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The Effects of Whole Body Vibration Combined with L-Citrulline Supplementation on Arterial Stiffness, Pressure Wave Reflection, Endothelial Function and Body Composition in Overweight/Obese Postmenopausal Women

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THE EFFECTS OF WHOLE BODY VIBRATION COMBINED WITH L-CITRULLINE SUPPLEMENTATION ON ARTERIAL STIFFNESS, PRESSURE WAVE REFLECTION, ENDOTHELIAL FUNCTION AND BODY COMPOSITION IN OVERWEIGHT/OBESE POSTMENOPAUSAL WOMEN.

By
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ABSTRACT

Arterial stiffness (pulse wave velocity [PWV]) and endothelial dysfunction contribute to the development of cardiovascular disease (CVD), which is currently afflicting millions of individuals. The use of the amino acid L-citrulline has been suggested as a potential aid for the treatment of CVD by increasing endothelial production of Nitric Oxide (NO). Whole body vibration (WBV) is a new and effective form of exercise that is feasible for clinical populations and has been proven to decrease BP and arterial stiffness and increase muscle mass and strength. Therefore, the purpose of this study was to investigate if the combination of WBV training (WBVT) plus L-citrulline supplementation would induce positive additive effects and would be an effective means to ameliorate arterial stiffness, endothelial function, BP and muscle strength/mass in postmenopausal women.

PURPOSE: The aim of this study was threefold; 1) to evaluate the effects of 8 weeks of WBVT combined with L-citrulline supplementation on hemodynamics and arterial function; 2) to evaluate the effects of WBVT combined with L-citrulline supplementation on muscle mass and strength; and 3) to measure endothelial function by assessing plasma NOx in order to examine the potential mechanisms by which WBVT and/or L-citrulline, decreases BP, arterial stiffness and wave reflection.

METHODS: Forty-one postmenopausal women (age, 58 ± 3 years, body mass index; 34 ± 2 kg/m²) were randomized into 3 experimental intervention groups, L-citrulline, WBVT+L-citrulline and WBVT+Placebo for 8 weeks. WBVT consisted of 3 supervised training sessions a week. Subjects used 6 grams/day of oral L-citrulline or Placebo (maltodextrin) as a supplement. Brachial systolic BP (SBP), brachial diastolic BP (DBP), brachial mean arterial pressure (MAP), brachial pulse pressure (PP), aortic SBP, aortic DBP, aortic MAP, aortic PP, heart rate, augmented pressure (AP), augmentation index (Alx), Alx adjusted to 75 beats per minute (Alx@75), carotid-femoral PWV (aortic PWV), brachial-ankle PWV (baPWV), femoral-ankle
PWV (legPWV), plasma NO metabolites (NOx), leg fat mass (FM), arm FM, total FM, leg lean mass (LM), arm LM, total LM, leg strength and arm strength were measured before and after 8 weeks of the assigned intervention.

**RESULTS:** The WBVT+L-citrulline group significantly decreased (-0.9 ± 0.2 m/sec, p< .05) aortic PWV compared with no changes after WBVT+Placebo and L-citrulline groups. The WBVT+L-citrulline and WBVT+Placebo significantly decreased (p< .01) AIx and AIx @75 compared to baseline. However, the change in AIx@75 in the WBVT+L-citrulline was significantly (-10 ± 2%, p< .05) different compared with no changes in the L-citrulline group. In addition, the WBVT+L-citrulline and WBVT+Placebo groups significantly increased (p< .01) leg strength and this increase was significantly different than the no change seen after L-citrulline. All 3 groups significantly (p< .05) decreased BSBP,BDBP, BMAP, ASBP, ADBP, AMAP, AP, NOx, leg PWV and baPWV compare to baseline, but no difference among groups was found. Leg LM significantly increased (0.9 ± 0.3 Kg, p< .05) in the WBVT+L-citrulline only, but this change was not different than the other groups. Arm strength, leg FM, arm FM, total FM, arm LM, total LM were not significantly (p>.05) affected by any of the 3 interventions.

**CONCLUSION:** We showed that WBVT combined with L-citrulline supplementation decreases both brachial and aortic BP, legPWV, baPWV and AIx but does not amplify these changes compared to the other interventions. The present study demonstrates that the combination of WBVT and L-citrulline supplementation decreases aortic PWV, an effect that was not accomplished by either intervention alone. In addition, the combination of WBVT and L-citrulline supplementation decreases AIx@75, which was significantly different than the L-citrulline group. Furthermore, an increase in leg lean mass was observed with the combination treatment. In conclusion, WBVT combined with L-citrulline supplementation may be a feasible adjuvant treatment to decrease arterial dysfunction and may have a potential role in the prevention and treatment of sarcopenia. Further research is warranted in order to evaluate the effects of the
combination of WBVT and L-citrulline in different populations at increased cardiovascular and sarcopenic risk.
CHAPTER 1
INTRODUCTION

There are 35 million people who are 65 years of age or older in the United States today. That number will double by the year 2030, a result of the aging of the Baby Boomer generation. Advancing age is a major risk factor for cardiovascular disease (CVD), which is the number one killer worldwide, through its negative effects on the arteries (1). These negative effects include structural and functional changes in the arterial wall, which lead to increased arterial stiffness. Structural changes of the arterial wall include decreases in elastin as well as increases in collagen deposition, interstitial cell adhesion molecules and vascular smooth muscle thickening (2,3). Functional changes that contribute to arterial stiffness include endothelial dysfunction and increased sympathetic nervous system activity.

Endothelial dysfunction with aging is triggered by a decrease in antioxidative capacity and an increase in oxidative stress, resulting in an imbalance between endothelial vasodilatory and vasoconstrictory factors such as nitric oxide (NO) (4) and endothelin-1 (ET-1) (5,6). Decreased NO and increased ET-1 results in impaired vasodilation via increased vasomotor tone that enhances blood pressure (BP), arterial stiffness (pulse wave velocity [PWV]) and wave reflection (augmentation index [AIx]) (7-9). Brachial to ankle PWV (baPWV) is used as an index of systemic arterial stiffness (10) which is known to increase with age and is mainly influenced by aortic PWV (58%) and leg PWV (legPWV) (23%) (11). Both aortic PWV and legPWV are also known to increase with age (12,13). However, previous research suggests that aging affects the elastic arteries (carotid and aorta) more than the muscular arteries (ex: brachial, femoral, popliteal) (12,14,15). Therefore, aging increases aortic PWV to a greater extent than legPWV (13).
Increased aortic PWV, legPWV, baPWV and total peripheral resistance lead to an early return of the reflected pressure wave from peripheral arterial sites to the aorta during late systole instead of diastole, thereby increasing the second systolic peak of the aortic waveform and aortic systolic pressure. The augmented aortic systolic and pulse pressures increase left ventricular afterload, which has negatives effect on the arterial wall and left ventricular function (16,17). Increase arterial stiffness is also associated with a decrease in leg blood flow in older individuals (18), and may play an important role in the development of sarcopenia since several studies have reported a negative association between muscle mass and aortic PWV (19) as well as baPWV in older individuals (20-22). Thus, sarcopenia and arterial dysfunction may share a common pathway and interact with each other to facilitate mutual abnormalities and increase the risk for CVD.

Since endothelial dysfunction is a well-recognized putative factor in the pathogenesis of CVD, it may serve well as a therapeutic target. However, current therapeutic strategies intended to ameliorate endothelial dysfunction and arterial stiffness are limited or involve a high medical expense. Although a wide array of therapeutic agents are available, targeting arterial stiffness attributed to endothelial dysfunction deserves more attention as a potential therapy. Although the mechanisms associated with arterial stiffening are not completely understood, it has been shown that the individual and concomitant use of exercise training and L-arginine is an effective means to improve overall vascular function (23-25). Indeed, extracellular L-arginine is quickly taken up by endothelial cells and be converted to NO by the enzyme NO synthase (NOS) (26-29). NO is a molecule, which is widely known to promote vasodilation, regulate platelet activation and inhibit smooth muscle cell proliferation (26,27). Recently, this group demonstrated that 6 weeks of watermelon supplementation drink (which is a rich source of L-citrulline, a precursor for L-arginine) decreases BP and AIx in middle aged pre-hypertensive individuals (30) as well as baPWV in obese hypertensive postmenopausal women (31).
In addition, animal studies have shown the beneficial effects of L-arginine and L-citrulline supplementation on endothelial function through increased NO bioavailability (32,33). Increased NO levels through L-citrulline rich supplements leads to decreases in BP and AIx independently of aortic stiffness (aortic PWV) (30).

In regards to exercise training, recent research suggests that whole-body vibration (WBV) may be a therapeutic exercise modality for the treatment of sarcopenia (34), hypertension and arterial stiffness (35-37). WBV training (WBVT) has been shown to increase leg muscle mass and leg strength in the young (35,38,39) as well as in clinical populations (34,36,37,40-45). Moreover, increases in strength in older individuals are similar in extent to those observed after resistance training (RT) (45,46). In previous studies by this group, WBVT decreased AIx, brachial and aortic BP in addition to baPWV in overweight/obese young (35) and postmenopausal women (36,37). These studies suggest that WBVT and L-citrulline supplementation may be effective therapies for impaired arterial function. In addition, muscular function may be improved with these two therapies together since WBV has been shown to improve muscle mass and strength in several populations (34-45); and L-citrulline supplementation has been found to improve vasodilation and blood flow in the skeletal muscle (33,47,48), which may potentially have an additional effect on muscle function. However, the potential cardioprotective and anti-sarcopenic effects of the combination of WBVT with L-citrulline supplementation have yet to be evaluated. This is an important gap in knowledge that warrants a line of research in order to understand the effects of the combination of WBVT with L-citrulline supplementation as a potential therapeutic agent to reduce the risk for cardiovascular complications and sarcopenia.
Statement of the problem

The aim of this study was threefold; 1) to evaluate the effects of 8 weeks of WBVT combined with L-citrulline supplementation on hemodynamics and arterial function; 2) to evaluate the effects of WBVT combined with L-citrulline supplementation on muscle mass and strength; and 3) to measure endothelial function by assessing plasma markers of NO in order to examine the potential mechanisms by which WBVT and/or L-citrulline, decreases BP, arterial stiffness and AIx.

Significance of the Study

Approximately 80% of all CVD deaths are associated with dysfunction in the vasculature (49). Since aging is major risk factor for CVD, evidence suggests that aging negatively affects the arteries (1). It is likely that several adverse changes contribute to arterial dysfunction with aging. However, a recent line of research suggests that endothelial dysfunction and structural changes in the arterial wall lead to arterial stiffness and play an important role in the development of CVD in older adults (1,2,5).

Although, the beneficial effects of WBVT or L-citrulline supplementation on BP, arterial stiffness and AIx have been previously described (30,31,35-37,50); to the best of our knowledge, the additive effects of WBVT combined with L-citrulline supplementation are unknown and deserve further investigation. In addition, WBVT has been found to improve muscle function in several populations (34-45). However, the effects of WBVT combined with L-citrulline supplementation or L-citrulline alone on muscle function were yet to be evaluated. Therefore, the study was important for the following reasons:

• This study provided information on the effects of L-citrulline supplementation on legPWV, which was previously unknown. In addition, this study was the first to evaluate the effects of WBVT alone or combined with L-citrulline supplementation on endothelial
function. It added to our understanding of the physiological mechanisms behind the improvements in BP, arterial stiffness, and Alx after WBVT, L-citrulline or their combination.

• This study provided information on the effects of WBVT combined with L-citrulline supplementation or L-citrulline alone on body composition (muscle mass and fat mass) and muscle strength.

• The results of this study can aid in the development of an adjunct therapy for the prevention and management of CVD in individuals who may find high-intensity or prolonged aerobic exercise unattractive or may need a less time consuming alternative, such as older and obese adults.

**Main Hypothesis**

The central hypothesis of this study was that 8 weeks of WBVT combined with L-citrulline supplementation would reduce BP, arterial stiffness, wave reflection, and improve endothelial function and leg muscle mass and strength to a greater extent than WBVT or L-citrulline alone in overweight and obese postmenopausal women. In addition, we anticipated that WBVT would improve arterial function through increased NO and decreased ET-1 levels in plasma. Furthermore, we also anticipated that legPWV would improve after L-citrulline supplementation, which had not been previously studied.

**Specific Aims**

The central hypothesis of the study was tested using the following specific aims:

Aim 1: To determine the additive effect of 8 weeks of WBVT combined with L-citrulline supplementation on brachial and aortic BP, Alx, legPWV and baPWV using arterial tonometry.
Aim 2: To measure plasma markers of endothelial function (NO) to determine whether improved endothelial function is in part responsible for the protective effects of WBVT and L-citrulline on arterial function. In order to test this aim plasma samples were collected before and after 8 weeks of the intervention and were analyzed for NO metabolites (NOx) using enzyme-linked immunoabsorbent assay.

Aim 3: To determine the additive effects of WBVT and L-citrulline supplementation on leg muscle mass, fat mass, and muscle strength. Leg lean and fat mass were assessed using whole-body dual-energy X-ray absorptiometry (DXA) scans. Muscle strength was determined by the eight-repetition maximum (8-RM) test for the leg press and chest press exercises.

**Assumptions**

The following assumptions were considered for our proposed research:

1. All measurements and laboratory equipment was accurate and reliable.
2. The participants gave their best efforts during training and performed the assigned exercises.
3. The participants followed all supplementation guidelines set forth by the researcher.
4. All participants followed pre-test recommendations since they influence cardiovascular measurements such as alcohol consumption, exercise, and medication.
5. During the 8 week study period the participants did not change their daily activities or lifestyle.

**Delimitations**

The delimitations for this study included:

1. Forty-six postmenopausal women were recruited for this study. All the participants were asked not to change their lifestyles during the duration of the study.
2. Participants were excluded from the study if they were smokers, L-citrulline users or regular exercisers (defined as more than 120 min per week) in the last 6 months. Participants were also excluded if they had BP ≥160/90 or ≤ 120/80 mmHg, body mass index (BMI) > 40 or < 25 kg/m² and chronic or conditions including known heart disease, peripheral vascular disease, epilepsy, gallstones, kidney stones, acute inflammations, joint implants, recent thrombosis, recent operative wounds, intense migraines, tumors, hernias, uncontrolled diabetes and individuals on hormones or with recent medication changes.

3. A diverse ethnicity of the participants.

Limitations

Like any other research project, there are limitations to this study.

1. The biggest limitation in this study was that the interventions were not completely blinded. A WBVT exercise program (with or without L-citrulline) is nearly impossible to blind versus no exercise at all, which is the case for the L-citrulline alone group.

2. Our population consisted of only postmenopausal women. A complete spectrum of separate age groups would be ideal to better describe the effects of our interventions, but 50 to 70 years old individuals were chosen to more clearly see the effects of aging. Those affected by a disease are the ones that have more benefits from appropriate interventions. Due to the normal aging process, many older individuals presented the dysfunctions of interest in this study. Also, arterial stiffness (51), arterial pressure waveforms (52) and arterial compliance (53) are all influenced by the menstrual cycle. To avoid such variations, and the uncertainty of perimenopausal physiological variations, our female participants were comprised of postmenopausal women.

3. We decided not to include a real control group in the present project, which may be considered a limitation. However, recent debate has taken place regarding the universal
relevance of intervention studies that involve such groups. It has been suggested that research should transition from determining efficacy (for example, inclusion of an inactive control group taking placebo supplementation) to assessing comparative clinical effectiveness of active interventions (54). In this study, we considered a control group (inactive with placebo supplementation) not to be redundant and indispensable in order to prove our research hypotheses. In addition, there have also been some previous parallel designed studies on the impact of WBVT on hemodynamics (37), arterial stiffness (36), body composition (34) and strength (34,36,37) in postmenopausal women which have included a non-exercising control group and observed no change on any of the aforementioned variables.

