated vascular responses during CPT. Therefore, L-citrulline may elicit cardioprotective effects from cold exposure in older adults with and without prediabetes.

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INTRODUCTION

Aging is an independent risk factor for cardiovascular disease (CVD) and diabetes. Prediabetes, marked by chronic elevated glucose in the blood, increases stress on the arteries which can lead to injury of the endothelium (Fowler, 2008). With increasing age, there is a significant reduction in vascular function via arterial stiffening due to a build-up of plaque or shift in smooth muscle composition over time (Benetos, Laurent, Hoeks, Boutouyrie, & Safar, 1993; Lakatta & Levy, 2003; Boutouyrie, et al., 1992). The stiffening of arteries and injury of the endothelium are associated with a greater risk for cardiovascular disease and mortality (Zieman, Melenovsky, & Kass, 2005). These changes may exaggerate vascular reactivity in cold temperatures. Cold exposure augments sympathetic response on the body. Increased sympathetic activity induces vasoconstriction and may lead to greater stiffness in the arterial system, which can lead to a higher frequency of adverse cardiovascular events (Edwards, Gauthier, Hayman, Lang, & Kenefick, 2005). A cold weather spell increases risk for sudden cardiac deaths by 19% each day that temperature remains lower than the average (Ryti et al., 2017). Therefore, attenuation of augmented vasoconstriction to cold exposure is meaningful.

The inner lining of the arterial wall is composed of endothelial cells, which can react to shear stress from changes in flow of blood via specialized mechanoreceptors. Shear stress is the primary stimuli for the release of nitric oxide (NO) from the endothelial cells as the diameter of the lumen increases (Alberts et al., 2002). A potent vasodilator and an anti-inflammatory molecule, NO, diffuses into the smooth muscle cells inducing an efflux of calcium which allows for smooth muscle relaxation which leads to vasodilation (Evans et al., 2000). This process of vascular reactivity allows for appropriate blood flow to the tissue, mainly during exercise

(Sandoo et al., 2010). Reduced bioavailability of NO may be a symptom of endothelial dysfunction (Shemyakin et al., 2012), a primary cause for the development of CVD (Benetos et al., 1993; Vlachopoulos et al., 2010). Furthermore, endothelial dysfunction is a common link between diabetes and CVD and may be due to the lumen-thickening effects of hyperglycemia (Grundy, 2012). Fowler (2008) stated that an increase in free radical production and reactive oxygen species are stimulated by hyperglycemia which causes an increase in oxidative stress and injury of endothelial cells. This stress and injury is enough to stimulate the thickening of these membranes. Additionally, Hiroaki et al. (1999) reported that an elevated concentration of blood glucose can acutely induce endothelial dysfunction as measured via flow-mediated endothelial-dependent vasodilation. Insulin resistance has a vasoconstrictive effect on the arteries due to the release of cytokines. These cytokines inhibit endothelial NO synthase (eNOS) resulting in a decrease in NO availability (Kobayashi, 2015). This decrease in NO reduces the vasodilatory response of the endothelium. Insulin is suggested to stimulate the release of both NO and endothelin 1 (ET-1), a potent vasodilator and vasoconstrictor, respectively (Cardillo, Campia, Bryant, & Panza, 2002). The balance between the two substances determine the final effect on the artery. Augmented levels of ET-1 have been detected in patients with type 2 diabetes which may elucidate the relationship between endothelial dysfunction and arterial stiffness in individuals with elevated levels of blood glucose (Cardillo et al., 2002).

L-arginine is a precursor for NO synthesis (Breuillard, Cynober, & Moinard, 2015). Oral L-arginine improves endothelial function and vascular elasticity; however not all studies have shown this (Siasos et al., 2009). The effectiveness of arginine is controversial given the sequestration of arginine by several gut and liver enzymes. When arginine reaches the gut, it is utilized by several enzymes before reaching the liver which also has a high presence of arginase and several other enzymes to break down arginine into urea and several other substances. (Cynober, Le Boucher, & Vasson, 1995; Figueroa et al., 2016; van de Poll et al., 2007). Figueroa,

Sanchez-Gonzales, Perkins-Veazie, and Arjmandi (2011) reported that L-citrulline, an amino acid that is a precursor to L-arginine, supplementation improved aortic hemodynamics. L-citrulline aids in vasodilation by increasing NO production (Figueroa, Wong, Jaime, & Gonzales, 2017). Short-term (two weeks) L-citrulline caused a 21% increase in NO production (Bailey et al., 2015). Furthermore, L-citrulline also has been shown to decrease blood pressure (BP) (Massa et al., 2016; Wong et al., 2015) and arterial stiffness measured by pulse wave velocity (PWV) (Ochiai et al., 2012). Lastly, previous literature supports short-term supplementation (two weeks) of L-citrulline to reduce BP (Figueroa, Wong, & Kalfon, 2014) and PWV (Figueroa, Alvarez-Alvarado, Jaime, & Kalfon, 2016) during a cold pressor test (CPT) in young, healthy men.

Evidence suggests that L-citrulline is helpful for mainly hypertensive adults at rest and during a stressor test. It is therefore the purpose of the present study to determine if L-citrulline supplementation is a viable treatment for individuals with abnormal glucose regulation who are seeking an additional preventative option for elevated BP and cardiovascular disease.

METHODS

Twenty 20 adults (60-85 years of age) volunteered to participate in the study. Exclusion criterial prior to participation included those with any arrythmias, a pacemaker, or were unable to complete all tests. Three participants were excluded due to poor compliance (<80%) during the placebo or L-citrulline supplementation period. One participant was excluded due to the age becoming a statistical outlier. Of the 16 remaining subjects, eight participants were classified as prediabetic (HbA1c $\geq$ 5.7 or fasting glucose of 100-125 mg/dL of blood) and eight were classified as healthy (HbA1c of < 5.7 or fasting glucose of <100 mg/dL of blood). All participants were non-smokers and were free of chronic disease. Participants maintained their normal exercise and dietary habits. The study was approved by the University of Wisconsin- La Crosse Institutional Review Board for the Protection of Human Subjects and participants gave their written consent prior to participation.

Study design

The present study utilized a randomized, double-blind, placebo-controlled design. Participants were familiarized with the protocols. The cardiovascular parameters were evaluated after an overnight fast and abstinence from caffeine, alcohol, or medications for 12 hours and abstinence from any intense or prolonged physical activity for 24 hours. The participant wore an eye mask and ear phones to prevent any visual or auditory stimuli from affecting blood pressure.

After 10 minutes of supine rest, cardiovascular parameters were measured and averaged at rest and measured during the last minute of the CPT. After baseline measurements, participants were randomly assigned to placebo (maltodextrin) or L-citrulline for 14 days, followed by a 14-day

washout period and then were crossed over to the other supplement for 14 days. Measurements were repeated before and after each trial.

Measurements

Anthropometrics.

Body weight and height were measured using a stadiometer to the nearest 0.25 kg and 0.01 m respectively. Waist and hip circumference were measured using a tape measure to the nearest 0.5cm at the midpoint from the bottom of the ribcage to the superior border of the iliac crest and around the greatest girth of the hips, respectively. A fingerstick test was completed during the first session to determine HbA1c (Afinion AS100 Analyzer System, Medline Industries, Inc., Northfield, IL) for grouping criteria; during each session, fasting blood glucose (FBG) and a full lipid profile was measured (Alere Cholestech LDX Analyzer System, MedTek, Sandy, UT). A bioelectrical impedance (BIA) test was performed in a supine position to determine body composition (RJL Systems, Clinton Township, MI). Fat free mass (FFM) was calculated using the equations (Eq. 1) from the National Health and Nutrition Examination Survey (NHANES) (Chumlea et al. 2002).