4. The duration of the study intervention was short. Although lifestyle modifications are meant to be kept for a lifetime in order to provide continuing protection against chronic diseases and their progression; short-term interventions have value as they can provide a starting point for more involved and targeted interventions. To maximize the effects of the exercise intervention, a session frequency of 3 times per week was used. Nevertheless, longer interventions are appropriate for future and larger studies.

5. Another limitation was the accuracy of the self-reported data collected from the subjects and the subjects’ ability to comply adequately with the assigned interventions. This is not a problem for the training intervention since training sessions were supervised and took place in our facilities; but to minimize dietary variability, the subjects were required to submit 3-d food records before and on the last week of their assigned intervention. Any greater requirement from the subjects in reporting information increases the likelihood of decreased adherence over a period of 8 weeks.
Definition of Terms

Whole Body Vibration (WBV)- A relatively new training method employing an oscillating platform that produces sinusoidal vibrations that mechanically stimulate musculoskeletal structures for the improvement of muscle mass and strength (34-45) and more recently cardiovascular health (35-37,55).

Frequency- The number of complete oscillations per second of energy.

Amplitude- The maximum displacement of a vibrating body from its position to rest.

Augmented pressure (AP)- Defined as the difference between the first and the second systolic peak (56,57).

Augmentation Index (Alx) - Defined as the differences between the first and second systolic peak divided by the pulse pressure and multiplied by 100. The Alx is considered a marker of wave reflection (56).

First Systolic Peak (P1) - Forward pressure wave caused by stroke volume ejection (57).

Second Systolic Peak (P2) - Peripheral reflection of the pressure wave is also considered a marker of arteriolar vascular tone (56).

Time of reflection (Tr) - Transit time of the reflected wave indicates the round-trip travel of the forward wave to the peripheral reflecting sites and back to the aorta (58).

Pulse wave velocity (PWV)- an index of arterial stiffness (10,11).

Brachial to ankle (baPWV)- an index of systemic arterial stiffness (10).

Carotid to femoral (aortic PWV)- an index of aortic arterial stiffness (11).

Femoral to ankle (legPWV)- an index of leg arterial stiffness (11).

Endothelin 1 (ET-1) – A peptide produced by vascular endothelial cells which is known to have a potent vasoconstrictor effect (59).

Nitric Oxide (NO) - Is an important signaling molecule involved in the regulation of vascular tone. NO is known to have a potent vasodilatory effect (60).
CHAPTER 2
REVIEW OF LITERATURE

Background

Aging is accompanied by a number of life changes such as decrease in health, mobility and quality of life. These changes have been associated to declines in cardiovascular (Table 1) as well as musculoskeletal functions. Among them are increased BP, sarcopenia (the loss of muscle mass with aging) and the gain of adipose tissue which may decrease physical activity and contributes to chronic diseases such as CVD; the number one killer worldwide. Approximately 80% of all CVD deaths are associated with dysfunction in the vasculature (49). Since aging is major risk factor for CVD, evidence suggests that aging negatively affects the arteries (1). It is likely that several adverse changes contribute to arterial dysfunction with aging. However, a current line of research suggests that endothelial dysfunction and structural changes in the arterial wall lead to increases in arterial stiffness and BP, playing an important role in the development of CVD in older adults (1,2,5,61). Therefore, endothelial dysfunction is a well-recognized putative factor in the pathogenesis of CVD in the elderly that may serve as a therapeutic target. Therefore, research aimed to prevent endothelial dysfunction and arterial stiffness is warranted since it may have potential clinical applications for the treatment and prevention of hypertension, CVD, and sarcopenia.

Arterial Stiffness, Wave Reflection, and Central Blood Pressure

Arterial stiffness can be described mathematically by equations which express arterial diameter changes relative to pressure changes. While the terms distensibility, elasticity and compliance may all refer to the same general functional characteristic of an artery, mathematically they are distinct. Distensibility is the relative diameter or area change for a given
pressure increment, elasticity is the pressure step required for a theoretical 100% stretch from resting diameter, and compliance is the absolute diameter or area change for a given pressure step. When methods are used to determine arterial diameter and pressure, they are referred to as direct measures of arterial stiffness (62). Non-invasive direct measures of arterial stiffness use diagnostic ultrasound for imaging of the artery of interest and conventionally measured brachial blood pressures or the technique of applanation tonometry. Applanation tonometry is based on the principle that if a pressure containing vessel can be flattened by a pressure sensor, then the circumferential wall stresses are balanced and intra-arterial pressure can be accurately measured (63).

PWV is an indirect measure of arterial stiffness which has recently been identified as the best available technique to assess arterial stiffness (62). As an artery becomes stiff, the velocity of arterial wave propagation increases. To measure aortic PWV, the arterial pulse wave is recorded at a proximal site, such as the carotid artery and at a distal site, such as the femoral artery (64). The time delay from the arrival of the proximal pulse wave to the distal site is divided by the distance between the two sites to determine PWV. Thus PWV can be a good estimate of arterial stiffness in a large segment of an artery, such as the aorta, where atherosclerosis is often quite patchy (64). The direct measures of arterial stiffness are limited to a single point on the artery (62).

A method for measuring wave reflection is pulse wave analysis (PWA), which is calculated from an arterial pressure waveform (57,65). A pressure waveform consists of a forward moving pressure wave (incident wave) produced by the stroke volume ejection and a reflected wave returning from the periphery to the aorta (56,57). “Colliding” or summation of the reflected wave with the incident wave causes the resultant pressure waveform to have a characteristic appearance. When a reflected wave returns during systole, late systolic pressure is “augmented” and a “shoulder” is seen on the front of the pressure waveform (56). AIx is
calculated as the height of the peak above the shoulder relative to the height of the entire pressure wave (pulse pressure) (56,63). The timing of the reflected pressure wave is influenced by several factors, how fast the incident wave is moving (PWV) how far the reflective site is from the point of measurement, heart rate and left ventricular ejection characteristics (62).

**Menopause, Obesity, Aging and Arterial Dysfunction**

Women have a lower risk of CVD compared to male peers of similar age. Decreases in estrogen levels and activity are important mediators of arterial dysfunction which may explain the progressive and characteristic increase in baPWV after menopause. Estrogen modifies endothelial function through the modulation of NO activity and the attenuation of vascular response to injury (66). First, estrogen promotes vasodilation through stimulation of NO and increases NOS activity (67). Second, estrogen acts at the level of the mitochondria in the vascular endothelium to promote oxidative phosphorylation and reduce mitochondrial production of reactive oxygen species (ROS) (67). In addition to the effect of ROS on vasodilation, the action of estrogen on the mitochondria may play a role in age related changes in the vascular endothelium. A decrease in ROS may reduce the accumulation of ROS-induced mitochondrial DNA mutations which are thought to be associated with age-related disease (68).

Although anecdotal evidence accumulated over many years had supported the idea that estrogen in postmenopausal women reduced cardiovascular events (69), several studies have shown increased risk of cardiovascular events with the use of hormone replacement therapy (70-74). It appears that hormone replacement therapy changes estrogen’s hepatic gene expression and can potentially effect expression of genes involved in coagulation (69). Therefore, non-pharmacological interventions such as this investigation may have a great impact on the development of therapy against arterial dysfunction and consequently decreasing cardiovascular risk.
Overweight and obesity may be another important contributor to endothelial dysfunction in postmenopausal woman. Weight gain is accelerated after menopause. Decreases in estrogen levels are associated with an increase in abdominal fat, which is associated with an increased arterial dysfunction (20). Obesity has traditionally been viewed as a passive increase in adipose tissue and body weight, but emerging evidence points to adipose tissue as a metabolically active with capacity to promote inflammation (75). Indeed, there is increasing evidence that adiposity is associated with an increased vascular inflammation and oxidative stress (76), which play a role through number of substances including leptin, adiponectin, and other adipokines (77). Therefore, in postmenopausal women abdominal fat is negatively associated with increased aortic and legPWV, which explains the increases in baPWV after menopause (78).

Aging is widely known to affect arterial function and is mostly characterized by dilation, increase in wall thickness and stiffening of the major elastic arteries (carotid and aorta) (12,15). However, the arterial tree is not a homogenous system and is composed of the large elastic arteries and the small size muscular arteries (ex: brachial, femoral, popliteal). Therefore, several differences in structure and function exists that may explain why aging affects the elastic arteries more than the muscular arteries (12,14,15). Thus, aging increases aortic PWV to a greater extent than legPWV, and legPWV more than arm PWV (measured at the carotid-brachial segment) (13).

There is a direct link between adipose tissue, aging, menopause and vascular disease in women. Adipose tissue activity together with menopause and aging may indeed drive and create a chronic inflammatory state in obese women and contribute to arterial dysfunction and to the development of CVD.
Effects of Aging and Obesity on Strength and Muscle Mass

Aging is associated with a remarkable number of changes including an inevitable loss of muscle mass (79). Some of the loss in muscle mass is due to physical inactivity, but even those individuals that are physically active like athletes experience the lose muscle mass and strength with age, known as sarcopenia (80,82).

Even though RT (often referred as weight training) has shown to prevent or counteract sarcopenia, the effectiveness of exercise interventions to increase muscle mass and strength is less efficient in elderly than in the young individuals, due to multiple cellular and biochemical changes (83,84). Although obesity has been shown to maintain muscle mass itself, muscle weight and even muscle fiber size measurements can be misleading in aging because muscle becomes infiltrated with fat (82,85). Furthermore, obesity prevents muscle gain in response to functional overload, possibly through skeletal muscle mammalian target of rapamycin (mTOR) hyperactivation (86,87), which initiates messenger RNA translation that controls muscle peptide/protein formation (88,89). Importantly, the combination of obesity and sarcopenia carries high health risks (82). Among them arterial dysfunction, since several studies have reported a negative association between muscle mass and aortic PWV (19) as well as baPWV in older individuals (20-22). In addition, a recent study showed a negative association between leg strength and AIx in older individuals (90). Thus, sarcopenia and arterial dysfunction may share a common pathway and interact with each other to facilitate mutual abnormalities and increase the risk for CVD.

Table 1. Summarizes the detrimental effects of aging on cardiovascular function

<table>
<thead>
<tr>
<th>Effects of aging and obesity on the Cardiovascular System</th>
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<tbody>
<tr>
<td>• Increased arterial stiffness</td>
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<td>• Increased endothelial dysfunction</td>
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<tr>
<td>• Increased myocardial stiffness</td>
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<tr>
<td>• Decreased baroreceptor responsiveness</td>
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<tr>
<td>• Decreased Sinus node function</td>
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<tr>
<td>• Impaired beat-adrenergic responsiveness</td>
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</table>
Endothelial Dysfunction

The endothelium is a thin layer of specialized cells that lines the internal surface of blood vessels (91). These specialized cells are called endothelial cells and they play a crucial role in vascular function since they are essential for vascular tone regulation (91). Endothelial cells can be activated by different physical and chemical stimuli (Example: shear stress, hormones and BP) to stimulate the release of endothelium derived relaxing and constricting factors that act on vascular smooth muscle to control its tone and thus controlling peripheral resistance and BP (92). Among these factors released by the endothelium, NO is considered one of the most important due to its capacity to produce smooth muscle relaxation and consequently vasodilation (60).

NO is a gaseous molecule, that is synthesized endogenously from L-arginine (a semi-essential aminoacid) by the enzyme NOS in the endothelial cells of the arterial wall (26,27). In addition, NO synthesis requires a combination of substrates and cofactors such as oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) in addition to activation of endothelial NOS (92). L-arginine is the main substrate for NO production via hydroxylation to \( N \)-hydroxy-L-arg and then it is further oxidized to L-citrulline. NO stimulates soluble guanylate cyclase in vascular smooth muscle cells (28). This stimulation elevates cyclic guanosine 3' ,5'-monophosphate (cGMP) and activates cGMP-dependent protein kinase G (PKG), which consequently causes vascular relaxation by phosphorylating potassium channels and decreasing calcium levels in the cytosol of vascular smooth muscle cells (28).

While NO is release from endothelial cells, vasoconstrictor substances may be also released from the endothelial cells specially when NO is reduced. For instance, the vasoconstrictor peptide ET-1 is produced by endothelial cells leading to increase in vascular smooth muscle tone (91,93). Therefore, appropriate balance of vasodilatory substances (NO) to vasoconstrictors (ET-1) is pivotal for vascular health and BP control. When the balance between
vasodilators and vasoconstrictors shifts towards the vasoconstrictors (endothelial dysfunction), the endothelium is unable to properly regulate vascular smooth muscle tone, which increases arterial stiffness, wave reflection, and BP is due to become elevated.

**Exercise and the Improvement of Endothelial Dysfunction and Arterial Stiffness**

Physical activity increases the expression of NOS in the arteries of both animals and human beings (94-97). Several studies indicate that increases in upregulation of NOS are related to increases in physical forces within the arterial wall, especially shear stress (force generated by flowing blood, which acts in frictional shear at endothelial cell surface). Acute exercise induced increases in heart rate augments cardiac output and vascular shear stress, leading to increased expression of NOS and therefore NO (94). Increased NO synthesis secondary to amplified shear stress induces extracellular superoxide dismutase expression in a positive feedback manner so as to inhibit the degradation of NO by ROS (98).

Another parallel mechanism that participates to this harmony is upregulation of NOS through exercise induced ROS production, since exercise-induced increases in shear stress stimulates vascular production of ROS by an endothelium dependent pathway (99). Indeed, resting NO metabolites levels in plasma have been recently shown to increase after 8 weeks of aerobic and RT in young prehypertensives individuals (100).

Another putative mechanism is exercise-induced increases in arterial compliance (decrease in arterial stiffness) which is mediated by reduction of plasma ET-1 concentration as well as vascular tone. Beck and colleagues (100) reported decreases in plasma ET-1 levels after 8 weeks of aerobic and RT in young individuals. In addition, twelve weeks of aerobic exercise training results in increased arterial compliance, which was accompanied by decreased plasma ET-1 levels (101).
In fact, moderate intensity aerobic exercise has been shown to endothelium-dependent vasodilatation in several studies. A few studies have investigated the vasodilatory response to RT (102,103). Alomari and colleagues (102) noted a significant improvement in endothelial-dependent vasodilation after 4 weeks of handgrip training in healthy men. A similar flow-mediated vasodilatory capacity in endurance- and resistance-trained men after a bout of maximal treadmill exercise suggests that both training modes may improve endothelial function (103). However, Baynard et al. (103) demonstrated that aerobically-trained men have a greater capacity for vasodilation compared to resistance-trained men at rest.

It is interesting to point out that high-intensity RT may increase aortic PWV, legPWV, and baPWV (104,105). These adverse effects of high-intensity RT on baPWV appear to be mediated by upper-body but not lower-body exercises (104). Alternatively, low-intensity RT decreases baPWV (106) in young healthy adults while it still improves muscle mass and strength (107). Contrary to RT, aerobic training does not improve strength and muscle mass, but is widely known to decrease central and peripheral arterial stiffness (105). Considering that muscle mass and strength, and other modifiable risk factors for CVD as arterial stiffness may be improved using a combination of aerobic and RT (108,109), it is of clinical importance to find interventions that produce the same benefits as this combination. The primary reason for using WBV as training modality is that it offers the benefits related to these exercise modalities while is suitable for special populations who cannot perform high-intensity or prolonged aerobic exercise, such as the elderly.

**Resistance Training for Improvement of Strength and Muscle Mass**

RT promotes increases in muscle strength and size (110,111). Usually, RT is performed with coupled shortening (concentric) and lengthening (eccentric) muscle actions, while the number of sets and repetitions vary depending on the specific aim of the training. Therefore, RT
programs may vary from very high-intensity and ballistic exercises (112,113) to improve strength and power in athletes, and moderate- to high-intensity to improve strength and muscle mass in older individuals (109). The greatest increases in muscle strength and size have resulted from protocols using medium to high intensity RT (training at 75% to 100% of the one repetition maximum) (114). In recent years, RT has gain popularity among the general population, by improving functional strength and benefiting health in recreationally active for general fitness and health promotion (115,116), sedentary people (117,118), and elderly (119-121), and as an adjunct in injury prevention and rehabilitation programs (122,123).