Eq. 1.

Males: $FFM = -10.678 + 0.262\ weight + 0.652\ height^2 / Resistance + 0.015\ Resistance$

Females: $FFM = -9.529 + 0.168\ weight + 0.696\ height^2 / Resistance + 0.016\ Resistance$

Estimates for total body fat (TBF) and percent body fat (%BF) were then derived from the equations $TBF = weight - FFM$ and $\%BF = TBF / weight$.

Cardiovascular Measures.

Following a 15-min rest period, pulse wave analysis (PWA) was measured using the SphygmoCor XCEL (Atcor Medical, Sydney, AU). A blood pressure cuff was placed on the

brachial artery on the right arm. The device measured 20 consecutive waveforms and was repeated three times. The average of the second and third values was recorded. The waveforms are composed of the wave caused by the initial wave and a reflected wave that returns to the aorta from peripheral arteries (Nichols and Singh 2002). Augmented pressure (AP) is the difference between the two systolic peaks (Wilkinson, et al., 2000). Augmentation index (AIx) is expressed as a ratio of AP and pulse pressure (PP). PP is the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP). A greater PP suggests arterial stiffness (Zieman, Melenovsky, & Kass, 2005). PWV was evaluated using a SphygmoCor XCEL (Atcor Medical, Sydney, AU). A blood pressure cuff was positioned around the right thigh and a high-fidelity tonometer (SphygmoCor XCEL) was placed on the carotid artery. Carotid-femoral pulse wave velocity was measured as the time it takes for the pulse wave to travel between the points, divided by the distance between the arterial segments (m/s). A cold pressor test was performed using methods consistent with previous literature. The participant briefly submerged his/her left hand into the 4°C water while laying supine (Figueroa, Wong, Kalfon, 2014; Wood, Sheps, Elveback, & Schirger, 1984; Sanchez-Gonzales, Koutnik, Ramirez, Wong, & Figueroa, 2013). During the CPT, the PWA was conducted using the brachial cuff. After the PWA was completed, the subject was allowed to take the hand out of the ice water and initiate warming.

L-citrulline supplementation.

Participants were instructed to ingest four capsules of 6g L-citrulline or placebo twice a day (at breakfast and before bed) for 14 days. After a 14 day wash-out period without supplementation, the participant crossed-over to the other supplement. Participants returned unused capsules and records to assess their compliance.

Statistical Analysis.

Utilizing previous research (Figueroa et al., 2010), a power calculation done a priori determined a population of 24 subjects with an effect size of 0.30 and power of 80% to observe a significant

difference (α=0.05) in SBP (5%) between placebo and L-citrulline treatments to CPT. Normality was confirmed using the Shapiro-Wilk test for all measurements. Independent t-tests were used to measure potential differences between groups at baseline. Analysis of variance with repeated measures was used to determine differences within and between interventions in the cardiovascular parameters at rest and during CPT. When significance was identified, an independent t-test was used to determine significant interaction.

RESULTS

All data are presented as mean ± standard deviation. Participant characteristics are presented in Table 1. Weight, % BF, cholesterol levels, and glucose levels remained unaltered throughout the study. Compliance with placebo and L-citrulline supplementation was 96.7 ± 4.9% and 93.5 ± 6.0%, respectively.

Table 2 summarizes the cardiovascular and aortic hemodynamic parameters at baseline and after the supplementations at rest. The present study evaluated the changes from pre to post supplementation of brachial and central SBP and DBP, central PP, HR, central MAP, AP, AIx, and PWV. There were no significant differences between groups or between supplements at baseline. The changes that were seen from the supplementation were not significant at rest with either group ($P > 0.05$).

Table 3 summarizes the CV responses of the intervention during a CPT. The magnitude of change between pre and post measurements in response to the CPT was recorded for central SBP and DBP, central PP, HR, central MAP, AP, and AIx. There was a significant difference between pre and post-testing in cPP in normal individuals and in AP in both healthy and prediabetic individuals. L-citrulline supplementation, compared with placebo, decreased cPP in normal individuals ($P = 0.0.31$) (Figure 1a), but not in prediabetics (Figure 1b). AP decreased in both normal individuals ($P = .046$) and prediabetics ($P = .027$) with L-citrulline supplementation compared with placebo (Figure 2). L-citrulline also decreased Alx compared with placebo in prediabetics ($P = .028$), but not in normal individuals (Figure 3). Although not a significant

difference, the change in brachial SBP from rest to CPT decreased by 30% with L-citrulline supplementation.

Table 1. Descriptive characteristics of participants (n=16).

Variable	Mean	Standard Deviation
Age	71.5	7.10
Weight (kg)	74.7	14.25
Height (m)	1.7	0.08
BMI	26.9	4.38
BF%	35.3	8.12
TC	208.5	53.32
TRG	140.3	78.66
HDL	58.9	19.94
LDL	120.5	43.04
GLU	105.5	12.42

Abbreviations: BMI, body mass index; BF%, body fat percentage; TC, total cholesterol; TRG, triglycerides; HDL, high density lipoproteins; LDL, low density lipoproteins; GLU, glucose.

Table 2. Hemodynamic parameters at rest before and after placebo.

	Normal		Prediabetic	
	Pre	Post	Pre	Post
bSBP (mmHg)	136.6 ± 17.26	135.5 ± 14.38	143.5 ± 13.32	140.4 ± 8.23
bDBP (mmHg)	76.6 ± 7.35	75.3 ± 5.26	78.9 ± 8.77	79.8 ± 7.72
HR (bpm)	60.3 ± 7.78	60.1 ± 6.81	59.5 ± 8.05	60.6 ± 11.3
cSBP (mmHg)	125.3 ± 14.10	124.0 ± 12.07	132.9 ± 13.86	130.4 ± 7.71
cDBP (mmHg)	78.0 ± 7.52	76.8 ± 5.42	80.0 ± 8.73	80.8 ± 7.91
cPP (mmHg)	47.3 ± 9.74	47.3 ± 9.91	52.9 ± 10.38	49.6 ± 8.86
cMAP (mmHg)	94.8 ± 9.53	93.5 ± 7.87	98.8 ± 10.19	98.9 ± 7.79
AP (mmHg)	14.3 ± 4.98	14.4 ± 8.75	36.8 ± 8.17	17.8 ± 5.99
AIx (mmHg)	30.6 ± 10.45	29.4 ± 8.75	36.8 ± 8.17	35.5 ± 7.89
PWV (m/s)	7.8 ± 1.29	7.9 ± 0.75	8.4 ± 1.41	8.4 ± 1.42

Values represent mean ± standard deviation.
Abbreviations: bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; HR, heart rate; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cPP, central pulse pressure; cMAP, central mean arterial pressure; AP, augmentation pressure; Alx, augmentation index; PWV, pulse wave velocity.

Table 3. Hemodynamic parameters at rest before and after L-citrulline supplementation.

	Normal		Prediabetic	
	Pre	Post	Pre	Post
bSBP (mmHg)	134.3 ± 13.57	135.0 ± 21.57	139.3 ± 13.13	143.3 ± 11.95
dDBP (mmHg)	75.3 ± 7.78	73.8 ± 6.92	79.3 ± 8.70	79.5 ± 6.28
HR (bpm)	61.8 ± 10.40	59.0 ± 5.98	61.0 ± 8.55	58.4 ± 9.96
cSBP (mmHg)	122.6 ± 11.01	123.3 ± 18.81	128.9 ± 11.97	132.9 ± 10.06
cDBP (mmHg)	76.5 ± 7.98	75.1 ± 7.20	79.9 ± 8.46	80.4 ± 6.26
cPP (mmHg)	46.1 ± 6.38	48.1 ± 13.14	49.0 ± 11.17	52.5 ± 11.69
cMAP (mmHg)	93.1 ± 9.17	92.3 ± 12.01	98.1 ± 8.61	99.1 ± 5.84
AP (mmHg)	13.9 ± 11.03	5.3 ± 8.10	19.9 ± 7.34	19.4 ± 5.71
AIx (mmHg)	29.9 ± 11.03	30.4 ± 11.36	35.3 ± 9.07	37.5 ± 9.10
PWV (m/s)	7.9 ± 1.32	8.0 ± 1.15	8.5 ± 1.15	8.7 ± 1.43

Values represent mean ± standard deviation.
Abbreviations: bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; HR, heart rate; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cPP, central pulse pressure; cMAP, central mean arterial pressure; AP, augmentation pressure; Alx, augmentation index; PWV, pulse wave velocity.

Table 4. Hemodynamic parameters during the CPT before and after placebo.

	Normal		Prediabetic	
	Pre	Post	Pre	Post
bSBP (mmHg)	29.8 ± 15.43	30.3 ± 16.97	29.0 ± 8.49	32.4 ± 18.17
dDBP (mmHg)	17.9 ± 6.42	18.6 ± 7.71	21.1 ± 8.46	19.5 ± 8.54
HR (bpm)	6.5 ± 4.28	7.3 ± 3.92	4.6 ± 3.62	5.3 ± 4.77
cSBP (mmHg)	26.5 ± 14.00	26.9 ± 14.23	26.6 ± 7.84	29.3 ± 16.46
cDBP (mmHg)	19.3 ± 8.66	17.9 ± 7.41	21.4 ± 8.63	19.9 ± 8.68
cPP(mmHg)	7.3 ± 10.55	9.0 ± 8.83	5.3 ± 8.94	9.4 ± 11.43
cMAP (mmHg)	23.5 ± 10.36	22.6 ± 9.69	24.8 ± 7.85	25.0 ± 12.35
AP (mmHg)	2.5 ± 5.35	2.5 ± 3.59	-0.6 ± 4.37	3.5 ± 7.13
AIx (mmHg)	0.4 ± 8.21	0.4 ± 5.07	-4.4 ± 4.17	-0.3 ± 6.78

Values are mean ± standard deviation.
Values represent the change from rest to CPT.
Abbreviations: bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; HR, heart rate; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cPP, central pulse pressure; cMAP, central mean arterial pressure; AP, augmentation pressure; Alx, augmentation index.

Table 5. Hemodynamic parameters during the CPT before and after L-citrulline supplementation.

	Normal		Prediabetic	
	Pre	Post	Pre	Post
bSBP (mmHg)	32.6 ± 18.87	21.6 ± 7.67	27.5 ± 12.26	19.3 ± 13.96
bDBP (mmHg)	18.6 ± 9.58	17.5 ± 6.35	19.3 6.30	22.5 ± 10.20
HR (bpm)	7.8 ± 7.92	9.3 ± 4.65	5.8 ± 8.43	5.3 ± 2.71
cSBP (mmHg)	28.6 ± 16.24	19.3 ± 6.23	25.8 ± 11.21	18.6 ± 10.29
cDBP (mmHg)	19.0 ± 10.28	17.6 ± 5.42	20.3 ± 6.11	23.3 ± 10.39
cPP (mmHg)	9.6 ± 8.05	1.6 ± 8.10*ɬ	5.5 ± 8.68	-4.6 ± 16.16
cMAP (mmHg)	24.3 ± 13.39	19.9 ± 5.44	23.6 ± 9.72	23.0 ± 7.56
AP (mmHg)	3.0 ± 3.89	-0.6 ± 4.90*ɬ	0.6 ± 7.31	-4.0 ± 8.25*ɬ
AIx (mmHg)	1.4 ± 7.31	-2.4 ± 9.64	-3.1 ± 11.29	-6.8 ± 13.65ɬ

Values are mean ± standard deviation.
Values represent the change from rest to CPT.
Abbreviations: bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; HR, heart rate; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cPP, central pulse pressure; cMAP, central mean arterial pressure; AP, augmentation pressure; Alx, augmentation index.
* $P < 0.05$ different from pretest.
ɬ $P < 0.05$ different from placebo.

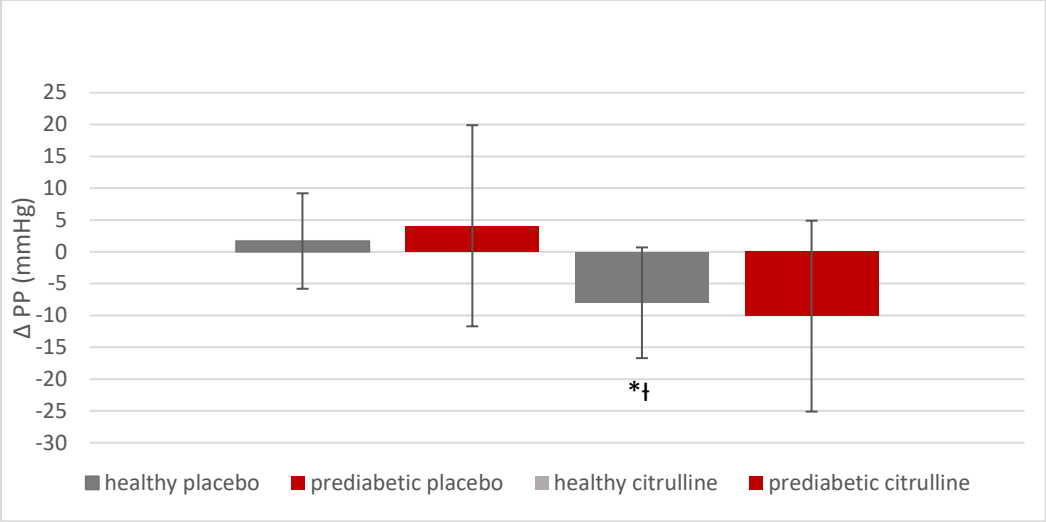


Figure 1. The change in pulse pressure (PP) between placebo and L-citrulline during the CPT in healthy and prediabetic individuals. Values are the means ± SEM.
* $P < 0.05$ vs. before; ǂ $P < 0.05$ vs. placebo.