Although RT has been around for centuries and extensive research on it has been done for over 6 decades (124,125), controversy still prevails regarding e.g. training loads, number of sets and repetitions, rest periods etc (110). It is generally held that muscle strength gains are predominantly explain by neural adaptations during the first 3 weeks of RT programs (126,127) while the contribution of muscle hypertrophy is evident after approximately 6 weeks of training (128-132), gradually contributing to improved strength (127).

**Strength Gains with Resistance Training: Neuromuscular and Muscular Mechanisms**

After RT programs, the increase in maximal strength typically exceeds the accompanied rate of hypertrophy (128,129). This response has been attributed to neural adaptations such as increased ability for recruitment (128-135) of fast motor units (127), enhanced ability to coordinate muscle agonists (133-136) and reduced neural inhibition (137). According to the concept of specificity (133,138), strength increases are most evident after the particular mode of training performed. For example, dynamic RT produces greater increases in dynamic strength than isometric strength (136,139). Similarly, changes in strength are most apparent at the specific joint angle used during training (140). As a result of neural adaptations a given force could be produced with less muscle involvement (134,139,141,142). Hypertrophy after RT is
reflected by increases in cross-sectional area of whole muscle or individual muscle fibers (143). The number of sarcomeres in parallel and in series increases (144,145). This effect causes the improvement in both force production and ability to withstand stress (145). Moreover, the subsequent increase in fiber pennation angle (146) may contribute to improved neuromuscular efficiency (139).

It has been shown that the cross sectional area of particularly the Type II fibers increases following RT (130,141,143,147-150), and hypertrophy of Type II fibers appear to be preferentially enhanced by eccentric contractions (130,147). Therefore, eccentric training induces a greater rate of hypertrophy than concentric training (147,151).

**Whole Body Vibration**

WBV is a novel training modality that is been proposed as a supplement to traditional exercise programs such as RT. The principles of WBV lie in the law of motion as stated by Sir Isaac Newton; mainly, that the force of an object is equal to the mass multiplied by its acceleration \( f = m \times a \). Thus, functional force can be improved by either applying more mass or more acceleration to a body. Normal conventional RT uses additional mass, for example, free weights. Whole body vibration machines on the contrary, utilize acceleration by keeping the body weight constant (152). This exercise mode uses an oscillating platform (Fig 1) that delivers sinusoidal vibrations (153) that evoke reflexive muscle contractions (154) while the person performs steadily controlled dynamic and static exercises. The mechanical action of vibration causes changes in the length of the muscle-tendon complex (152). These length changes are detected by sensory receptors that modulate muscle contraction through reflex muscular activity via the stretch reflex loop and activation of the muscle spindles (152). These contractions explain the increased levels of electromyography in working muscle during WBV exercise (155).
(156), and the increased energy output that is shown to result from the addition of vibration to squat exercises (157).

The effect of vibration is dependent upon the amplitude and frequency of oscillations, and therefore the rate and length of muscular stretch. The amplitude is determined by the peak by peak displacement of the platform, with the repetition rate of cycles of oscillation is denoted by the frequency of the vibration (measured in Hz) (152). The vibration effects on leg muscle activity and have been shown to vary with different frequencies of vibration. Greater vibration frequencies (35-45Hz) have been shown to increase greater muscle activity than lower frequencies (<35Hz) for both static and dynamic exercises (158).

There are three types of vibration platforms commonly used in clinical studies; and oscillating side to side action of the platform (Galileo), uniformly up and down action (Powerplate) and triplanar in which the vibration is applied in anterior/posterior, side to side, and up and down directions (Powerplate).

Fig 1. Image of a whole body vibration platform

**WBVT for Cardiovascular Health**

The beneficial effects of WBV on the cardiovascular system have been mostly observed following acute bouts of WBV. These changes include local increases in skin blood flow in the upper (159) and lower extremities (160) after a session of passive vibration (PV), defined as
exposure of the limbs to continuous vibration without performing voluntary muscle contractions. The vasodilatory response in the previous studies was found to be related to increases in NO production (161). In addition to NO, other endothelial factors such as reduced ET-1, a vasoconstrictor, may be implicated in the vasodilatory response to PV (162). Another line of studies by our group concluded that AIx (163), legPWV and baPWV (164) decrease while aortic PWV did not change (164) after a 10 min session of PV on the legs in healthy young men.

Similar results to PV have also been seen after a WBV session. Otsuki et al. found decreases in baPWV after a session of ten 1-min static squat exercises with WBV separated by 1-min inter-set rest periods (165); In addition, a study by our group showed a decrease in AIx and legPWV (166) following the same protocol used by Otsuki et al. (165). Increases in blood flow after an acute session of WBV were demonstrated by Kerschan-Schindl et al. (167) who found increased blood flow in the popliteal artery after 9 minutes of static squat with WBV. Increases in blood flow were also demonstrated by Yamada et al. (2005) who found that blood volume in the vastus lateralis acutely increases after WBV with a dynamic squat exercise (168). Research on the chronic effects of WBVT on the cardiovascular system is limited to studies by our group. Recently, we reported promising findings that may lead to the application of WBVT as a method of cardiovascular therapy. In this investigation, 10 overweight/obese normotensive women underwent 6 weeks of WBVT using leg exercises. WBVT resulted in a decrease in brachial systolic BP (SBP), aortic SBP, baPWV, AIx and sympathovagal balance (35). In another line of studies by our group in obese postmenopausal women, similar decreases were reported in aortic SBP, aortic DBP and AIx after 6 weeks of WBVT (37), and brachial SBP and DBP and baPWV after 12 weeks of WBVT (36). The decreases in baPWV in postmenopausal women were attributed to decreases in legPWV, since there were no changes in aortic PWV (36). Hence, the effect of WBVT on arterial stiffness is localized to the trained legs.

Furthermore, Sanudo et al. reported increases in femoral artery blood flow and mean velocity
suggesting improvements in leg artery vasodilation after 12 weeks of WBVT in individuals with type 2 diabetes mellitus (55). However, our previous studies have not investigated the mechanisms behind the improvements in arterial function after WBVT, which represents a gap in knowledge. Since the elderly have increased SBP, wave reflection and arterial stiffening the likelihood of cardiovascular events may be increased in these individuals. This is important to point out since the chronic adaptations to WBVT reported in the previously mentioned studies could translate into health benefits for the elderly.

**WBVT and Muscle Mass**

Research on the effects WBVT on body composition is more extensive than in the cardiovascular system. Several studies have shown increases in muscle mass in the young (35,38,39) as well as in clinical populations (34,41). Following 24 weeks of WBVT, which utilized static and dynamic leg and arm exercises 3 x week, Roelants et al. (39) showed increases in total muscle mass in a group of 13 young females. A recent project by our group in young overweight/obese women (35), 6 weeks of WBVT using four leg exercises resulted in improvements in leg lean mass, assessed by DXA, in comparison to baseline. Although the changes in the WBV group over time were not significantly different compared to the control group, these changes have clinical significance since the increase in leg muscle mass of ~0.4 Kg (0.9 lb) seems to be important considering the short amount of time of the intervention. In a study by Milanese et al. (38), WBVT completed over an 8 week intervention period resulted in increased total body lean mass in young non-obese women. In another 10 week study, Machado et al. (34) found a significant increase in thigh muscles cross sectional area in healthy elderly women. The increased in cross sectional area (assessed by computed tomography) was a result of increases in the vastus medialis and bicep femoris by 8.7% and 15.5%, respectively. Based on the previous results, we conducted a study of 12 weeks of WBVT in postmenopausal
women with a similar protocol as the one used by Machado et al. (34). Although we saw no significant changes in leg lean mass (assessed by DXA), there was a trend for it to increase after WBVT (~0.3 Kg, p=0.06). The impact of WBVT on muscle mass over a period longer than 6 months has only been studied once. In this study by Bogaerts et al. (41), elderly men trained 3 times weekly over a period of 1 year. These individuals were split into one of two groups that performed a WBVT or a Fitness training (aerobic, resistance, balance and flexibility exercises) routine. At the end of this investigation, leg muscle mass was increased 3.4% and 3.8% in the WBVT and Fitness training groups, respectively. Therefore it was concluded that WBVT is as efficient as a Fitness intervention in increasing muscle mass in elderly men.

**WBVT and Fat Mass**

Previous animal research in rats suggests that the chronic exposure to WBV decreases adipogenesis (169,170). Vissers et al. (171) found that WBVT would have a greater potential to reduce visceral adipose tissue than a program consisting of combined aerobic and RT in middle-aged obese adults. It has been previously reported that a combination of RT and WBVT, but not RT alone, was effective for decreasing total body fat percentage after 8 months of training in postmenopausal women (172) and after 8 weeks of training in young adults (173). Alternatively, Verschueren et al. suggested that 6 months of RT and WBVT produce similar decreases (-3.1 and -2.3%) in fat mass measured by DXA in older women (45). Furthermore, Lamont et al. (174) reported decreases in leg fat% after 6 weeks of combined RT and WBVT using squat exercises, an effect that was not achieved by RT alone. In a recent study by Milanese et al. (38) it was found that 8 weeks of WBVT decreases leg and total body fat mass in young non-obese women. However, a recent study by our group showed no changes in total fat mass in overweight/obese premenopausal women after 6 of WBVT (35). In another study by
our group, 12 weeks of WBVT did not change total body fat percentage in obese postmenopausal women (36). Although our results failed to show decreases in total fat mass and percentage, we did not measure leg fat mass or percentage, which has been previously shown to improve after 8 weeks of WBVT (38) or WBVT combined with RT (173) in young adults. Therefore, based on these previous results, we expect to see decreases in leg fat mass and fat percentage in our intervention.

**WBVT and Strength**

Recent evidence suggests that WBV exercise could be an alternative exercise modality for eliciting muscle strength in older adults (34,41-45,175). Findings of improved strength have also been shown following WBVT in younger individuals (35,39). The addition of vibration to exercise programs has been shown to increase strength and power more than exercise programs without vibration (42,176). Moreover, recent studies have shown that WBVT using a frequency of 25-40 Hz and an amplitude of 1-2 mm for 6 months can increase muscle strength at a similar extent than a RT program in postmenopausal women (45,46). In a recent study that investigated the effects of 1-year WBVT in elderly men, this modality proved to be as effective as a fitness program (combination of aerobic, resistance and flexibility exercises) to increase knee extension strength (41). According to Roelants et al. (39) a significant 24.4 % net benefit increase in isometric knee extensor strength was observed after 6 months of WBVT in young untrained females. The results of this previous study in young women were corroborated by another study by our group, which showed improvements in leg extension strength after a 6 weeks WBVT program in young overweight and obese women (35). Additionally, increases in isometric (15%) and dynamic strength (16%) of the knee extensor muscles have been shown after 6 months (44,45) of WBVT in postmenopausal women. Shorter duration interventions have also demonstrated that WBVT as a feasible intervention to increase muscle strength in
postmenopausal women. Following 10 weeks of WBVT using leg exercises with a frequency of 3 x week, Machado et al. (34) demonstrated increases (38.8%) in maximal voluntary isometric contraction of the leg extensors. In a study by our group, leg strength increased after 6 (9%) (37) and 12 weeks (21%) (36) of WBVT in postmenopausal women. Based on our previous results after 6 and 12 weeks of WBVT in the aforementioned population, we expect to see increases in leg strength of 10-20% in our 8-week intervention.

Proposed Mechanisms of WBVT for Improved Strength, Body Composition and Cardiovascular Health

There have been a number of mechanisms proposed for the increases in strength with WBV including the tonic vibration reflex (152), increased hormone secretion (177-179), muscle hypertrophy (180) and stimulation of proprioceptive pathways (180). Tonic vibration reflex happens when vibration causes a reflex muscle contraction by exciting the muscle spindles in the exposed muscle (152). Previous studies in rats have shown enlargement of fast and slow twitch muscle fibers following short-term WBV exposure (181). In addition, several human studies have shown a vibration induced muscle hypertrophy in healthy individuals as well as in clinical populations (34,35,38-41), which cannot be overlooked as possibility for strength improvement. It is possible that WBVT may improve the efficiency of the proprioceptive feedback loop. Proprioceptive pathways are used during isometric contractions to produce force (182). A more efficient proprioceptive feedback loop, caused by WBV, could lead to increases in force production.

There are some mechanisms proposed for the improvement in body composition (increase in muscle mass and decrease fat mass) with WBVT, which are the same mechanisms that cause improvements following RT. Previous studies in rats have shown enlargement of type I and II muscle fibers following vibration (181). Interestingly, it was previously reported that
the additional gravitational load that an individual experiences when exposed to WBV elicits an anabolic hormonal response (152). This would be comparable to mechanistic studies using conventional RT, which found a positive relationship between increasing blood levels of anabolic hormones and increased muscle mass and strength (183-185). In fact, an acute session of WBV has been shown to acutely increase serum levels of testosterone and human growth hormone and decreases of cortisol levels (179). In addition, previous animal research in rats suggests that chronic exposure to WBV stimulates a lipolytic effect (170). Rittweger et al. reported that metabolic power increased with vibration training and that energy requirements during vibration training were similar to those of moderate intensity walking (186). Indeed, an acute bout of WBV transiently reduces plasma glucose, possibly by increasing glucose uptake and utilization by contracting muscle (187). These findings suggest that WBVT has potential as a treatment for obesity and sarcopenia by inducing a predominance of anabolic hormones along with increased energy expenditure that may lead to an increase in lean mass and reduction in fat mass. This is very important to mention since the progression of arterial stiffness in older individuals is related to muscle tissue loss (188).

It is possible that the same mechanisms that explain improved vasodilation following aerobic training and RT are those causing vascular improvements with WBVT. It has been speculated that when the body is exposed to vibration it evokes rhythmic muscle contractions (157,167) which may induce changes in peripheral arteries. Widening of the capillaries in the gastrocnemius and quadriceps facilitates the exchange of nutrients, metabolic byproducts and delivery of oxygen between the capillaries and the fluid surrounding body cells (167). WBV has been shown to increase mean velocity of the popliteal artery, which indicates vasodilation of the small vessels in the exposed muscles. This increase in blood flow may be due to vibration-induced reduction in blood viscosity (167). Another factor that may play a role in the increased peripheral blood flow might be an improved endothelial function. The underlying mechanism for
the significant increase in blood flow following vibration may be due to pulsatile endothelial stress resulting in increased circulating NO concentration as a result of increased endothelial NOS activity (160,161). In addition to NO, other endothelial factors such as reduced ET-1, a vasoconstrictor, may be implicated in the vasodilatory response to vibration (162).