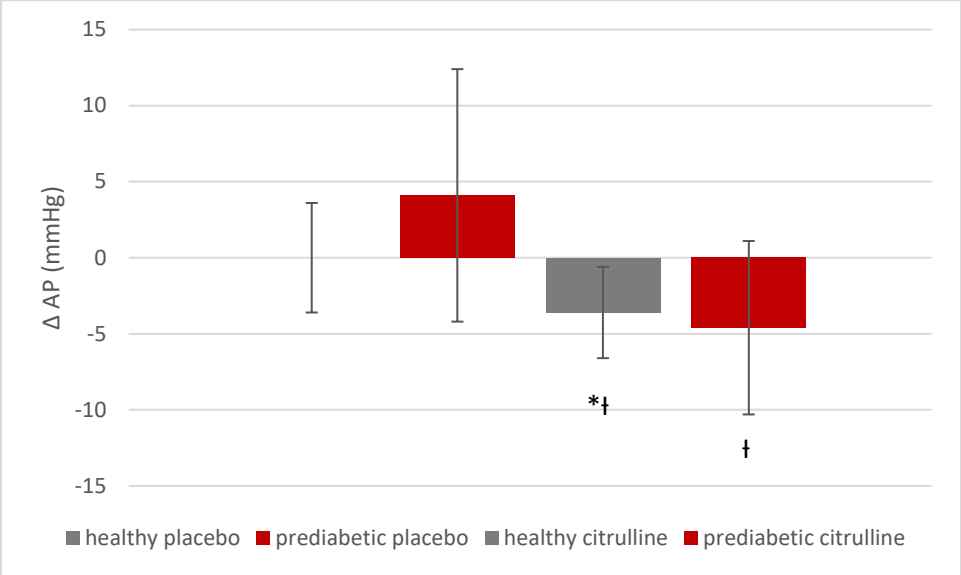


Figure 2. The change in augmented pressure (AP) between placebo and L-citrulline during the CPT in healthy and prediabetic individuals. Values are the means ± SEM.
* $P < 0.05$ vs. before; ǂ $P < 0.05$ vs. placebo.

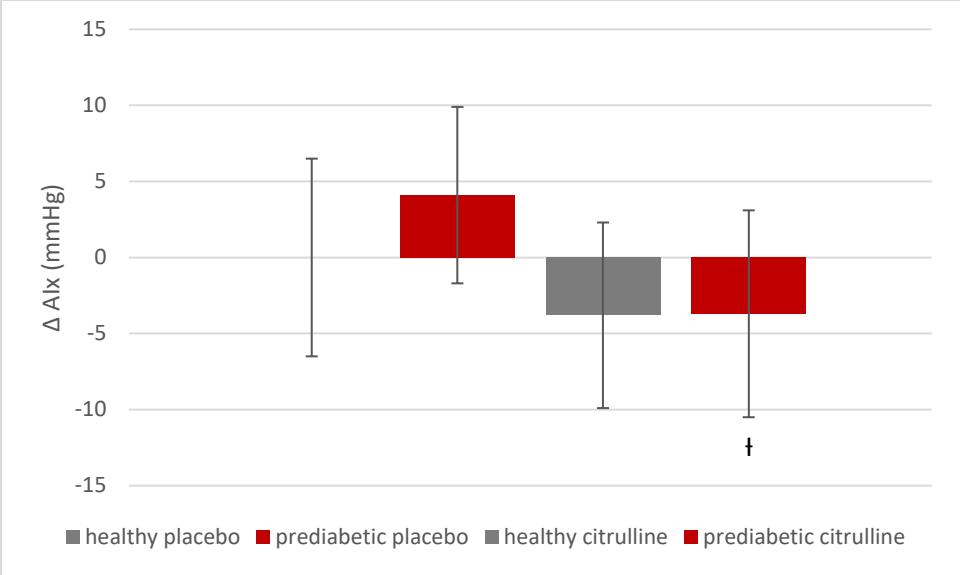


Figure 3. The change in augmentation index (Alx) between placebo and L-citrulline during the CPT in healthy and prediabetic individuals. Values are the means ± SEM. ł $P < 0.05$ vs. placebo.

DISCUSSION

Although there were no significant findings at rest, the main effects of this study are that L-citrulline attenuated central PP and AP responses to the CPT in normal individuals and AP and AIx responses to the CPT in prediabetics. These are markers for vascular reactivity and arterial stiffness (Zieman et al, 2005). These findings suggest that L-citrulline effectively decreased the vascular reactivity effects from cold-induced conditions but not at rest. This agrees with previous data showing the benefits of L-citrulline with cold exposure involving PP, AP, and AIx (Figueroa et al, 2014; Figueroa et al, 2010).

Cardiovascular measures did not change significantly from L-citrulline supplementation at rest. These results were similar to those by Figueroa et al. (2010) and Figueroa et al. (2016), wherein a 2-4 week supplementation period of L-citrulline did not change brachial or aortic systolic or diastolic blood pressures at rest. A 1-week study also showed no change in brachial systolic blood pressures in normotensive men (Ochiai et al., 2012). However longer periods of supplementations have apparently promising results as Wong et al. (2015) presented an 8-week supplementation of L-citrulline was successful in decreasing brachial and aortic systolic and diastolic blood pressures in hypertensive and prehypertensive obese postmenopausal women. Additionally, 8-week supplementation of L-citrulline significantly reduced brachial systolic and diastolic pressures in heart failure patients (Orozco-Gutierrez et al., 2010).

Acute cold exposure has been observed to increase BP, AIx, and PWV (Figueroa et al., 2010). The present study found a non-significant decrease in brachial systolic blood pressure between placebo and L-citrulline in healthy individuals (6.0% decrease) during the CPT. Figueroa et al. (2016) found significant ($P < 0.05$) decreases in BP (10.7% decrease). A potential

explanation for the difference in percent decrease between the present study and the study conducted by Figueroa may be attributable to variance. This attenuation of blood pressure with cold exposure exemplified the release of NO with L-citrulline. Other studies found similar results in systolic blood pressure. With L-citrulline supplementation, Figueroa (2016) found a 37% decrease in the rise of SBP from the CPT test. Similarly, Figueroa (2010) found a decrease of 26%. Variance in the present study could explain the non-significant differences even with similar decreases. This improved peripheral vasodilation was thought to be beneficial to reduce MAP and BP during acute exposure to cold (Figueroa et al., 2016). The present study found that markers for arterial stiffness (AIx, PP, and AP) had significant changes during the CPT with L-citrulline supplementation compared to placebo. In prediabetics, the changes in AIx and AP significantly decreased during the CPT. In healthy individuals, the changes in cPP and AP significantly decreased during the CPT. Similarly, supplementation with L-citrulline appeared to attenuate the increase in AIx and AP and of central PP (Figueroa et al., 2013; Figueroa et al., 2010) during cold exposure. In contrast, MAP and PWV were decreased significantly with a two week L-citrulline supplementation (Figueroa et al., 2016). These measures were not seen in the present study.

This study found that L-citrulline helped to attenuate the increases in cPP, AP, and AIx during the CPT. These markers for arterial stiffness all improved compared to placebo. When there was an increase in central PP, there was also an increase in pulsatile flow and pressure causing more stress on the arteries (Zieman et al., 2005). This stress can cause atherosclerotic changes and lead to a cardiac event or disease. A decrease in cPP would decrease the amount of stress on the arteries and decrease the stiffness of the arteries over time (Zieman et al., 2005). Augmentation pressure is the pressure from the reflected waveform that contributes to systolic BP. An increase in AP will increase SBP, causing an increase in LV afterload. AIx is very similar to AP as an increased AIx can also lead to strain of the LV due to an increased afterload

(Takazawa, 2005). This increase of afterload will cause an increase in myocardial oxygen demand, placing more stress on the heart (Fantin, Mattocks, Bulpitt, Banya, Rajkumar, 2007). AIx is meaningful in clinical environments as it predicts events independent of peripheral pressures (Vlachopoulos et al., 2010). This shows that L-citrulline aids in proper functioning of the arteries and stimulates an increase in NO production, allowing for an increase in vascular reactivity and vasodilation. A cold environment increases vasoconstriction to shunt blood to the core to maintain core temperature; doing this increases blood pressure and arterial stiffness. These cardiovascular responses to the cold make cardiac related events two times as likely in the winter or during a cold spell. (Sartini, Wannamethee, Lennon, Whincup, & Morris, 2015). It is important to decrease this sympathetic response during times of cold exposure in hopes to decrease cardiac events. Previous studies have shown that L-citrulline has reduced peripheral and central BP, PWV, and is found to be beneficial to attenuate the increase in SBP and PP during a CPT (Sanchez-Gonzalez et al., 2012; Figueroa et al., 2016; Figueroa et al., 2014; Figueroa et al., 2010). This study sought to expand the study population to prediabetics. The present study may be the first to demonstrate that L-citrulline supplementation improves arterial functioning in prediabetics during a CPT.

Based on the present study's findings, it can be suggested that older adults supplement with L-citrulline to reduce the constriction of the arteries in times of cold exposure. For those who have abnormal glucose levels, such as prediabetics, it appears to be beneficial to supplement with L-citrulline to help maintain vascular reactivity and endothelial function since increased glucose levels tend to cause endothelial injury (Fowler, 2008). This injury to the endothelial cells can cause dysfunction and decrease the vasodilatory response to NO in the endothelium (Cardillo et al., 1999). L-citrulline can reduce the adverse effects from hyperglycemia and endothelial dysfuncion. Vasodilation causes a reduction in peripheral resistance which decreases left ventricular afterload. This reduction in afterload can reduce systolic BP and decreases myocardial

oxygen demand. The reduction in peripheral resistance and the increase in NO also decreases the pressure on the organs which prevents end organ damage (Bataineh & Raij, 1998).

Potential limitations of the present study include the use of a small sample size and a lower than expected compliance of participants with taking the supplements. Although the minimum requirements calculated in the power analysis was met, it is important to note that the sample size was calculated using young, healthy men. Given the potential disease states of the subjects in the present study, a larger sample size was likely needed. It could be that the present study did not have sufficient statistical power to detect significant changes in blood pressure response and pulse wave velocity within the 2-week supplementation period in prediabetics. The shortened supplementation time and the amount of variance may also explain why BP and PWV were not significantly affected by the supplementation. This study primarily focused on individuals with prediabetes while previous research examined healthy individuals or those with hypertension. More research with prediabetics is warranted to see the effects of L-citrulline supplementation at rest and during cold exposure. Increasing the supplementation duration may elicit the hypothesized effects that this study was unable to show in this population, such as the attenuation of PWV and BP. Further research is also needed regarding the short term supplementation of L-citrulline and the effects on hemodynamics and arterial functioning at rest, during cold exposure, and during other times of increased sympathetic stimulation. A longitudinal study examining the supplementation of L-citrulline with the prevalence of CVD, prediabetes, and hypertension would help to solidify the evidence that this supplement increases endothelial function and decreases the risk of CVD and other chronic diseases. Prospective studies are warranted to examine the long term cardiovascular protective effects of L-citrulline supplementation.

CONCLUSION

This study demonstrated that a 14 day course of 6g daily dosage of L-citrulline reduced the responses of central AP and AIx in prediabetics during a CPT (Nichols & Singh, 2002; Zieman et al., 2005). These changes decrease the amount of stress on the arteries and increases the load on the left ventricle. Central PP affects arterial health and can lead to atherosclerosis, while AIx increases LV afterload and requires more effort from the heart muscle (Zieman et al., 2005; Fantin et al., 2007;Takazawa, 2005). There were no significant changes with L-citrulline supplementation during resting cardiovascular measures. There was, however, a non-significant attenuation of brachial BP with L-citrulline supplementation during the CPT. The present study's findings suggest that L-citrulline is a beneficial supplement to improve arterial functioning and aid in vasodilation during times of cold exposure. We speculate that this may decrease cardiac related events in the cold due to less stress on the cardiovascular system. Because of the predisposition for endothelial injury and dysfunction in prediabetics, L-citrulline may be even more beneficial for these individuals to maintain proper endothelial function.

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APPENDIX A

INFORMED CONSENT

INFORMED CONSENT FOR "The effects of short-term L-citrulline supplementation on arterial function in prediabetic adults"

Principal Investigator:
Salvador Jaime, PhD UW-La Crosse 142 Mitchell Hall La Crosse, WI 54601
(608)785-6518

1. I, _____, give my informed consent to participate in this study designed to determine the effectiveness of L-citrulline as a natural supplement to attenuate vascular dysfunction in prediabetic individuals. I have been informed that the study is under the overall direction of Salvador Jaime, Ph.D. who is an Assistant Professor in the Department of Exercise and Sport Science at the University of Wisconsin-La Crosse. I consent to the presentation, publication and other release of summary data from the study which is not individually identifiable.
2. I have been informed that my participation in this study will require 5 total visits to the human performance laboratory (Mitchell 225). Each visit to the lab requires fasting for at least 12 hours (food, alcohol, and caffeine), no strenuous exercise for at least 48 hours, and no morning vasoactive medication (I will bring to the lab for consumption following the tests). The subsequent measures will be taken before and after each treatment (L-citrulline and placebo) and are as follows:

   The first visit will involve the following:

   a. You will be given further detail regarding the nature of the study and then complete the written informed consent. Following the agreement, we will take your height and weight.
   b. Next, we will take a small amount of blood, from a finger-stick blood draw or venous blood draw from the antecubital vein, to evaluate HbA1c and plasma nitrate levels.
   c. Next, you will be asked to lie supine on an athletic training table for 10 minutes of silent rest. During this time, we will place four electrodes, two on your right hand and two on your right foot. A small and imperceptible electrical current will be sent through your body to measure body composition (amount of fat and fat free mass).
   d. Following 10 minutes of rest, blood pressure cuffs will be placed around your upper left thigh and arm. We will also use a pencil-like pressure sensor (tonometer) to measure the pulses in your neck area (carotid artery).
   e. Following the resting measures, we will place your hand in a bucket of ice water (4°C) for 3 minutes (cold pressor test). During the last minute, we will take your blood pressures of your arm and leg.
   f. Steps 2-5 will be repeated during visits 2-5.

Supplementation:

a. After your second and fourth visit, you will receive an unlabeled bottle of capsules [Lcitrulline (6g/day) or placebo (maltodextrin)].
b. You will consume 8 capsules per day for 14 days. 4 capsules before breakfast, 4 capsules before bed.
c. Following the 14-day period, your post-treatment visit will take place within 48-72 hours.
d. Between your third and fourth visit, you will have 14 days with no capsule consumption. This is known as the wash-out period.

3. Due to the nature of the cold pressor and deoxygenation protocols, you may feel sensations of pain. This will subside as soon as the stimulus is removed with no lasting effects.
4. You will receive information regarding your current cardiovascular health. You will also receive $50 in monetary compensation following the completion of the study.
5. I have been informed that the investigator will answer questions regarding the procedures throughout the course of the study.
6. I have been informed that I am free to decline to participate or to withdraw from the study at any time without penalty.
7. Concerns about any aspects of this study may be referred to Dr. Salvador Jaime at (608)785-6518. Questions about the protection of human subjects may be addressed to the Chair of the UW-L Institutional Review Board (608)785-6892.

Signature of Participant _____ Date _____

Printed Name of Participant _____

Signature of Witness _____ Date _____

APPENDIX B

REVIEW OF LITERATURE

Importance

The baby boomer generation is starting to reach the age of retirement, which in turn, has caused the elderly population in the United States to increase. Currently 13.7% of the population is over the age of 65; it is predicted that by the year 2030 the percent of the elderly population will exceed 20% of the total US population (Ortman, Velkoff, & Hogan, 2014). With aging comes a decline of health and mortality and an increase in medical bills; up to 36% of the U.S. health care expenses were due to those older than age of 65 (Stanston, 2006). The World Health Organization (2015) stated that 80% of heart disease, stroke, and diabetes can be prevented; this prevention is necessary to improve quality of life.