L-Arginine

L-arginine is a semi-essential amino acid which is mainly produced by the kidney from L-citrulline (189). Some of the substrates participating in the synthesis of L-arginine include glutamine, glutamate, and proline (190). Dietary L-arginine intake by the average American individual is ~5 grams per day. Because of a relatively high arginase activity in the intestines, ~40% of dietary L-arginine is degraded during absorption and the remainder enters the portal vein (191). Thus, it has a bioavailability of approximately 60% (190). Endothelial L-arginine is derived from plasma, intracellular synthesis from L-citrulline and the net degradation of intracellular proteins (191). Although ingestion and synthesis of L-arginine is sufficient to maintain cardiovascular health, L-arginine may become a nutritionally essential amino acid especially during disease states including those affecting vascular tone and hemodynamics (29,190). L-arginine has been shown to be effective to increase endogenous NO production as suggested by several studies showing improvements in vascular function, exercise capacity, erectile dysfunction, endothelial dysfunction, and arterial stiffness (23-25,32,192-194). These studies suggest that oral L-arginine supplementation may be effective for attenuating the age-induce vascular effects which include increased BP, arterial stiffening, aortic hemodynamics as a consequence of endothelial dysfunction.
L-citrulline Supplementation for Cardiovascular Health

Even though L-arginine supplementation has been shown to effectively enhance NO production (24,25,193,194) resulting in improved endothelial function (195-201), lower doses of L-citrulline may be more effective than L-arginine since it bypasses hepatic metabolism and/or intestinal elimination, resulting in higher circulating L-arginine levels (29). Certain enzymes such as arginases and arginine decarboxylase have been shown to convert excessive L-arginine into L-ornithine, urea and agmatine (200). Agmatine is known to bind to the enzyme NOS (which converts L-arginine into NO), inhibiting its activity (29). Therefore, the effectiveness of oral L-arginine supplementation is compromised by extensive pre-systemic elimination and increased intestinal arginase activity (47). On the other hand, low doses of oral L-citrulline (4-6 grams/per day) are efficiently converted to L-arginine, increasing NO production (47,48) and resulting in improved endothelial function (33,47,202). Previous studies by our group showed that 6 weeks of watermelon supplementation (rich in L-citrulline) reduced AIx, brachial and aortic BP in prehypertensive individuals (30) and aortic BP in postmenopausal women (31). In addition, oral L-citrulline either synthetic (50) or from watermelon (31) has been found to decrease baPWV in different populations. In a previous study by Ochiai et al., despite the positive effect of a 7-day L-citrulline supplementation on baPWV, brachial BP was not reduced in young normotensive men (50). A possible explanation for the discrepancy between our results and those of the study by Ochiai et al. may be the population used in the previous study (prehypertensive and hypertensive individuals vs normotensive men) and the duration of the L-citrulline supplementation (6 weeks vs 1 week). Therefore, it is possible that durations of at least 6 weeks might be needed in order to decrease BP after L-citrulline supplementation. Together, all these studies indicate that oral L-citrulline supplementation efficiently improves baPWV in young and middle-aged adults by increasing NO production. This is important to point out since evidence shows an association between decrease NO levels with aging and vascular dysfunction (203).
Since the femoral to ankle arterial section is a large component of baPWV (11) (64) and aortic PWV has been previously found not change after watermelon supplementation (30), we might speculate that the reductions in baPWV after L-citrulline supplementation might be related to decreases in legPWV. However, the effect of L-citrulline supplementation on legPWV is currently unknown.

L-arginine Supplementation and Body Composition

Although the effect of L-citrulline supplementation on body composition has not been studied in humans, a few studies have evaluated the effects of L-arginine supplementation on body composition in animals. Studies in rats have shown decreases in fat mass (32) and adiposity (33) after 4 weeks of L-arginine ingestion and watermelon pomace respectively. Additionally, a study on pigs found a significant decrease in total fat mass and an increase in total muscle mass after chronic L-arginine supplementation (204). Previous research in obese diabetic patients have shown that L-arginine maintained lean body mass and decrease fat mass during a 3 week intervention involving a caloric restricted diet with exercise training (25); interestingly, the maintenance of lean body mass was not achieved in another group of patients exercising but not taking L-arginine (25). Since in the previous study a group of patients taking L-arginine without exercise training was not included, the decrease in fat mass of patients in both exercise groups (with and without L-arginine) might be due to the effects of exercise. Therefore, the effects of L-arginine supplementation on body composition in the previous study are not completely clear. In a recent study, Borsheim et al. (205) evaluated the effects of an essential aminoacids supplementation plus L-arginine taken between meals in elderly individuals. At 12 weeks of the intervention, it was reported an increase in lean body mass as well as leg muscle strength (205). Although it is not clear if these effects are the result of L-arginine or the essential amino acids, it is of remark to mention that none of the elderly
individuals change their physical activity levels. It is possible that L-arginine may exert its
anabolic effect on muscle via angiotensin converting enzyme (ACE) inhibition (206). ACE
inhibitors, as well as L-arginine, are known to improve endothelial function and glucose uptake
as well as to stimulate insulin-like growth factor 1 (IGF-1) secretion (207), all of which could
result in an improved muscle mass and arterial function (208-211). In fact, a greater leg muscle
mass (212) and slower decline in overall muscle strength (213) have been shown in older adults
taking ACE inhibitors than those on other hypertensive medications. All this evidence suggests
that oral L-arginine supplementation may positively affect fat and lean mass. Since the oral
administration of L-citrulline may be more efficient in increasing L-arginine levels than oral
administration of L-arginine itself (47,48), we propose to examine the effects of L-citrulline
supplementation on fat mass, lean mass, and muscle strength in postmenopausal women.
Therefore, is reasonable to investigate this supplementation as an adjunct treatment for the
decline in muscle mass and strength associated with age (29).
CHAPTER 3
METHODS

Sample Size Determination

An estimation of an appropriate sample size was conducted using the G*Power analysis software (214,215). Our rationale for sample size was based on a couple previous studies by our lab on WBV training and watermelon supplementation in postmenopausal women. First, a recent study by our group (36) showed a significant decreased in baPWV after WBV training in 13 postmenopausal women. Using the equation for effect size \( ES = \frac{\text{mean after control} - \text{mean after WBV training}}{\text{the pooled standard deviation of baPWV}} \) (in meters/seconds), our study(36) revealed an ES of 0.78 = [(14.0-12.8)/1.52]. Also, we observed a significant reduction in baPWV after six weeks watermelon supplementation in postmenopausal women (31). Using the equation for \( ES = \frac{\text{Mean after placebo} - \text{mean after watermelon group}}{\text{the pooled standard deviation of baPWV}} \), the study revealed an ES of 0.80 = [(5.2-4.0)/1.49]. Despite the large effect sizes in our previously mentioned studies, we used a medium effect size (0.50) in order to increase sample size and expect more achievable results. Based on \( \alpha = 0.05 \), a power (1- \( \beta \)) of 0.80, and an ES =0.50, a total sample size of 42 subjects (14 subjects per group) was needed to have sufficient power to detect significant changes in baPWV among the three groups in the study. However, we recruited an extra 10% subjects (N=46, ~15 per group) to protect for possible attrition.

Subjects

A total of 46 postmenopausal women (body mass index [BMI] >25 or <40 kg/m2, 50-70 years of age and > 1 year after menopause) with resting blood pressure between 121-159/81-99 mmHg were enrolled in this study. Subjects were non-smokers, L-citrulline users or regular...
exercisers (defined as more than 120 min per week) in the previous 6 months of the study. The exclusion criterion was based on contraindications to exercise. Individuals with ages <50 or >70 years, body mass index (BMI) <25 or >40 kg/m$^2$, known heart disease, peripheral vascular disease, epilepsy, gallstones, kidney stones, acute inflammations, joint implants, recent thrombosis, recent operative wounds, intense migraines, tumors, hernias, uncontrolled diabetes and individuals on hormones or with recent medication changes were excluded from the study. In addition, women were excluded if they have any restriction that significantly interfered with compliance with the L-citrulline supplementation (e.g., allergies) or whole body vibration training. Subjects with diverse ethnic backgrounds were recruited from the Tallahassee metropolitan area by advertisement and direct communication. 41 out of the 46 subjects who started the study, completed it in its entirety (3 dropped for personal reasons and 2 were excluded for exercising outside their assigned intervention).

**Study Design and Experimental Protocol**

The study protocol was approved by the Florida State University Human Subject Committee (2012.9674). After completion of initial screening, subjects were randomly assigned to a WBVT+ Placebo (Maltodextrin), WBVT+L-citrulline or L-citrulline alone (L-citrulline supplementation with no exercise) groups for 8 weeks of treatment. Before baseline measurements, allocation was stratified for BMI (<32 or ≥32 kg/m$^2$) and for brachial SBP (<140 or ≥141 mmHg), and the sequence was generated by a computer-based number. Resting cardiovascular function, plasma NOx, body composition and strength were evaluated for the 3 groups at baseline and after 8 weeks of the assigned intervention. Physical activity levels were estimated by the use of the physical activity scale for the elderly questionnaire (PASE). In addition, to minimize dietary variability the subjects were required to submit 3-day food records.
Both the PASE and the 3-day food records were submitted by the subjects at baseline and at 8 weeks of the assigned intervention.

Scheduled Laboratory Visits

Subjects had the following laboratory visits:

Visit 1 (Screening and Familiarization) - All recruits were oriented to the study, and filled out health and exercise questionnaires. An appropriately-sized BP cuff was wrapped around the left arm (~2 cm above the antecubital fossa with the position mark aligned with the brachial artery) and arm blood pressure was measured twice after 5 min of rest in the seated position, the average value was recorded. Subjects gave informed consent if they qualified. Height and weight were measured. Subjects had a familiarization session with laboratory measurements and to learn the correct form to perform the exercises.

Visit 2 (Body Composition and 8-RM determination) - Subjects came to the laboratory with exercise clothes to undergo body composition analysis by a DXA scan. Thereafter, maximal strength was assessed by an 8 repetition maximum (8-RM) test (216) for the leg press and chest press exercises. Once the warm-up was completed, subjects progressed towards a maximal weight that they were able to lift a maximum of eight times through a full range of motion (8-RM) for the leg press and chest press exercises.

Visit 3 (Blood Draw and Cardiovascular Measurements) - The protocol was conducted in the morning after at least 8 hours of overnight fasting. Upon arrival to the laboratory, blood samples (10 mL) were drawn from a vein in the forearm by an experienced phlebotomist to measure a marker of endothelial function (NOx). Subjects were then tested for resting cardiovascular measurements in the supine position. Following resting cardiovascular measurements, muscle strength was re-evaluated as described in Laboratory Visit 2. Following muscle strength testing participants were randomized to one of the 3 groups.
**Visit 4-** It consisted of end of study testing (8 weeks of intervention). DXA scan, height and weight, blood samples, cardiovascular measurements and muscle strength were re-evaluated as described in Laboratory Visits 2 and 3 (above). All the visits took approximately one hour and 30 min.

**Additional Laboratory Visits**— Subjects in the L-citrulline group did not have any additional laboratory visits. Subjects in both WBVT+Placebo and WBVT+L-citrulline groups came 3 times per week for training. Subject had a total of 4 laboratory data collection visits and a total of 24 training sessions.

**Cardiovascular Measurements**

The day before each test, subjects refrained from any caffeine, alcohol, prescribed medications or any unaccustomed physical activity for 24 hours. On data collection days, all subjects came to the laboratory after at least 8 hours of overnight fast between 7 and 11 am.

All data collection was carried out at least 48 hours after the last training session (to avoid the influence of acute effects of exercise), at the same time of day (± 1 hour) and under the same conditions to avoid the effects of diurnal variation. Measurements were taken in a quiet, temperature controlled room (23 ± 1° C) with lighting kept to a minimum. Upon arrival to the laboratory, the subject went through a brief review of the procedures and then height and weight measurements were performed. After 10 min of supine rest, brachial BP, aortic hemodynamics and PWV was measured twice and the average value was recorded.

Brachial BP, HR and PWV was assessed by a semi-automatic device (VP-2000; Omron Healthcare, Vernon Hills, IL), which uses BP cuffs around both arms (brachial artery), and ankles (posterior-tibial artery) and tonometers over the right carotid and femoral arteries to obtain baPWV, aortic PWV (carotid-femoral) and legPWV (femoral-ankle). The feet of the pulse waves were related to the R-wave of the electrocardiogram (ECG) to calculate transit time. The
distance between sampling points for aortic PWV was measured with a non-elastic tape, whereas for baPWV and legPWV, this value was calculated automatically according to the subject’s height (10). PWV was calculated as distance/transit time (10) while HR was obtained from the ECG.

Radial applanation tonometry assessed wave reflection through pulse wave analysis (PWA). Brachial SBP and diastolic BP (DBP) were used to calibrate radial waveforms obtained from a 10 sec epoch using a high-fidelity tonometer (SPT-301B; Millar Instruments, Houston, TX) (217). Aortic pressure waveforms were derived using a generalized validated transfer function (SphygmoCor, AtCor Medical, Sydney, Australia). The incident wave (P1), created by stroke volume ejection, travels through the arterial tree until it hits points of reflection. Then, it reverses direction and returns as a reflected wave (P2) to the aorta. When the reflected wave arrives during late systole, it augments SBP and pulse pressure (PP = SBP-DBP). The AIx is defined as the augmented pressure (AP = P2-P1) expressed as a percentage of the aortic PP x 100. AIx was normalized to a heart rate (HR) of 75 beats/min (AIx@75) since it is negatively influenced by HR (65). Transit time of the reflected wave (Tr) indicates the round-trip travel of the forward wave to the peripheral reflecting sites and back to the aorta (58). AIx and Tr are commonly used markers of wave reflection and estimated aortic stiffness, respectively (65).

Figure 2. Image of the aortic pulse waveform. Abbreviations: AIx, augmentation index; AP, augmentation pressure, DBP, diastolic blood pressure; P1, first systolic peak; PP, pulse pressure; SBP, systolic blood pressure; Tr, transit time of reflected wave.
**Blood Samples**

Blood was collected for biochemical analysis by venipuncture. Plasma was collected using EDTA as an anticoagulant for NOx (circulating levels of nitrite + nitrate) determination, which represents the final and stable end-product of the metabolic pathway. Samples were centrifuged for 15 minutes at 3500 rpm within 30 minutes of collection and were stored at -20° C before being analyzed. The samples were analyzed in duplicate by means of enzyme linked immunosorbent assays (ELISA) using commercially available kits (R&D Systems, Inc., Minneapolis, MN, USA), which converts all nitrate to nitrite using nitrate reductase.

**Anthropometry and Body Composition**

Height was measured to the nearest 0.5 cm using a stadiometer and body weight was measured to the nearest 0.1 kg using a seca scale (Sunbeam Products Inc., Boca Raton, FL, USA). Fat mass (total body, percent, arm and leg) and lean mass, (total body, arm, and leg) was determined from whole body DXA scans (GE Lunar DPX-IQ, Madison, WI, USA). The appendicular skeletal muscle mass is the lean mass of the arms and legs. Appendicular skeletal muscle mass index (ASMI) was defined as appendicular lean mass/height$^2$ (kg/m$^2$) (218).

**Muscle Strength**

Subjects performed an 8-RM test (defined as the highest weight the individual is able to lift for only 8 times) (216) 3 times. This was measured twice on non-consecutive days before the start of training and once at the end of the study. The leg press and chest press exercises were used for the 8-RM and the results recorded as a value for lower and upper body strength, respectively. Individuals completed the 8-RM test within 5 attempts and had 2-3 min of rest between attempts. The test was terminated when the subject could no longer maintain good form or perform the necessary repetitions. The highest weight attempted and performed with
good form for the first 2 tests was recorded as the initial 8-RM. The highest weight attempted and performed with good form at the end of the study was recorded as the final 8-RM.

**WBVT Protocol**

Subjects in both WBVT groups completed 3 supervised training sessions per week for 8 weeks. The subjects performed different exercises for the legs on a vibration platform (pro5 AIRdaptive; Power Plate International, Northbrook, IL). The exercises were performed without shoes (wearing socks only) in order to standardize the damping of the vibration and to better stimulate sensory receptors in the feet and consequently maximize muscle contractions through the tonic vibration reflex. The exercises consisted of static and dynamic squats with a 90° knee angle (considering 180° as full knee extension), semi-squats with 120° knee angle, wide-stance semi-squats and calf raises (Figure 3). All dynamic exercises were performed with slow controlled movements starting from an upright position into the assigned degree of knee flexion (squat, semi-squat and wide-stance semi-squat) and maximal heel elevation (calf-raise). These movements were performed at a rate of 3 seconds eccentric/2 seconds concentric phases and were controlled with the use of a metronome. The selected rate for the exercise movement was based on a previous study that showed that cardiovascular risk immediately after low intensity exercise trials with slow movement, like we used in the present study, is very low (219). Static exercises were performed without movement in the joint angles described previously. The training volume was increase progressively over the 8 week training period by increasing the intensity of vibration (25 to 40 Hz of frequency and 1 mm to 2 mm of amplitude), duration of the exercise set (30-60 sec), number of sets (1-5), and total duration of the training session (11-60 min), and decreasing the duration of rest periods (60 sec to 30 sec). The selected training protocol was similar to those used in previous research that has shown significant vascular and body composition changes after WBVT (34-37).
Figure 3. Whole body vibration training exercises. A, squat (90° knee angle); B, semi-squat (120° knee angle); C, wide-stance semi-squat (120° knee angle); D, calf-raise (maximal heel elevation).
Table 2. Whole Body Vibration Training Protocol

<table>
<thead>
<tr>
<th>Period (week)</th>
<th>Volume</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training Frequency (Sessions/week)</td>
<td>Number of sets</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

**L-citrulline Supplementation**

L-citrulline and WBVT+L-citrulline protocols used 6 grams/day [before breakfast (3 grams) and before sleeping (3 grams)] of the L-citrulline that was taken in the form of 750 mg capsules, for a total of 4 capsules (3 grams) each time is ingested. The WBVT+Placebo protocol used 8 capsules (4 capsules before breakfast and 4 capsules before sleeping) of maltodextrin (a complex carbohydrate derived from corn) a day to match the number of capsules used by the L-citrulline and WBVT+L-citrulline protocols. All three groups consumed their capsules for a total of 8 weeks (56 days). The selected dose and times of ingestion were based on previous studies that showed lower baPWV after L-citrulline or watermelon supplementation (31,50). The last dose of L-citrulline and Placebo was ingested 48 hours before the last visit. The subjects were required to return records at 4 and after 8 weeks for assessment of compliance.
**Statistical Analysis**

A 2x 3 Analysis of variance (ANOVA) with repeated measures (time [baseline vs 8 weeks] x group [L-citrulline vs WBVT+L-citrulline vs WBVT+ Placebo]) was used to determine differences between the treatments followed by the appropriate Scheffe’s post-hoc test when a significant treatment and treatment-by-time interaction was revealed. Statistical significance was considered at p<.05. SPSS 21.0 was used to analyze the data.
CHAPTER 4

RESULTS

All data are presented as mean ± SD in the text and tables, and mean ± SE in the figures.

Physical activity levels (see Table 3) and dietary composition (see Table 4) in all groups were similar at baseline and were maintained after 8 weeks. Compliance to the assigned supplementation, either placebo or L-citrulline, was 97.5 ± 2.7%, 95.8 ± 3.0% and 96.4 ± 3.6% for the L-citrulline, WBVT+Placebo and WBVT+L-citrulline groups, respectively. All subjects in the WBVT+L-citrulline and WBVT+Placebo groups completed at least 95% of the designated training protocol, which consisted of 24 training sessions. Attendance to the exercise sessions for WBVT+L-citrulline and WBVT+Placebo was 99.4 ± 1.6% and 99.7 ± 1.1%, respectively.

Table 3. Physical activity scale for the elderly (PASE) scores at baseline and after 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>PASE score</td>
<td>125 ± 16</td>
<td>127 ± 15</td>
<td>126 ± 13</td>
</tr>
</tbody>
</table>

Data are mean ± SD

Table 4. Dietary composition at baseline and after 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>Total Kcal/Day</td>
<td>2718 ± 310</td>
<td>2751 ± 298</td>
<td>2639 ± 297</td>
</tr>
<tr>
<td>Fat, g/day</td>
<td>117 ± 6</td>
<td>119 ± 5</td>
<td>111 ± 6</td>
</tr>
<tr>
<td>Fat, %</td>
<td>38.7 ± 2.0</td>
<td>38.9 ± 1.7</td>
<td>38.1 ± 1.9</td>
</tr>
<tr>
<td>Protein, g/day</td>
<td>51 ± 7</td>
<td>52 ± 8</td>
<td>51 ± 8</td>
</tr>
<tr>
<td>Protein, %</td>
<td>17.4 ± 2.5</td>
<td>17.3 ± 2.5</td>
<td>17.4 ± 2.6</td>
</tr>
<tr>
<td>CHO, g/day</td>
<td>133 ± 8</td>
<td>134 ± 8</td>
<td>129 ± 6</td>
</tr>
<tr>
<td>CHO, %</td>
<td>43.7 ± 2.7</td>
<td>43.6 ± 2.7</td>
<td>44.4 ± 2.2</td>
</tr>
</tbody>
</table>

Data are mean ± SD. CHO, carbohydrates; %, percentage of calories per day.
Hemodynamics

Hemodynamic values before and after 8 weeks are presented on Table 6. BSBP (Fig. 6A), BDBP (Fig. 6B), BMAP (Fig. 6C), BPP, ASBP (Fig. 6D), ADBP (Fig. 6E), AMAP (Fig 6F), APP, P2 (Fig. 7B) and AP (Fig. 7C) decreased significantly over time with no treatment by time effect among the groups (see Table 5,6 and 8). Alx (Fig 8A) significantly decreased and Tr (Fig. 7D) significantly increased in the WBVT+L-citrulline and WBVT+Placebo groups over time, but these increases were not different among the three group (see Table 6 and 8). A significant time by intervention interaction (p<.05) was detected for Alx@75 (see Fig 8B). The WBVT+L-citrulline (d=0.92) decreased compared with the lack of change after the L-citrulline group (0.33). There were no significant changes in P1 (Fig. 7A) and HR after 8 weeks in all 3 groups (see Table 8).

Table 5. Blood pressure values at baseline and after 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSBP (mmHg)</td>
<td>141 ± 12</td>
<td>137 ± 13</td>
<td>140 ± 9</td>
</tr>
<tr>
<td>BDBP (mmHg)</td>
<td>80 ± 8</td>
<td>77 ± 6</td>
<td>78 ± 7</td>
</tr>
<tr>
<td>BMAP (mmHg)</td>
<td>103 ± 10</td>
<td>101 ± 7</td>
<td>102 ± 8</td>
</tr>
<tr>
<td>BPP (mmHg)</td>
<td>61 ± 8</td>
<td>56 ± 13</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>ASBP (mmHg)</td>
<td>135 ± 13</td>
<td>132 ± 13</td>
<td>133 ± 9</td>
</tr>
<tr>
<td>ADBP (mmHg)</td>
<td>51 ± 9</td>
<td>78 ± 6</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>AMAP (mmHg)</td>
<td>103 ± 10</td>
<td>95 ± 9</td>
<td>102 ± 8</td>
</tr>
<tr>
<td>APP (mmHg)</td>
<td>54 ± 9</td>
<td>49 ± 14</td>
<td>54 ± 7</td>
</tr>
</tbody>
</table>

Data are mean ± SD. BSBP, brachial systolic blood pressure; BDBP, brachial diastolic blood pressure; BMAP, brachial mean arterial pressure; BPP, brachial pulse pressure; ASBP, aortic systolic blood pressure; ADBP, aortic diastolic blood pressure; AMAP, aortic mean arterial pressure; APP, aortic pulse pressure. *p < .05, # p< .01 different than baseline.
Table 6. Wave reflection and heart rate values at baseline and after 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>Alx (%)</td>
<td>42 ± 8</td>
<td>36 ± 8††</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>Alx@75 (%)</td>
<td>37 ± 9</td>
<td>31 ± 8††</td>
<td>36 ± 8</td>
</tr>
<tr>
<td>Tr (ms)</td>
<td>123 ± 13</td>
<td>134 ± 13† †</td>
<td>122 ± 8</td>
</tr>
<tr>
<td>P1 (mmHg)</td>
<td>112 ± 9</td>
<td>107 ± 14</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>P2 (mmHg)</td>
<td>135 ± 13</td>
<td>125 ± 17*</td>
<td>132 ± 15</td>
</tr>
<tr>
<td>AP (mmHg)</td>
<td>23 ± 7</td>
<td>18 ± 6†</td>
<td>22 ± 8</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>65 ± 9</td>
<td>64 ± 7†</td>
<td>64 ± 8</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Alx, augmentation index; Alx@75, augmentation index adjusted at heart rate of 75 bpm; Tr, time of reflection; P1, first systolic peak pressure; P2, second systolic peak pressure; AP, augmented pressure. *p < .05, †p < .01 different than baseline. ††p< 0.05 different than L-citrulline.

Table 7. Effect Size (Cohen’s d) for blood pressure, wave reflection and heart rate variables after 8 weeks of each intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSBP</td>
<td>0.64</td>
<td>0.50</td>
<td>0.88</td>
</tr>
<tr>
<td>BDBP</td>
<td>0.59</td>
<td>0.42</td>
<td>0.77</td>
</tr>
<tr>
<td>BMAP</td>
<td>0.63</td>
<td>0.74</td>
<td>1.00</td>
</tr>
<tr>
<td>BPP</td>
<td>0.40</td>
<td>0.31</td>
<td>0.46</td>
</tr>
<tr>
<td>ASBP</td>
<td>0.66</td>
<td>0.60</td>
<td>1.11</td>
</tr>
<tr>
<td>ADBP</td>
<td>0.54</td>
<td>0.22</td>
<td>0.77</td>
</tr>
<tr>
<td>AMAP</td>
<td>0.60</td>
<td>0.74</td>
<td>1.00</td>
</tr>
<tr>
<td>APP</td>
<td>0.50</td>
<td>0.36</td>
<td>0.71</td>
</tr>
<tr>
<td>Alx</td>
<td>0.75</td>
<td>0.47</td>
<td>1.21</td>
</tr>
<tr>
<td>Alx@75</td>
<td>0.70</td>
<td>0.33</td>
<td>1.16</td>
</tr>
<tr>
<td>Tr</td>
<td>-0.85</td>
<td>-1.02</td>
<td>-1.37</td>
</tr>
<tr>
<td>P1</td>
<td>0.43</td>
<td>0.33</td>
<td>0.52</td>
</tr>
<tr>
<td>P2</td>
<td>0.66</td>
<td>0.55</td>
<td>1.11</td>
</tr>
<tr>
<td>AP</td>
<td>0.76</td>
<td>0.53</td>
<td>1.00</td>
</tr>
<tr>
<td>HR</td>
<td>0.12</td>
<td>-0.13</td>
<td>0</td>
</tr>
</tbody>
</table>

BSBP, brachial systolic blood pressure; BDBP, brachial diastolic blood pressure; BMAP, brachial mean arterial pressure; BPP, brachial pulse pressure; ASBP, aortic systolic blood pressure; ADBP, aortic diastolic blood pressure; AMAP, aortic mean arterial pressure; APP, aortic pulse pressure; Alx, augmentation index; Alx@75, augmentation index adjusted at heart rate of 75 bpm; Tr, time of reflection; P1, first systolic peak pressure; P2, second systolic peak pressure; AP, augmented pressure; HR, heart rate.
Table 8. Repeated Measures ANOVA results for blood pressure, wave reflection and heart rate variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Wilk’s $\lambda$</th>
<th>$F$ (df)</th>
<th>$P$-value</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSBP</td>
<td>Time</td>
<td>0.52</td>
<td>(1,38)=35.78</td>
<td>0.001</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.237</td>
<td>0.790</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.164</td>
<td>0.850</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>BDBP</td>
<td>Time</td>
<td>0.68</td>
<td>(1,38)=17.60</td>
<td>0.001</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.187</td>
<td>0.830</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.559</td>
<td>0.576</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>BMAP</td>
<td>Time</td>
<td>0.49</td>
<td>(1,38)=39.26</td>
<td>0.001</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.122</td>
<td>0.886</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.379</td>
<td>0.687</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>BPP</td>
<td>Time</td>
<td>0.76</td>
<td>(1,38)=12.22</td>
<td>0.001</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.300</td>
<td>0.743</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.201</td>
<td>0.818</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>ASBP</td>
<td>Time</td>
<td>0.44</td>
<td>(1,38)=48.17</td>
<td>0.001</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.128</td>
<td>0.880</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.243</td>
<td>0.785</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>ADBP</td>
<td>Time</td>
<td>0.68</td>
<td>(1,38)=17.88</td>
<td>0.001</td>
<td>0.320</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.152</td>
<td>0.859</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.436</td>
<td>0.650</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>AMAP</td>
<td>Time</td>
<td>0.49</td>
<td>(1,38)=39.26</td>
<td>0.001</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.122</td>
<td>0.886</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.379</td>
<td>0.687</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>APP</td>
<td>Time</td>
<td>0.68</td>
<td>(1,38)=17.93</td>
<td>0.001</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.028</td>
<td>0.972</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.050</td>
<td>0.951</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>AIx</td>
<td>Time</td>
<td>0.55</td>
<td>(1,38)=30.85</td>
<td>0.001</td>
<td>0.448</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.033</td>
<td>0.968</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=2.582</td>
<td>0.089</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>AIx@75</td>
<td>Time</td>
<td>0.54</td>
<td>(1,38)=31.65</td>
<td>0.001</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.012</td>
<td>0.988</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=3.252</td>
<td>0.050</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>Tr</td>
<td>Time</td>
<td>0.58</td>
<td>(1,38)=28.12</td>
<td>0.001</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.039</td>
<td>0.962</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=1.013</td>
<td>0.373</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>Time</td>
<td>0.76</td>
<td>(1,38)=11.48</td>
<td>0.072</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.102</td>
<td>0.903</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.202</td>
<td>0.818</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>Time</td>
<td>0.46</td>
<td>(1,38)=44.47</td>
<td>0.001</td>
<td>0.539</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.109</td>
<td>0.897</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.400</td>
<td>0.673</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>Time</td>
<td>0.44</td>
<td>(1,38)=47.52</td>
<td>0.001</td>
<td>0.556</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.039</td>
<td>0.962</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=1.061</td>
<td>0.356</td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>Time</td>
<td>0.996</td>
<td>(1,38)=1.153</td>
<td>0.698</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>(2,38)=0.277</td>
<td>0.760</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>(2,38)=0.840</td>
<td>0.440</td>
<td>0.042</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: $\lambda$, Lambda; df, degrees of freedom; $\eta^2$, eta squared. BSBP, brachial systolic blood pressure; BDBP, brachial diastolic blood pressure; BMAP, brachial mean arterial pressure; BPP, brachial pulse pressure; ASBP, aortic systolic blood pressure; ADBP, aortic diastolic blood pressure; AMAP, aortic mean arterial pressure; APP, aortic pulse pressure; AIx, augmentation index; AIx@75, augmentation index adjusted at heart rate of 75 bpm; Tr, time of reflection; P1, first systolic peak pressure; P2, second systolic peak pressure; AP, augmented pressure.
Figure 4. Peripheral and aortic blood pressure variables at baseline and after 8 weeks of each intervention. Mean and SE for brachial systolic blood pressure (A), brachial diastolic blood pressure (B), brachial mean arterial pressure (C), aortic systolic blood pressure (D), aortic diastolic blood pressure (E) and aortic mean arterial pressure (F). *p < .05, #p < .01 different than baseline.
Figure 5. Selected pulse wave analysis variables at baseline and after 8 weeks for each intervention. Mean ± SE for first systolic peak pressure (A), second systolic peak pressure (B), augmented pressure (C), time of reflection (D), augmentation index (E) and augmentation index adjusted at heart rate of 75 bpm (F). *p < .05, †p < .01 different than baseline. ‡p < .05 different than L-citrulline. NS, non-significant.
Figure 6. Changes in augmentation index and augmentation index adjusted at heart rate of 75 bpm after 8 weeks for each intervention. Mean and SE for changes in augmentation index (A) and augmentation index adjusted at heart rate of 75 bpm (B). *p < 0.01 different than baseline. †p < .05 different than L-citrulline.