Risk of Aging

Aging is a strong risk factor for cardiovascular disease (CVD) and diabetes. With age, the dynamics of arterial function can change by increasing arterial wall thickness and stiffen the major elastic arteries due to a build-up of plaque over time (Benetos, Laurent, Hoeks, Boutouyrie, & Safar, 1993; Lakatta & Levy, 2003; Boutouyrie, et al., 1992). Chronic diseases, such as cardiovascular disease (CVD) and diabetes, usually appear mid-life after years of an unhealthy lifestyle (Prasad, Sung, & Aggarwal, 2012). Heart disease, the most common type of CVD, is the leading cause of death for both men and women in the U.S. (Centers for Disease Control and Prevention [CDC], 2017). High blood pressure, high low-density lipoprotein (LDL) cholesterol, and smoking are three major risk factors for the development of heart disease; about half of the U.S. population currently has one of these three risk factors (CDC, 2017). Poor functioning of the

vasculature can account for up to 80% of deaths associated with CVD (Mozaffarian, et al., 2016).

Another risk factor for the development of CVD is diabetes (American College of Sports Medicine [ACSM], 2018). According to the American Diabetes Association (ADA) in 2015, 9.4% of the population had diabetes and 25% of seniors are living with this metabolic disease (2018). Diabetic patients are at a higher risk for cardiovascular incidents than those free from diabetes (Mittleman, at al., 1993). Once again, this highlights the importance of finding preventative measures for the development of these chronic diseases.

Prevention

Although aging is an inevitable risk factor, there are many modifiable ones. The first line of prevention is lifestyle modifications. Absence of smoking, being physically active, and maintaining a healthy body weight are three controllable risk factors. Smoking increases damage to the arterial system. Smoking was associated with lower fitness levels, both cardiorespiratory and muscular fitness (Conway & Cronan, 1992). Chau, Reyes-Marcelino, Burnett, Bauman, & Freeman (2018) explained that prolonged sitting may be just as detrimental to health as smoking is. A study by Mahmud and Feely (2003) explained that acute and chronic smoking increases aortic and diastolic blood pressure, augmented pressure, and pulse wave velocity. These values are all measures of arterial elasticity and stiffness (Mahmud & Feely, 2003). Sandoo, van Zanten, Metsios, Carroll, & Kitas (2010) explained that physical activity is key to reduce the development of CVD by maintaining vascular reactivity. Physical activity and exercise lowers oxidant

stress which lowers the degree of arterial stiffness (Lessiani et al, 2016). In the sedentary population, the degree of arterial stiffness is higher due to oxidative stress. Sandoo et al. goes on to explain that vascular reactivity is the process in which the blood vessels dilate or constrict to allow for appropriate blood flow to the tissue. This process helps the endothelium maintain their functioning with age. Maintaining a healthy diet consisting of low levels of saturated fats, trans fats, and sodium can help maintain a healthy body weight and maintain healthy levels of cholesterol, blood pressure (BP), and glucose (Grundy, 2012). A study by Wing et al (2011) examined the relationship between body weight and risk of CVD. A 5-10% decrease in body weight in obese and diabetic adults resulting in an improvement in blood pressure, glycemic control, and lipids, all of which are risk factors for CVD. It may be possible that a lowering of glucose could reduce atherogenesis. ACSM (2018) states that a BP exceeding 140/90mmHg and fasting blood glucose (FBG) above 126mg/dL are considered risk factors for the development of chronic disease. Physical inactivity, regardless of BP and FBG levels, tends to have the most impact on mortality (Lee, et al., 2012). Grundy (2012) explains that if lifestyle modifications fail to provide the preventative measures, drug therapy can be used. Blood pressure lowering agents including beta-blockers, calcium channel blockers, and angiotensin-converting enzyme inhibitors can be used for those who have high blood pressure. Beta-blockers must be used in caution for those with borderline to high blood glucose levels as this drug can increase the amount of glucose in the blood. A safe and effective supplement to decrease blood pressure would be beneficial for prediabetic and diabetic patients to avoid the increase of glucose from the blood pressure lowering agent.

Macrovasculature

Endothelium is the innermost layer that lines all blood vessels. This wall is composed of endothelial cells which are capable of detecting shear stress, from changes in flow of blood via mechanoreceptors. Shear stress acts as a stimulus and causes the endothelial cells to change the diameter of the lumen (Alberts et al, 2002). Sheer stress stimulates the release of NO from the endothelium which diffuses into the smooth muscle cells allowing for vessel relaxation, or vasodilation (Evans et al, 2000). Reduction in endothelial function can lead to chronic diseases (Benetos, Laurent, Hoeks, Boutouyrie, & Safar, 1993; Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Changes in the arterial structure and function, such as endothelial dysfunction, sympathetic modulation, and stiffening of the arterial smooth muscle, precede CVD. These conditions can increase the risk of disease (Shirwany & Zou, 2010; Hadi, Carr, & Suwaidi, 2005; Gomez-Sanchez, et al., 2017). Shemyakin, et al. (2012) explained that a reduced availability of vasodilator and anti-inflammatory molecule nitric oxide (NO) can allow for the development of endothelial dysfunction. In a healthy individual, a decrease in sympathetic stimulation would cause the release of NO from the endothelium for vasodilation and relaxation of the endothelial wall (Alberts et al, 2002)

Arterial stiffness, can be measured with the carotid-femoral pulse wave velocity (PWV), using applanation tonometry, which is considered the gold standard. This method measures the central BP as well as aortic pressure waves. (AlGhatrif et al., 2013). The two systolic peaks on the PWV are representing the pressure waves. The first inflection point, P1, is the pressure wave travels to the periphery. This wave returns during diastole to aid in coronary blood flow; this is the second inflection point, P2 (Sutton et al, 2005).

One measure from PWV, the augmentation index, shows the aortic pressure waveform and is found from the difference between P_2 and P_1, the two systolic peaks. This can be shown in Figure 1 (Wilkinson, et al., 2000).

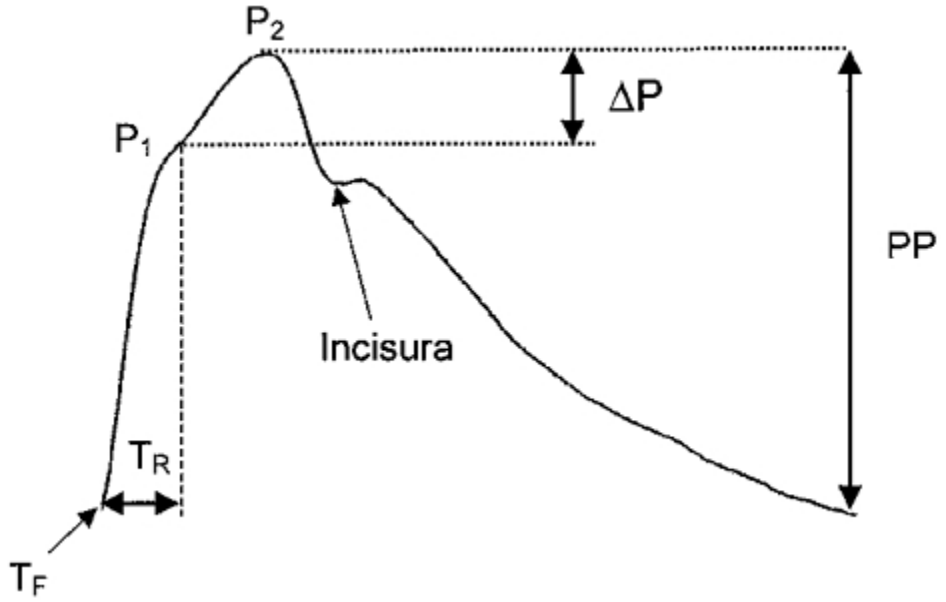


Figure 1: Pulse wave analysis. P1, first systolic peak; P2, second systolic peak; P, augmented pressure; PP is pulse pressure; TR, reflection time from foot of wave to first inflection point; TF, foot of wave; Ejection duration, the time from TF to incisura.