Arterial Stiffness and Endothelial Function

Participant’s arterial stiffness and NOx values before and after 8 weeks of their assigned intervention are presented in Table 9. There were significant time effects for NOx. NOx increased after 8 weeks in all 3 groups, but the increase did not differ among groups. Furthermore, there were significant time effects for legPWV and baPWV (p<.01) (Table 9 and 11). legPWV (Fig. 9B) and baPWV (Fig. 9C) significantly decreased after 8 weeks in all 3 groups, but the decrease did not differ among groups (Table 9 and 11). A significant time-by-intervention interaction (p<.048) was revealed for aortic PWV (Table 9 and 11, Fig 9A). The L-citrulline and WBVT+Placebo groups did not change through time compared to the decrease after the WBVT+L-citrulline (d=−1.12 and d=−0.78 among the two groups and WBVT+L-citrulline).
Table 9. Plasma levels of nitrite+nitrater (NOx) and arterial stiffness values at baseline and after 8 weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>NOx (µmol/L)</td>
<td>28.9 ± 8.8</td>
<td>40.1 ± 22.3*</td>
<td>28.2 ± 7.3</td>
</tr>
<tr>
<td>Aortic PWV (m/sec)</td>
<td>11.4 ± 1.4</td>
<td>11.2 ± 1.4</td>
<td>11.5 ± 1.4</td>
</tr>
<tr>
<td>legPWV (m/sec)</td>
<td>10.2 ± 0.8</td>
<td>9.6 ± 1.2*</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td>baPWV (m/sec)</td>
<td>14.5 ± 1.6</td>
<td>13.5 ± 2.0*</td>
<td>14.1 ± 1.8</td>
</tr>
</tbody>
</table>

Data are mean ± SD. NOx; plasma levels of nitrite+nitrate concentration; PWV, pulse wave velocity; legPWV, leg PWV; baPWV, brachial to ankle PWV. *p < .05, # p < .01 different than baseline. †p < .05 different than L-citrulline and the WBVT+Placebo groups.

Table 10. Effect Size (Cohen’s d) for plasma levels of nitrite+nitrate (NOx) and arterial stiffness (PWV) variables after 8 weeks of each intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx</td>
<td>-0.66</td>
<td>-0.81</td>
<td>-0.58</td>
</tr>
<tr>
<td>Aortic PWV</td>
<td>0.14</td>
<td>0.20</td>
<td>0.95</td>
</tr>
<tr>
<td>legPWV</td>
<td>0.59</td>
<td>0.71</td>
<td>0.70</td>
</tr>
<tr>
<td>baPWV</td>
<td>0.55</td>
<td>0.65</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Table 11. Repeated Measures ANOVA results for plasma levels of nitrite+nitrate (NOx) and arterial stiffness (PWV) variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Wilk’s λ</th>
<th>F (df)</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx</td>
<td>Time</td>
<td>0.69</td>
<td>(1,34)=14.75</td>
<td>0.001</td>
<td>0.326</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.97</td>
<td>(2,34)=0.103</td>
<td>0.902</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.73</td>
<td>(2,34)=0.512</td>
<td>0.604</td>
<td>0.032</td>
</tr>
<tr>
<td>Aortic PWV</td>
<td>Time</td>
<td>0.73</td>
<td>(1,33)=12.16</td>
<td>0.001</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.82</td>
<td>(2,33)=0.024</td>
<td>0.977</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.53</td>
<td>(2,33)=3.510</td>
<td>0.042</td>
<td>0.180</td>
</tr>
<tr>
<td>legPWV</td>
<td>Time</td>
<td>0.53</td>
<td>(1,33)=28.74</td>
<td>0.001</td>
<td>0.466</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.95</td>
<td>(2,33)=0.445</td>
<td>0.644</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.43</td>
<td>(2,33)=0.884</td>
<td>0.423</td>
<td>0.051</td>
</tr>
<tr>
<td>baPWV</td>
<td>Time</td>
<td>0.43</td>
<td>(1,38)=49.73</td>
<td>0.001</td>
<td>0.567</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.96</td>
<td>(2,38)=0.209</td>
<td>0.812</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.96</td>
<td>(2,38)=0.883</td>
<td>0.422</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Abbreviations: λ, Lambda; df, degrees of freedom; η², eta squared.
Figure 7. Selected pulse wave velocity variables and plasma levels of nitrite+nitrate (NOx) at baseline and after 8 weeks for each intervention. Mean and SE for NOx (A), aortic pulse wave velocity (B), brachial to ankle pulse wave velocity (C) and leg pulse wave velocity (D). *p < .05, †p < .01 different than baseline. ‡P < 0.05 different than L-citrulline and the WBVT+Placebo groups.
Figure 8. Changes in pulse wave velocity variables after 8 weeks of each intervention. Mean and SE for changes in aortic pulse wave velocity (A), leg pulse wave velocity (B) and brachial to ankle pulse wave velocity (C). *p < .05, †p < .01 different than baseline. ‡P < 0.05 different than L-citrulline and the WBVT+Placebo groups.
**Body Composition and Strength**

Mean body composition and strength values before and after 8 weeks of their assigned intervention are presented on Table 11. There were no significant differences in weight, BMI, arm fat mass, leg fat mass, total fat mass (Fig. 4A), arm lean mass and arm strength among the 3 groups (see Table 3 and 5). Leg Lean mass (~0.9 kg), total lean mass (Fig 4B) and ASMI significantly (p<.05) increased after 8 weeks of WBVT+ L-citrulline, but the increase was not significantly different from L-citrulline or WBVT+Placebo groups (see Table 3 and 5, Figure 5A). A significant treatment-by-time interaction (p<.001) was detected for leg strength (see Table 3 and 5, Figure 5B). The WBVT+L-citrulline (d=3.72) and WBVT+Placebo (d=2.21) groups increased leg strength more than the L-citrulline group (d=0.19).

Table 12. Body composition and strength variables at baseline and after 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Baseline</td>
</tr>
<tr>
<td>Arm fat mass (kg)</td>
<td>4.1 ± 1.1</td>
<td>4.0 ± 1.1</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Leg fat mass (kg)</td>
<td>14.6 ± 4.3</td>
<td>14.4 ± 4.3</td>
<td>13.3 ± 2.6</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>42.2 ± 8.2</td>
<td>41.7 ± 8.5</td>
<td>39.8 ± 7.2</td>
</tr>
<tr>
<td>Arm lean mass (kg)</td>
<td>5.0 ± 0.9</td>
<td>5.0 ± 1.0</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>Leg lean mass (kg)</td>
<td>15.9 ± 3.0</td>
<td>16.0 ± 2.6</td>
<td>14.8 ± 1.6</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>45.4 ± 5.7</td>
<td>45.7 ± 5.0</td>
<td>42.6 ± 4.1</td>
</tr>
<tr>
<td>ASMI (kg/m²)</td>
<td>8.14 ± 1.2</td>
<td>8.16 ± 1.1</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>Arm strength (kg)</td>
<td>58.1 ± 16.1</td>
<td>58.6 ± 15.5</td>
<td>56.2 ± 7.3</td>
</tr>
<tr>
<td>Leg strength (kg)</td>
<td>238 ± 72</td>
<td>316 ± 95†</td>
<td>227 ± 56</td>
</tr>
</tbody>
</table>

Data are mean ± SD. BMI, body mass index; ASMI, appendicular skeletal muscle mass index. *p< .05, # p< .01 different than baseline. †p< 0.01 different than L-citrulline group.
Table 13. Effect Size (Cohen’s d) for body composition and strength variables after 8 weeks for each intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT+ PLAC (n=14)</th>
<th>CIT (n=14)</th>
<th>WBVT + CIT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>-0.05</td>
<td>-0.19</td>
<td>-0.06</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.03</td>
<td>-0.14</td>
<td>-0.07</td>
</tr>
<tr>
<td>Arm fat mass</td>
<td>0</td>
<td>-0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Leg fat mass</td>
<td>0.05</td>
<td>0</td>
<td>-0.03</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>0.06</td>
<td>-0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Arm lean mass</td>
<td>0</td>
<td>-0.06</td>
<td>-0.10</td>
</tr>
<tr>
<td>Leg lean mass</td>
<td>-0.06</td>
<td>-0.16</td>
<td>-0.33</td>
</tr>
<tr>
<td>Total lean mass</td>
<td>-0.06</td>
<td>-0.16</td>
<td>-0.16</td>
</tr>
<tr>
<td>ASMI</td>
<td>-0.02</td>
<td>-0.11</td>
<td>-0.28</td>
</tr>
<tr>
<td>Arm Strength</td>
<td>-0.03</td>
<td>-0.06</td>
<td>-0.05</td>
</tr>
<tr>
<td>Leg Strength</td>
<td>0.93</td>
<td>-0.19</td>
<td>-1.72</td>
</tr>
</tbody>
</table>

Table 14. Repeated Measures ANOVA for the body composition and strength variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Wilk’s λ</th>
<th>F (df)</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Time</td>
<td>0.93</td>
<td>(1,38)=2.646</td>
<td>0.112</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,38)=0.980</td>
<td>0.385</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.97</td>
<td>(2,38)=0.624</td>
<td>0.541</td>
<td>0.033</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>Time</td>
<td>0.96</td>
<td>(1,38)=1.762</td>
<td>0.193</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,38)=1.000</td>
<td>0.378</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.98</td>
<td>(2,38)=0.320</td>
<td>0.728</td>
<td>0.017</td>
</tr>
<tr>
<td>Arm fat mass</td>
<td>Time</td>
<td>0.98</td>
<td>(1,35)=0.586</td>
<td>0.449</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.002</td>
<td>0.998</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.98</td>
<td>(2,35)=0.416</td>
<td>0.663</td>
<td>0.024</td>
</tr>
<tr>
<td>Leg fat mass</td>
<td>Time</td>
<td>1.00</td>
<td>(1,35)=0.007</td>
<td>0.936</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.345</td>
<td>0.711</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.98</td>
<td>(2,35)=0.309</td>
<td>0.736</td>
<td>0.018</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>Time</td>
<td>0.94</td>
<td>(1,35)=2.168</td>
<td>0.150</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.230</td>
<td>0.796</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.97</td>
<td>(2,35)=0.518</td>
<td>0.600</td>
<td>0.030</td>
</tr>
<tr>
<td>Arm lean mass</td>
<td>Time</td>
<td>0.97</td>
<td>(1,35)=1.186</td>
<td>0.284</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.537</td>
<td>0.589</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.95</td>
<td>(2,35)=0.890</td>
<td>0.420</td>
<td>0.050</td>
</tr>
<tr>
<td>Leg lean mass</td>
<td>Time</td>
<td>0.89</td>
<td>(1,35)=4.155</td>
<td>0.049</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.682</td>
<td>0.512</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.87</td>
<td>(2,35)=2.640</td>
<td>0.086</td>
<td>0.134</td>
</tr>
<tr>
<td>Total lean mass</td>
<td>Time</td>
<td>0.83</td>
<td>(1,35)=7.107</td>
<td>0.012</td>
<td>0.173</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=0.700</td>
<td>0.503</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.97</td>
<td>(2,35)=0.527</td>
<td>0.595</td>
<td>0.030</td>
</tr>
<tr>
<td>Appendicular Skeletal Mass Index</td>
<td>Time</td>
<td>0.88</td>
<td>(1,35)=4.298</td>
<td>0.046</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,35)=1.191</td>
<td>0.316</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.89</td>
<td>(2,35)=2.211</td>
<td>0.125</td>
<td>0.115</td>
</tr>
<tr>
<td>Arm Strength</td>
<td>Time</td>
<td>0.93</td>
<td>(1,38)=2.822</td>
<td>0.101</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,38)=0.180</td>
<td>0.836</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>1.00</td>
<td>(2,38)=0.005</td>
<td>0.995</td>
<td>0.001</td>
</tr>
<tr>
<td>Leg Strength</td>
<td>Time</td>
<td>0.22</td>
<td>(1,38)=128.64</td>
<td>0.001</td>
<td>0.777</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>(2,38)=1.878</td>
<td>0.167</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>Time by treatment</td>
<td>0.42</td>
<td>(2,38)=25.48</td>
<td>0.001</td>
<td>0.579</td>
</tr>
</tbody>
</table>

Abbreviations: λ, Lambda; df, degrees of freedom; η², eta squared.
Figure 9. Weight and Body Mass Index (BMI) at baseline and after 8 weeks of each intervention. Mean and SE for Weight (A) and BMI (B). NS, not significant. Data are mean ± SE.
Figure 10. Fat mass variables at baseline and after 8 weeks of each intervention. Mean and SE for arm fat mass (A), leg fat mass (B) and total fat mass (C). NS, not significant.
Figure 11. Lean mass variables at baseline and after 8 weeks of each intervention. Mean and SE for arm lean mass (A), leg lean mass (B), total lean mass (C) and appendicular skeletal muscle mass index (D). NS, not significant. *p<.05 different than baseline. Data are mean ± SE.
Figure 12. Leg strength at baseline and after 8 weeks of each intervention. *p < .01 different than baseline. ‡p < .01 different than L-citrulline group. NS, not significant. Data are mean ± SE.

Figure 13. Changes in leg lean mass and leg strength after 8 weeks of each intervention. Mean and SE for changes in leg lean mass (A) and leg strength (B). *p < .01 different than baseline. ‡p < .01 different than L-citrulline group.
CHAPTER 5

DISCUSSION

The research undertaken in this study was envisioned to determine the additive effect of WBVT combined with L-citrulline supplementation on arterial and endothelial function, strength and body composition. Our ultimate goal was to test and develop a non-pharmacological intervention (WBVT combined with L-citrulline supplementation) which would amplify the positive effects of WBVT alone and L-citrulline alone in the aforementioned parameters. In order to accomplish this, we sought to evaluate peripheral and central BP, arterial stiffness, wave reflection, endothelial function and leg muscle mass and strength before and after 8 weeks of WBVT combined with L-citrulline supplementation, WBVT alone and L-citrulline alone.

Accordingly, the main findings of the present study are summarized as follows: 1) WBVT combined with L-citrulline reduces aortic PWV, which is not attained by WBVT or L-citrulline alone, 2) WBVT combined with L-citrulline reduces AIx@75 and this reduction tends to be significantly larger from that observed after WBVT alone or L-citrulline alone, 3) WBVT combined with L-citrulline increases leg and total lean mass compared to baseline, which is not attained by WBVT or L-citrulline alone, 4) There are no additive effects of WBVT combined with L-citrulline on brachial and aortic BP, AIx, legPWV and baPWV, fat mass and strength. Together these findings suggest that the combination of WBVT and L-citrulline supplementation imposes additional benefits on central arterial stiffness and aortic wave reflection than either intervention alone. Moreover, the combination of WBVT and L-citrulline supplementation was effective in increasing leg lean mass, an effect that was not induced after 8 weeks of WBVT+placebo or L-citrulline supplementation.
Arterial Stiffness

A progressive increase in baPWV occurs after menopause, and both aortic PWV and legPWV may be the main contributors (78,220). In particular aortic PWV appears to be of prognostic importance as it is considered the gold standard for arterial stiffness (221). Several studies have demonstrated a strong association between increased aortic PWV and worse prognosis in the general population (222-224) and in specific groups of patients such as those with hypertension (64,225). In postmenopausal women, important determinants of aortic PWV are obesity and hypertension (78,220). Therefore, reducing arterial stiffness and specifically aortic PWV may result in significant decreases in CV events and mortality in our study population.

Consistent with previous studies (31,50), the current investigation found a significant decrease in baPWV (-1.1 m/s) following L-cit supplementation. In addition, aortic PWV did not change following the L-citrulline intervention in the current study, which is in accordance with our previous findings in individuals with prehypertension (30). Previous studies have reported that vasodilatory drug treatment with the angiotensin receptor blocker Valsartan reduced baPWV (226) but had no effect on aortic PWV in older persons with hypertension (227). Since baPWV is influenced by both the aortic PWV and legPWV (11), and legPWV significantly decreased in the current study, we can conclude that L-citrulline supplementation exerts its effects mainly through improvements in peripheral arterial stiffness. Therefore, the current study re-emphasizes the idea that peripheral arteries are more responsive to antihypertensive therapies than central arteries (228).