Macrovascular effects of aging.

The cardiovascular system changes with age due to a decrease in the availability of NO, a powerful vasodilator that helps to maintain proper function of the endothelium. Without the proper supply, vascular endothelial dysfunction increases as well as total peripheral resistance (Seals, Jablonski, & Donato, 2011). Augmentation index (AIx) and age have a curvilinear relationship and has an increase risk of CVD (Janner, Godtfredsen,

Ladelund, Vestbo, & Prescott, 2010). PWV increases significantly after the age of 50, occurring more in men than women (AlGhatrif et al., 2013). In a young adult with a healthy aorta, PWV is around 4-6 m/s; in an older adult with stiffer arteries, PWV can range from 8-10m/s and higher (Mitchell, et al., 2004). An increase in AIx and PWV can increase the risk of CVD due to an increase in arterial stiffness. A study by McEniery, et al. (2005) found that systolic blood pressure (SBP) increased with age. A higher SBP is associated with an accelerated increase of PWV (AlGratrif, et al., 2013). Dyslipidemia and diabetes are potential causes for an elevated PWV (Wilkinson, et al. 2002; Lacy, et al. 2004). Obesity can also be a cause for the increase in PWV. (Wildman, et al. 2005; Millasseau, Stewart, Patel, Redwood, & Chowienczyk, 2005). With age, arterial stiffness can arise which can degrade several factors in the body leading to conditions and disease.

Macrovascular effects of hyperglycemia.

Nearly half of the US population has either diabetes or prediabetes (American Diabetes Association, 2018). Most of these people do not die from the disease itself, but from the further complications of this disease. The number one cause of death for those with diabetes is cardiovascular disease (Wilkinson, et al 2000). Hyperglycemia is defined as elevated glucose levels in the blood, due to either insulin resistance or deficient insulin secretion. Endothelial cell walls are thickened by the impact of hyperglycemia (Grundy, 2012). Hiroaki et al (1999) reported that an elevated concentration of glucose can rapidly induce endothelial dysfunction as measured via flow-mediated endothelial-dependent vasodilation. In children with type 1 diabetes, Jarvisalo et al (2004) found that the intimae media of the carotid artery was thickened compared to those without diabetes. This shows

an impaired flow mediated dilation response in these children; this may mean that issues with glucose levels may play a role in increasing arterial wall thickness.

Paterson et al. (2007) explained that patients with hyperglycemia tend to have a higher resting heart rate (RHR) by about 8 beats per minute more than their non-hyperglycemic counterparts. Those with severe cardiovascular disease tends to have a higher RHR when compared to those free from disease, which suggests that an increase in RHR may increase the risk for CVD (Paterson, Rutledge, Cleary, Lachin, & Crow, 2007). Insulin resistance has a vasoconstrictive effect on the arteries (Kobayashi, 2015). The release of vasodilators from the endothelium are reduced in those with type 2 diabetes (Dipla, et al., 2017). Furthermore, Shin, Lee, & Lee (2011) reported that an increased FBG, even when not in a diabetic state, is correlated with an increase in PWV. Patients with diabetes have a greater risk for the development of endothelial dysfunction; these individuals are usually medicated to decrease their risk of developing CVD. The prediabetic range of FBG is 100-126 mg/dL and shows a significant increase in PWV. People with prediabetes are typically unaware of their condition so are unmedicated; this can cause a progression into diabetes and a higher risk for CVD (Campbell, Alberga, & Rosella, 2016).

In addition to the decrease the vasodilatory response from high glucose levels, the vasoconstrictive properties are heightened. Endothelin-1 (ET-1), a vasoconstrictor produced by the endothelial cells, is a strong vasoconstrictor (Plusczyk, Bersal, Menger, Feifal, 2001). A study by Cardillo et al in 1999 suggested that insulin stimulates the release of both NO, a powerful vasodilator, and ET-1, a powerful vasoconstrictor. The balance between the two substances determine the final effect on the artery. Elevated

levels of ET-1 have been detected in patients with type 2 diabetes; endothelial dysfunction has been associated with an increase in the formation of this vasoconstrictor (Cardillo, Campia, Bryant, Panza, 2002).

Aside from lifestyle modifications and medications, supplements have also been reported to lower PWV and improve arterial functioning. A study by Dangardt et al (2010) examined a supplementation of omega-3 fatty acids on vascular functioning. Omega-3 supplementation decreased inflammation and improved vascular functioning. Vitamins C and E also had beneficial effects on endothelium-dependent vasodilation and arterial stiffness. The effect reduced the plasma oxidative markers which, in turn, improved the modulation of oxidative stress (Plantinga et al, 2007). L-arginine, the byproduct from L-citrulline, is one amino acid responsible for aiding in NO synthesis (Breuillard, Cynober, & Moinard, 2015). A study by Siasos et al (2009) showed that oral L-arginine improves endothelial function and vascular elasticity. Understanding the relationship between L-arginine and L-citrulline, a study by Figueroa, Sanchez-Gonzales, Perkins-Veazie, and Arjmandi (2011) examined the benefits from L-citrulline, naturally found in watermelon. Figueroa found that watermelon supplementation improved aortic hemodynamics.

L-Citrulline

L-citrulline is a non-essential amino acid that is naturally found in watermelon (Figueroa, Wong, Jaime, & Gonzales, 2017). The rind of the watermelon offers 16.7 mg/g to 24.7 mg/g of L-citrulline; the red flesh of the watermelon contains only 7.4mg/g (Rimando & Perkins-Veazie, 2005). An appropriate dosage for L-citrulline is

6g/day.Fourteen ounces of watermelon rind would have to be eaten in order to ingest the recommended dosage of L-citrulline. In the urea cycle, L-citrulline is catabolized into L-arginine. Unlike L-arginine, L-citrulline inhibits arginase and bypasses the liver (Breuillard, Cynober, & Moinard, 2015; Moinard, et al., 2016). Figure 2 shows the difference in pathway between L-citrulline and L-arginine.