We noted reductions in baPWV and legPWV from baseline in both groups performing WBVT, regardless of supplementation. These results are consistent with our previous investigation showing decreases in legPWV and baPWV after WBVT in postmenopausal women (36). Additionally, aortic PWV was unaltered after WBVT+Placebo intervention which is
consistent with our findings in postmenopausal women after 12 weeks of WBVT (36) or low intensity resistance training (229). In the current investigation, the WBVT+L-Citrulline group decreased aortic PWV. This may be considered a major finding, since aortic PWV has an independent predictive value for cardiovascular events and all-cause mortality (223). The reduction in aortic PWV (-0.9 m/s) observed in the present study is greater than that observed after a diet-induced weight loss (-0.5 m/s) in postmenopausal women (229) and after treatment with antihypertensive drugs (-0.8 m/s) in middle-aged hypertensive women (230). Because the increase in aortic PWV after menopause is linked to the development of prehypertension and hypertension (220) our findings may have clinical importance.

**Hemodynamics**

Extensive epidemiological data suggests that hypertension is among the leading causes of morbidity and mortality in the United States (231). In fact, hypertension is the leading cause of morbidity and mortality in postmenopausal women (231,232). The age-related increase in BP, particularly SBP, is increased after menopause (78, 220,233,234) and is influenced to a great extent by increased aortic PWV, Alx, aging, and obesity (78,233,235-239). Thus, reducing BP and wave reflection may result in important decreases in morbidity and mortality in our study population.

In this study, all three groups significantly decreased resting brachial and aortic SBP and DBP to a similar extent. Therefore, the addition of L-citrulline supplementation to a WBVT program does not produce additional decreases in brachial or aortic SBP and DBP after an 8 week period in this population. Although the beneficial effects of supplemental L-citrulline on arterial health are well known (30,31,47,50,240), previous studies have reported no significant impact of L-citrulline (either synthetic or from watermelon) supplementation on brachial SBP in individuals with prehypertension (30), in the normotensive young (241) and in middle-aged men.
This discrepancy between our results and previous studies may be due, in part, to the difference in study populations (obese women vs non-obese individuals) and the duration of L-citrulline consumption (8 weeks vs 6 weeks or less). In contrast, aortic SBP decreases after 6 weeks of L-citrulline supplementation in individuals with prehypertension (~5 mmHg) (30) and in hypertensive postmenopausal women (~10 mmHg) (31), which are in agreement with our findings in the L-citrulline group (~8 mmHg). This is clinically important because aortic BP is a marker of left ventricular load, and a more relevant predictor of cardiovascular events than brachial BP (222).

In accordance with our findings, Figueroa et al. found reductions on brachial and aortic SBP in overweight/obese normotensive women who underwent 6 weeks of WBVT (35). In addition, another study by our group found significant decreases in brachial and aortic SBP and DBP after 6 (37) and 12 (36) weeks of WBVT in obese postmenopausal women. The changes in aortic SBP (~10 mmHg) and DBP (~6 mmHg) after WBVT+L-citrulline and WBVT+Placebo interventions in our study are strikingly similar to our previous findings after WBVT in postmenopausal women (36,37). Furthermore, these decreases in aortic BP in our current and previous studies with WBVT are higher in magnitude compared to decreases in aortic SBP/DBP after 12 (~7 mmHg/5 mmHg) (242) and 20 weeks (~6 mmHg/3 mmHg) (243) of RT in postmenopausal women and older adults, respectively. It is important to point out that reducing SBP and DBP by 10 and 5 mmHg, can reduce the risk of death due to stroke by 40% and due to heart or other vascular causes by 30% (244). Moreover, each 20/10 mmHg increment over 115/75 mmHg doubles the risk of heart attack, heart failure and stroke in individuals 40-70 years old (244,245).

The pulse waves contain an initial incident wave (P1), which originates from the blood ejected during ventricular systole (stroke volume). P1 travels from the aorta to the peripheral arteries and meets bifurcations that are mainly at the small muscular arteries and arterioles...
and cause a second wave that is reflected back to the aorta (P2) during ventricular diastole (myocardial relaxation) in normal individuals. However, in older and diseased individuals with increased arterial stiffness, P2 arrives earlier to the aorta during late systole which causes increases in aortic SBP, and consequently increases the difference between the magnitudes of P1 and P2 (AP) and Alx (247,248). Therefore, pressure wave reflection can be examined by the Alx or in absolute amounts of AP. The increased Alx observed in our participants before they were assigned to the interventions may be explained by age, obesity and female gender (233,249,250), which has been associated with higher rates of cardiovascular morbidity and mortality (220,251) due to an increased left ventricular afterload and peripheral resistance (252). In the current study, Alx and Alx@75 were not affected by the L-citrulline intervention, despite reductions in the magnitude of P2. This is probably due to the decrease in PP, which along with P1 and P2, are the main determinants of Alx (57). Our findings are consistent with a previous study that demonstrated no effect of L-citrulline supplementation on Alx in postmenopausal women (31). Contrary to our findings, Alx and Alx@75 decreased after 6 weeks of L-citrulline supplementation in prehypertensives individuals (30). Although non-significant, the decreases in Alx@75 in the present study (~4 mmHg) are similar to the decreases in the previous study (30). Elevated Alx and aortic SBP are associated with AP (253) and P2 but not Tr in women (236). After the L-citrulline intervention, AP was reduced exclusively due to a decrease in P2. As no change in P1 and Tr occurred after L-citrulline, the decrease in P2 may explain the non-significant decreases (~4 mmHg) in Alx and Alx@75. Since AP shows a progressive increase with age, whereas Alx may decrease or plateau with older age (249,254), AP may be a more accurate indicator of wave reflection than Alx in middle aged and older adults, especially in women (254).

In contrast, the decreases in Alx found after WBVT+L-citrulline and WBVT+Placebo interventions in the present study are in agreement with our previous reports in premenopausal
and postmenopausal women (37) after WBVT. Also in the present study, AP and the magnitude of P2 were decreased while Tr increased and the magnitude of P1 was not affected in both the WBVT+L-citrulline and WBVT+Placebo interventions. Therefore, a concomitant decrease in the magnitude of P2 and an increase in the round-trip travel time of P1 to the peripheral reflecting sites and back to the aorta, may explain the reduction in AIx after both WBVT interventions.

An important finding in our study is that the combination of L-citrulline supplementation and WBVT produces additional decreases in AIx@75 which are significantly different from those seen in the placebo group, and almost double than those seen in the WBVT+Placebo group (Fig. 8B). Besides aortic SBP, P2 and Tr, the AIx can be negatively influenced by HR and aortic PWV (255-257). We noted no change in HR after the 3 interventions, while aortic PWV decreased in the WBVT+L-citrulline group only. Therefore, we can speculate that the additive benefits of WBVT+L-citrulline on AIx@75 are mediated by decreases in aortic stiffness.

**Endothelial Function**

Advancing age and estrogen withdrawal with menopause induce negative changes in endothelial function (66,258). Endothelial function may also be adversely affected by adiposity, and this is of great concern since the incidence of obesity is rising in older women (259). There is evidence that age, menopause and obesity may independently modify the risk of endothelial dysfunction and CVD beyond the traditional risk factors.

Endothelial dysfunction may be the early step in the development of atherosclerosis and occurs prior to any structural change in the arterial wall that leads to increased stiffness. The primary mechanisms of endothelial dysfunction include a decreased production and availability of NO, increase in the inactivation of NO by ROS, and an increase in vasoconstrictors such as ET-1 (260). In the current study we measured endothelial function by analyzing plasma NOx
levels (the sum of nitrite and nitrate), which represents the final and stable end-product of the metabolic pathway.

Although the improvement of endothelial function with exercise, as measured by non-invasive endothelium dependent vasodilation is well established (261-263), less is known about the effect of exercise training on NOx levels. In the current study, NOx levels increased by 39%, 36%, and 33% in the WBVT+Placebo, WBVT+L-citrulline, and L-citrulline groups, respectively. Consequently, the combination of WBVT with L-citrulline does not produce any additional effects on NOx levels.

NOx levels have been found to increase after 4-12 weeks of aerobic or RT (100,264-266), although this is not consistent in all studies (267,268). Previous investigations have reported increases in plasma NOx levels after 8-12 weeks of aerobic training in young healthy individuals (58%) (264) and postmenopausal women (54%) (265). Another study reported that 8 weeks of aerobic or RT increase plasma NOx levels by 23% and 19% in young prehypertensive individuals (100). In contrast, two previous studies have reported no changes in NOx levels after 6 and 12 weeks of RT in young healthy men (267,268). Differences in the training duration and age, but more importantly the health status of the study population may account for inconsistency of the results in the previous studies. However, a detailed discussion of these discrepancies may be beyond the scope of this investigation. Thus, while previous studies examined NOx levels after an aerobic or a RT intervention, this study provides the first examination of changes in plasma NOx after WBVT.

In the current study we observed increases in plasma NOx levels in the L-citrulline group. It is well known that L-citrulline is efficiently converted to L-arginine and consequently transformed to NO (47,48), suggesting and improved endothelial function (33,47,202). Only one previous study has investigated the effects of oral L-citrulline on NOx levels. In this investigation, Ochiai et al. reported that oral L-citrulline supplementation (~6g/day) for 7 days
increased plasma NOx levels by 37% in middle-aged men (mean age= 58 years) with increased arterial stiffness (50). This increase in NOx is very similar to the improvement noted in our study (33%) after L-citrulline supplementation alone. These findings indicate that L-citrulline supplementation may improve endothelial NO production in middle-aged adults with impaired arterial function. Therefore, it appears that the beneficial effects of oral L-citrulline supplementation on endothelial function are evident after short-term (1-8 weeks) consumption. Future trials might be needed in order to assess the long term efficacy of L-citrulline supplementation on endothelial function.

**Body Composition**

Our results do not support the use of 8 weeks of WBVT alone as an effective intervention for producing positive changes in body composition in overweight/obese postmenopausal women. This observation is in agreement with the findings of Tapp et al. who found no significant changes in total lean mass after 8 weeks of WBVT in obese postmenopausal women (175). In addition, recent projects by our group with overweight/obese pre and postmenopausal women found no impact of 6 weeks of WBVT on leg lean mass (35,37). In agreement with our finding, Verschueren et al. found no significant changes in total lean mass assessed by DXA after 6 months of WBVT in overweight postmenopausal women, which supports the lack of improvement in lean mass in the WBVT+Placebo group in the current study (45). Our results differ from those reported by Milanese et al. (38) and Figueroa et al. (35), who noted increases in total lean mass or leg lean mass in young non-obese (2%) and obese (3%) women after 6-8 weeks of WBVT. This discrepancy might be related to the study population characteristics (young non-obese vs obese postmenopausal women) in the present study compared to the previous study. Also in contrast to our results, Machado et al found increases in thigh cross sectional area in postmenopausal women after 10 weeks of WBVT (34),
although muscle hypertrophy was assessed by computed tomography which is known to be more sensitive to changes than DXA.

A novel finding of this study is that the WBVT+L-citrulline group saw an increase leg lean mass and total lean mass in comparison to baseline. Although the changes in the WBVT+L-citrulline group over time were not significantly different compared to the other groups, these changes have clinical significance since the increase in leg muscle mass of ~0.9 Kg (2 lb or 6%) seems to be important considering the short amount of time of the intervention. Since the increase in total lean mass is similar to the increase in leg lean mass (~0.9 Kg) we can concluded that total lean mass changed exclusively as an increase in lean mass in the legs. In addition, the increase in ASMI in the WBVT+L-citrulline group over time is as a result of an increase in leg lean mass. Of note, although the changes in leg and total lean mass were not different compared to the other groups, a moderate effect size was demonstrated (0.33), along with a P-value of 0.086. Perhaps replicating the study with a larger number of subjects would have resulted in significant values.

It appears that L-citrulline may exert its additive anabolic effect on WBVT through increases in L-arginine levels. L-arginine has been previously found to improve endothelial function, glucose uptake and to stimulate IGF-1 secretion (207) by the blockage of somatostatin (269). All of these, coupled with stimulus by WBVT may account for the improvement in leg muscle mass. Considering that leg blood flow declines with aging in part due to endothelial dysfunction and arterial stiffness, PWV may have a predictive role on muscle mass decline (19). Indeed, previous investigations by Suzuki et al. found that blood flow in the legs is negatively correlated with legPWV (18) and baPWV (270) in older patients. In addition, recent studies found a negative association between aortic PWV (19) or baPWV (21) with leg muscle mass in older individuals. Therefore, evidence points to a potential link between PWV and lean mass. In our study we demonstrated simultaneous increases in leg lean mass with decreases in aortic
PWV, legPWV and baPWV after the WBVT+L-citrulline intervention. This suggests that improvement in arterial stiffening may play a predicting role in counteracting the development of sarcopenia. Since older women have been shown to have lower levels of anabolic hormones, which limits their ability to increase lean mass with exercise (271), our findings offer WBVT combined with L-citrulline supplementation as a potential anti-sarcopenic therapy.

Our results also reflect those previously reported by our group in which there was no change in total fat mass in overweight/obese premenopausal women after 6 weeks of WBVT (35). In another study by our group, 12 weeks of WBVT did not change total body fat percentage in obese postmenopausal women (36). Our results are in agreement with previous studies by Roelants et al. who found no significant changes in body fat percentage after 8 weeks and 24 weeks of WBVT in postmenopausal (175) and young untrained women (39), respectively. Our results differ from those reported by Milanese et al., who noted significantly greater decreases in leg and total body fat mass in young non-obese women (38). However this discrepancy might be related to the study population as previously mentioned.

**Strength**

Existing findings from several studies indicate that WBVT positively impacts muscle strength in young individuals (35,38,39) as well as in older adults (41-45), including postmenopausal women (34,36,37,175). In line with our expectations, both the WBVT+L-citrulline and WBVT+Placebo groups increased leg strength. However, these increases were similar between groups. Thus, it appears that the addition of L-citrulline supplementation to a WBVT program does not elicit any benefits in muscle strength. The current study therefore re-emphasizes the notion that WBVT is an effective alternative to conventional resistance exercise programs to improve leg strength in untrained older females (39). Consistent with our present
findings, previous studies have shown increases in leg strength (~9-25 %) without significant changes in body composition after 6 (37), 8 (175),12 (36) and 24 weeks (44,45) of WBVT in postmenopausal women. Another study using RT in overweight postmenopausal women found significant improvement (~40%) in leg strength after 8 weeks of RT (272). Therefore, the increase in leg strength in the current study (WBVT+L-citrulline ~41% and WBVT+Placebo ~34%) may be explained by neural adaptations that typically occur within two months of beginning a RT program. These neural adaptations include increased ability for recruitment (128,129,133-135) of fast motor units (127), enhanced ability to coordinate muscle agonists (133-136) and reduced neural inhibition (137). Also, gains in muscle strength are associated with enhanced synchronization between motor cortex and spinal cord motor neurons after training (273). In addition, increased activity of the motor cortex causes the secretion of anabolic hormones such as growth hormone and testosterone that have been associated with improvements in neuromuscular function, muscle strength and mass (152) (179). These mechanisms may explain the increases in leg strength that occur throughout the study. In addition, the improvements seen in leg strength, but not upper body strength, in both groups performing WBVT in our study are likely the result of a decline in vibratory stimulus transmission across muscles and joints farther from the point of application of the vibration (274,275).

Muscle mass and strength are inversely related to aortic PWV, baPWV and AIx in older individuals (19-22,90). However, evidence suggests that RT may increase aortic PWV, legPWV, and baPWV (104,105). Our study corroborates previous findings by our group in which there were concomitant increases in leg strength with decreases in legPWV and AIx after WBVT (36,37), but not RT in postmenopausal women (229,242). Therefore, the findings of the present study suggest the use of WBVT to improve both muscle strength and vascular function in this population.
Limitations

Potential limitations of this study include a small sample size and the lack of measurements of plasma ET-1, catecholamines and anabolic hormones. We were only able to provide one mechanism (improved endothelial function through improved NOx levels) to explain our results without cardiac and vascular autonomic function measures. Our study evaluated cardiovascular function in postmenopausal women and hence we cannot generalize our results to other populations.