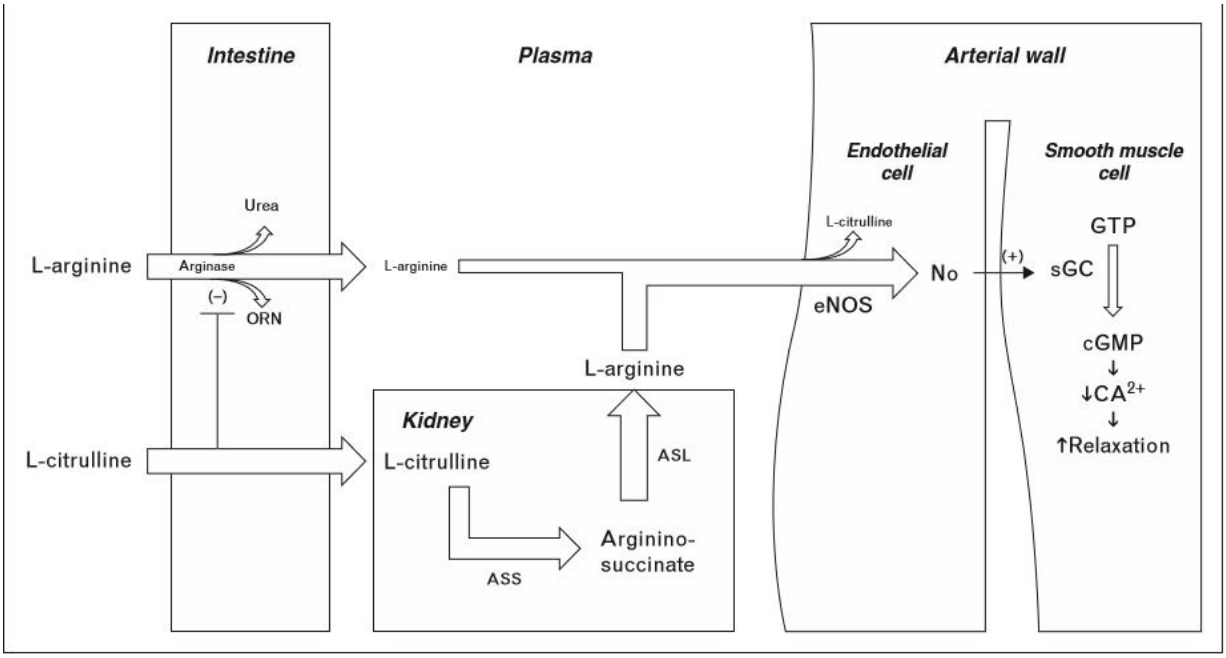


Figure 2: Figueroa, A., Wong, A., Jaime, S. J., & Gonzales, J. U. (2017); the pathway of L-citrulline compared to L-arginine in the body.

L-arginine supplementation is not as effective as L-citrulline because of the metabolism by enzymes (Van De Poll, et al., 2007). A decrease in availability of either of these two amino acids can cause NO deficiency and an upregulation of arginase. When NO is not present in endothelial cells, the development of CVD and endothelial dysfunction increases (Vanhoutte, Zhao, Xu, & Lueng, 2016; Shemyakin, et al., 2012). NO also serves a purpose in decreasing platelet aggression to preserve the vessel's

function and structure (Ignarro, Buga, Wood, Byrns, & Chaudhuri, 1987; Azuma, Ishikawa, & Sekizaki, 1986).

L-citrulline on arterial health

Several studies have examined the effects of arterial health with L-citrulline supplementation. Morita et al. (2013) showed that L-citrulline supplementation over 8 weeks resulted in a decrease in asymmetric dimethylarginine (ADMA). ADMA is an inhibitor of all forms of nitric oxide synthase (NOS); the decrease in NOS has been shown to cause endothelial dysfunction (Schwedhelm, et al, 2008). This decrease in ADMA could be a contributing factor for the improvement in endothelial functioning (Morita et al, 2013). Bailey et al (2015) found that a 7-day supplementation of L-citrulline increased endothelial functioning due to the 21% increase in plasma NO. This study also showed that L-citrulline was more effective than L-arginine at improving blood pressure. Acute supplementation of L-citrulline was shown to increase NO synthesis but not increase blood flow in healthy young adults and older adults with heart failure (Kim et al, 2015). Another study looked at short term L-citrulline supplementation; this study, conducted by Schwedhelm et al (2008), found no significant improvement of endothelium-dependent vasodilation with supplementation. Massa et al (2016) reported that 6 -weeks of L-citrulline supplementation lowered systolic and diastolic BP by ~10mmHg and 7mmHg, respectively. Ochiai et al (2012) showed that a decrease in PWV occurred after a one-week supplementation of L-citrulline. In a study by Wong et al. (2015), whole-body vibration training was used in conjunction with L-citrulline with obese postmenopausal women. The training with L-citrulline supplementation seemed to decrease hypertension in these women and improve pressure

wave reflections. L-citrulline decreased aortic pulse pressure by 5mmHg. Along with PWV, AIx can also be used as a marker for left ventricular afterload and a measure of arterial stiffness (Nichols, et al., 2008; Wilkinson, et al. 1998). Along with vasodilation, nitric oxide can also influence insulin secretions; a study by Hickner et al (2006) showed that L-citrulline reduced the nitric oxide-mediated insulin secretions from the pancreas following high-intensity exercise. This is particularly beneficial for those with an insulin abnormality to help to prevent the spike in levels of plasma insulin.

L-citrulline on sympathetic stimulation

L-citrulline has been shown to decrease BP and arterial stiffness at rest. When the heart and body are placed under stress, BP can rise from a resting state and other cardiovascular changes can take place (Korhonen, 2006). One way to stimulate a greater workload without exercise, is a pressor test. The cold pressor test (CPT) is one way to stimulate an increase in blood pressure by stimulating the sympathetic nervous system to cause vasoconstriction of arteries (Korhonen, 2006). The effects of L-citrulline on cardiovascular changes are yet to be completely understood in different populations. Figueroa, Alvarez-Alvarado, Jaime, & Kalfon (2016) showed that L-citrulline helped to reduce the stiffness of arteries during a CPT and has a protective effect on afterload. During a CPT, there is an increase in sheer stress due to the increase in coronary blood flow; this allows for vasodilation (Zeiher, Drexler, Wollschlaeger, Saurbier, & Just, 1989). With presence of endothelial dysfunction, a loss of vasodilatory response can occur and allows for vasoconstrictive responses to be permitted. The vasoconstrictor response from sympathetic stimulation is the release of thromboxane, serotonin, or histamine, all of which are vasoconstrictors (Zeiher et al, 1989).

In young, healthy men L-citrulline reduced the increase of BP during the cold pressor (Figueroa, Wong, & Kalfon, 2014). L-citrulline still increased NO production under stress with cold temperatures (Sanchez-Gonzales, et al. 2012). The results of these studies exemplified that the increased production of NO may be the cause of the reduction of arterial stiffness.

With endothelial dysfunction, it is important to reduce sympathetic stimulation because of the detrimental effects it has on the body. Sympathetic nerve activity can promote insulin resistance and a decrease in blood flow to the skeletal muscle (Jamerson, Julius, Gudbrandsson, Andersson, & Brant, 1993; Pollare, Lithell, & Berne, 1989; Thorp & Schlaich, 2015). Thorp also explains that a chronic sympathetic stimulation can augment the development of obesity, hyperglycemia, and hypertension (2015). By reducing the sympathetic nervous system stimulation, these conditions would regress or progress at a slower rate.

Conclusion

The need for an alternative to the prevention of CVD and diabetes is needed. Improving blood pressure during times of sympathetic stimulation can improve functioning of the arteries (Shirwany & Zou, 2010; Hadi, Carr, & Suwaidi, 2005; Gomez-Sanchez, et al., 2017). Improvement of the functioning in arteries can include a decrease in arterial stiffness and endothelial dysfunction. L-citrulline has been studied on the cardiovascular effects it has at rest and during stress (Korhonen, 2016; Figueroa, Wong, & Kalton, 2014; Sanchez-Gonzales, et al. 2012). The results of these studies suggest that L-citrulline may be beneficial for those in a prehypertensive or hypertensive state to

decrease resting BP and to improve arterial elasticity and responsiveness to stressors. L-citrulline effects on BP may be helpful in reducing arterial stiffness which may help to decrease risk for developing a chronic disease. For those with risk factors for CVD, this amino acid could be taken to help to reduce BP and arterial stiffness.

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