Future Directions

Previous studies have shown the effectiveness of WBVT and L-citrulline supplementation on vascular function. Our study adds to the current notion, by showing that besides improvements in vascular function the combination of WBVT and L-citrulline supplementation also has potential to improve aortic PWV and muscle mass. Future studies should evaluate the use of a combination treatment for reducing arterial stiffness, wave reflection and increasing muscle mass in populations at increased cardiovascular and sarcopenic risk such as older individuals with type II diabetes, obesity or heart disease. We propose the evaluation of the cardiac and vascular autonomic activity after the combination treatment, which could benefit the aforementioned populations and could explain some of the vascular improvements in our study. Finally, a longer research study may be needed in order to see significant changes in lean mass with the use of DXA, since most studies that used RT or WBVT in postmenopausal women for 12 weeks or less have produced little effects (36,37,175,227,276).

Conclusion

The present investigation was designed to define the hemodynamic, arterial stiffness, endothelial function, strength and body composition responses associated with the combination
of WBVT with L-citrulline supplementation. We accomplished this by evaluating the adaptations to 8 weeks of L-citrulline supplementation, WBVT or its combination in overweight/obese postmenopausal women. We showed here that WBVT combined with L-citrulline supplementation decreases both brachial and aortic BP, legPWV, baPWV and AIx but does not amplify these changes as opposed to our specific aim 1 working hypothesis. However, the present study demonstrates that the combination of WBVT and L-citrulline supplementation causes decreases in aortic PWV, which was not accomplished by either intervention alone. In addition, the increases NOx levels with all 3 groups show that endothelial function is in part responsible for the protective effects of WBVT and L-citrulline on arterial function; which is in accordance with our specific aim 2 working hypothesis.

Since our study was not limited to examine vascular adaptations, we evaluated the effectiveness of the combination treatment on body composition. Although most body composition parameters did not change, the data indicates that 8 weeks of WBVT combined with L-citrulline supplementation increases leg muscle mass. Even though this increase (0.9 Kg, ~2 lbs) was not significantly different compared (time effect only) to the WBVT or L-citrulline alone, it may be clinically significant since it demonstrates the anti-sarcopenic effects of the combined intervention. Therefore, WBVT combined with L-citrulline may be a feasible adjuvant treatment to provide vascularprotective and anti-sarcopenic effects in overweight/obese postmenopausal women.
Day: 3/7/2013
To: Arturo Figueroa, Alexei Wong
Address: 120 Convocation Way 1493
Dept.: Nutrition Food and Exercise Sciences
From: Thomas L. Jacobson, Chair
Re: Use of Human Subjects in Research
The effect of whole-body vibration training and L-cit supplementation on cardiovascular, autonomic function and body composition in postmenopausal women
The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 01/09/2013. Your project was approved by the Committee. The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.
If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.
If the project has not been completed by 1/8/2014 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.
You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others. By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations. This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is FWA00000168/IRB number IRB00000446.
Cc: Bahram Arjmandi <barjmandi@fsu.edu>, Chair
HSC No. 2012.9674
APPENDIX B

INFORM CONSENT

The effect of whole-body vibration training combined with L-cit supplementation on cardiovascular, autonomic function and body composition in postmenopausal women

INFORMED CONSENT FORM

1. I voluntarily and without element of force or coercion, consent to be a participant in the research study entitled “The effect of whole-body vibration training combined with L-cit supplementation on cardiovascular, autonomic function and body composition in postmenopausal women”. This study is being conducted by Dr. Arturo Figueroa and Alexei Wong MS, who are associated with The Florida State University in the Department of Nutrition, Food & Exercise Sciences.

2. The purpose of the study is to examine the chronic effects of 8 weeks of whole body vibration training combined with L-citrulline supplementation on arterial stiffness, wave reflection, endothelial function, cardiac autonomic responses and body composition in women 50-70 years of age with resting blood pressure below 160/100 mmHg.

3. My participation in this study will require coming to the Cardiovascular Physiology Laboratory at the Florida State University 5 testing sessions plus 36 training sessions to complete the experiments described below. I am aware that I cannot participate in this study if I answered yes to any of the exclusion criteria.

4. On the first visit, I will be oriented on the study, answer questions on my medical/exercise history, and to sign an informed consent. If I qualify, I will have my blood pressure, height, weight, skinfold, and waist circumference measured. I will have a familiarization session with laboratory measurements and will learn how to perform exercises in the vibration platform. The first visit should take approximately 60 minutes.

5. On the second visit, I will undergo body composition testing by dual energy X-ray absorptiometry (DXA). Thereafter, I will perform an 8 repetition maximum (8-RM) strength test. Once I complete the warm-up I will be progressed towards a maximal weight that I can lift eight times through a full range of motion for the leg press and chest press exercises.

6. On visit 3, I will have blood samples drawn and my cardiovascular function evaluated. Upon arrival, blood samples (about 4 tablespoons) will be drawn from a vein in the forearm by an experienced phlebotomist to measure nitric oxide metabolites, endothelin-1, IGF-1, leptin, adiponectin, insulin, glucose, and 8-isoprostane. Next I will undergo resting cardiovascular measurements. A total of 4 cuffs (around arms and ankles) and 2 tonometers (sensors applied to the skin to obtain pulse waves), one on the neck and the second on the inner thigh, will be used to measure pulse wave velocity (arterial stiffness). My blood pressure will be also monitored by placing a small cuff around the middle finger of the left hand and an additional pencil like tonometer on the wrist. Six electrocardiogram electrodes will be positioned on the skin of my chest and forearms to measure heart rate. Arm and leg blood flow will be measured
using vascular ultrasound positioned on my skin at rest and during increased blood flow after deflation of an arm cuff or leg cuff (5 minutes inflation). A pneumatic cuff will be placed around the upper third of my forearm and leg and inflated at least 50 mmHg above my systolic blood pressure for 5 minutes. Following the blood flow measurements, I will perform an exercise session on a vibration platform. The exercise session consists of 5 repetitions of a 1 minute squat exercise separated by 1 minute rest periods. Following the exercise session on the vibration platform, a second collection of blood samples (about 1 teaspoon) will be drawn.

On visit 4, my strength and body composition will be reevaluated as described on visit 2, and cardiovascular and endothelial function will be reevaluated as described on visit 3. I will be assigned to one of 4 groups: Control or L-citrulline with no WBVT or WBVT+L-citrulline or WBVT+Placebo. I will take 8 capsules of L-citrulline (750 mg each) or placebo per day for 8 weeks. Oral L-citrulline at the doses recommended in our study are safe and well tolerated. L-citrulline is an amino acid that is naturally produced in the body in small amounts and also found in some foods like watermelon, pumpkin and squash. Oral supplementation of L-citrulline has been shown to increase levels of L-arginine, in turn, L-arginine is converted to nitric oxide which is a potent vasodilator and antihypertensive substance. I will not change my lifestyle (diet and exercise) during the 8 week study period. At the end of the 8 weeks (visit 5), all the measurements conducted on visit 4 will be repeated.

7. I understand there is a possibility of a minimal level of risk involved if I agree to participate in this study. The risks associated with the non-invasive cardiovascular tests are minimal because they are obtained using devices applied on the skin. Blood pressure cuff inflation to 50 mmHg over my resting blood pressure may cause discomfort numbness, bruising, tingling and pain. The risk associated with body composition measurements by DXA is minimal and with no biological effects because the x-ray radiation received during the scan is less than that of an airline flight from California to New York. The risks associated with whole body vibration are minimal and the selected amplitudes have been previously used in other studies using human subjects. These include erythema (redness of the skin due to increased local blood flow), itching, and edema (swelling) of the legs. If the effects are severe, the frequency and or the amplitude of WBV may be reduced. If the problem persists, I will stop training, and will withdraw from the study. Oral L-citrulline at the doses recommended in our study are safe and well tolerated. In our previous study using L-citrulline from watermelon supplementation, none of the subjects complained from abdominal discomfort or diarrhea. Citrulline should not be taken concurrently with glutamine or glutamine-fortified products (whey proteins) and arginine-rich foods (nuts and chocolate). Citrulline is not recommended for those who have asthma, glaucoma or herpes simplex or have suffered heart attacks. I will complete a medical/exercise history before I can participate in the study.

8. The possible benefits of my participation in this study include learning about my cardiovascular health and how L-citrulline and/or WBVT may improve my cardiovascular, muscular function, and body composition (decrease fat mass and increase muscle mass). I will also be given a number of cardiovascular tests, L-citrulline supplementation and training at no charge.

9. The result of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number.
The only record with the subject’s name and code number will be kept by the principal investigator (Dr. Arturo Figueroa) in a locked drawer in his office. Data will be kept for 10 years and then destroyed.

10. I will not be paid for my participation in this study. In case of an injury, first aid (free of charge) will be provided to me by the laboratory and lifeguard personnel working on the research project. However, any other treatment or care will be provided at my expense.

11. Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Alexei Wong at [contact information] or Roy Kalfon at [contact information] or Dr. Arturo Figueroa at [contact information], for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

12. In case of an injury, or if I have questions about my rights as a subject/participant in this research, or I feel I have been placed at risk, I can contact the chair of the human subject committee (humansubjects@magnet.fsu.edu), Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633.

13. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

14. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies.

_________________________________________________
Subject Date
APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Cardiovascular/Exercise History

ID# __________
DATE __________

Answer the following questions, indicating the month and year of the event or diagnosis where appropriate.

1. Has a doctor ever told you that you have heart disease? ___ ___ ____/____

2. Have you ever had a heart attack? ___ ___ ____/____

3. Have you ever had chest pain? ___ ___ ____/____

4. Have you ever had cardiac catheterization? ___ ___ ____/____

5. Have you ever had balloon angioplasty? ___ ___ ____/____

6. Have you had coronary artery bypass graft surgery? ___ ___

If yes, list date and number of grafts:
7. Have you ever had a stroke? ___  ____/____

8. Do you have hypertension (high blood pressure)? ___  ____/____

If yes, how long have you had hypertension?

____ less than 1 year
_____ 1-5 years
_____ 6-10 years
_____ more than 10 years

9. Do you have diabetes mellitus? ___  ____/____

Yes No Month/Year

10. Do you take insulin for diabetes? ___ ___

If yes, how long have you taken insulin?

_____ less than 1 year
11. Do you take oral hypoglycemics for diabetes?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

12. Do you have a cardiac pacemaker?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

   If yes, how long have you had a cardiac pacemaker?  
   ______ less than 1 year  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

13. Have you had a carotid endarterectomy?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

14. Has your doctor ever told you that you have a heart valve problem?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

15. Have you had heart valve replacement surgery?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years

   If yes, what heart valves were replaced?  
   ______ mitral  
   ______ aortic

16. Have you had cardiomyopathy?  
   ______ 1-5 years  
   ______ 6-10 years  
   ______ more than 10 years
17. Have you had a heart aneurysm? ___ ___ ____/____

18. Have you had heart failure? ___ ___ ____/____

19. Have you ever suffered cardiac arrest? ___ ___ ____/____

20. Do you smoke?  Yes ___    No ___
    If yes, how long have you smoked   _____ years
    How many cigarettes per day   _____ per day
    If you stopped smoking, when did you do it?   _____ years ago

21. Are you pregnant?            Yes___ No____

22. Are you using any hormonal contraceptive method?  Yes___ No ____

23. Have you had vasculitis problems or Raynaud's phenomenon (finger blanching with cold exposure)
    ___ ___ ____/____

24. Are you taking medications for high blood pressure?
    Yes___ No____
    Name of drug                   Dosage Times/Day Duration of use
    _____          _____          _____            _____

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25. Other medical problems: Indicate if you have had any of the following medical problems:

<table>
<thead>
<tr>
<th>Past</th>
<th>Now</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>___</td>
<td>___</td>
<td>Alcoholism</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Allergies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anemia</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Arthritis</td>
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<tr>
<td>___</td>
<td>___</td>
<td>Asthma</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Back injury or problem</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Blood clots</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Bronchitis</td>
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<tr>
<td>___</td>
<td>___</td>
<td>Cirrhosis</td>
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<tr>
<td>___</td>
<td>___</td>
<td>Claudication</td>
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<td>___</td>
<td>___</td>
<td>Elbow or shoulder problems</td>
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<tr>
<td>___</td>
<td>___</td>
<td>Emotional disorder</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Eye problems</td>
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<tr>
<td>___</td>
<td>___</td>
<td>Gall bladder disease</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Glaucoma</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Gout</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Headaches</td>
</tr>
<tr>
<td>___</td>
<td>___</td>
<td>Hemorrhoids</td>
</tr>
</tbody>
</table>
Hernia
Herpes simplex
Hip, knee, or ankle problems
Intestinal disorders
Kidney disease
Liver disease
Lung disease
Mental illness
Neck injury or problem
Neuralgic disorder
OB/GYN problems
Obesity/overweight
Osteoporosis
Parkinson's disease
Phlebitis
Prostate trouble
Rheumatic fever
Seizure disorder
Stomach disease
Thyroid disease
Tumors or cancer - List type: ____________
Ulcers
Other - specify: ________________

List other medications / supplements you are taking below:

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Dosage</th>
<th>Times/day</th>
<th>Duration of drug use</th>
</tr>
</thead>
</table>

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Exercise

Do you exercise with weights regularly?

How long have you trained with weights?

Do you experience fatigue at the end of the set?

Do you do whole body routines?

If no, how do you divide your body to exercise with weights?

How many days per week do you train with weights?

How much aerobic (cardiovascular) exercise (walking, running, cycling, swimming, etc) do you do?

Days/week:

Duration in min/day:

Intensity of the workouts: low, moderate, high
How long have you trained with cardiovascular activities?

Have you interrupted your training for more than 2-3 weeks in the last year?

If yes, when was it?

Please describe the workouts of last week

   Number of repetitions per set, number of sets per exercise, and exercises

MONDAY

TUESDAY

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

SUNDAY
REFERENCES


BIOGRAPHICAL SKETCH

Alexei Wong

Education

2014  Ph.D in Exercise Physiology  
Florida State University, Tallahassee, Florida

2009  Master of Science in Exercise Physiology  
Florida International University, Miami, Florida

2007  Bachelor of Science in Applied Physiology and Kinesiology  
University of Florida, Gainesville, Florida

Professional Experience

01/2012-present  Research Assistant  
Cardiovascular Physiology Laboratory  
Department of Nutrition, Food and Exercise Sciences  
Florida State University. Tallahassee, Florida

09/2009-present  Instructor  
Department of Nutrition, Food and Exercise Sciences  
Florida State University. Tallahassee, Florida

02/2009-08/2009  Strength and Conditioning Coach  
Washington Nationals Baseball Club  
Washington Nationals Spring Training Complex. Viera, Florida

09/2007- 02/2009  Exercise Specialist/Wellness Coordinator  
Doral Golf Resort and Spa. Miami, Florida

05/2007-08/2009  Intern in strength and conditioning  
University of Florida Athletic Association. Gainesville, Florida

Activities

08/2006–12/2006  Volunteer  
Taught kids with mental disabilities in service of developing basic motor skills  
Terwilliger Elementary School, Gainesville, Florida
Awards

College of Human Sciences Dissertation Award Program (2014)

Florida State University Dissertation Research Grant Award (2013)

College of Human Sciences Research and Creativity Day Third Place Award (2012)

Scientific Publications


**Manuscripts Under Review**

1. Wong A, Figueroa A. The effects of stretching training in cardiac autonomic function in obese postmenopausal women.

**Manuscripts in Preparation**


**Abstracts and Presentations**


**Reviewership**

Journal of Human Hypertension

**Courses Taught**

PET 3102 Introduction to Exercise Sciences
PET 3322 Applied Anatomy and Physiology I
PET 3322L Applied Anatomy and Physiology I Laboratory

Professional affiliations

National Strength and Conditioning Association (NSCA)
American College of Sports Medicine (ACSM)

Certifications

Certified Strength and Conditioning Specialist (CSCS) through the NSCA
CPR and First Aid Certified through the American Heart Association (AHA).

Foreign Languages

Fluent in Spanish
Fluent in Russian