Impact of L-citrulline supplementation and whole-body vibration training on arterial stiffness and leg muscle function in obese postmenopausal women with high blood pressure

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Abstract

Aging is associated with increased arterial stiffness (pulse wave velocity, PWV) and muscle strength/mass loss. Exercise training alone is not always effective to improve PWV and lean mass (LM) in older women. To investigate the independent and combined effects of whole-body vibration training (WBVT) and L-citrulline supplementation on PWV and muscle function in women, forty-one postmenopausal women aged 58 ± 3 years and body mass index (34 ± 2 kg/m²) were randomly assigned to the following groups: WBVT, L-citrulline, and WBVT + L-citrulline for 8 weeks. WBVT consisted of four leg exercises three times weekly. Aortic (cfPWV) and leg (faPWV) PWV, leg LM index, leg strength, and body fat percentage (BF%) were measured before and after the interventions. WBVT + L-citrulline decreased cfPWV (−0.91 ± 0.21 m/s, P < 0.01) compared to both groups. All interventions decreased faPWV (P < 0.05) similarly. Leg LM index increased (2.7 ± 0.5%, P < 0.001) after 31 WBVT + L-citrulline compared with L-citrulline. Both WBVT interventions increased leg strength (−37%, P < 0.001) compared to L-citrulline while decreased BF% (−2.0%, P < 0.01). Reductions in cfPWV were correlated with increases in leg LM index (r = −0.63, P < 0.05). Our findings suggest that leg muscle strength and arterial stiffness can be improved after WBVT, but its combination with L-citrulline supplementation enhanced benefits on arterial stiffness and leg LM. Therefore, WBVT + L-citrulline could be an intervention for improving arterial stiffness and leg muscle function in obese postmenopausal women with prehypertension or hypertension, thereby reducing their cardiovascular and disability risk.

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Each visit, participants were asked to abstain from caffeine and alcohol consumption for 24 h. Participants were also asked to discontinue medication the night and morning before testing. Cardiovascular measurements were collected during the same time of day (± 1 h) in the morning following an overnight fast and between 48 and 72 h following the last exercise session. Participants rested in the supine position for at least 10 min before data collection in a quiet temperature-controlled room (23 °C ± 1 °C). Women were asked not to make any changes in their regular lifestyle other than the assigned intervention.

2.3. Interventions

Participants in the WBVT and WBVT + L-citrulline groups completed supervised training 3 times a week separated by at least 48 h for 8 weeks. Participants performed four static and four dynamic leg exercises on a vibration platform (pro5 AIRdaptive; Performance Health Systems, Northbrook, IL). Considering 180° as full knee extension, exercises were squats at 90° and 120° knee angle with normal stance, squats at 120° knee angle with wide stance, and calf raises with minimal heel elevation. Static exercises were performed maintaining the joint angles without movement (Machado et al., 2010). Dynamic exercises were performed with slow controlled movements starting from an upright position to the assigned knee angles (90° and 120°) described for the three types of squat and also moving the heels from maximal elevation to below the platform for the calf raises. The movement during dynamic exercises was controlled by the use of a metronome at a rate of 3 s eccentric/2 s concentric phases. The training volume was progressed 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 s), number of sets (1–5), total duration of the training session (11–60 min), and decreasing the duration of rest periods (60–30 s). The training protocol was adapted from others previously used in postmenopausal and obese women (Figueroa et al., 2012, 2014b; Machado et al., 2010).

Participants in the L-citrulline and WBVT + L-citrulline groups were supplemented with 6 g of L-citrulline/day. The L-citrulline was ingested in the form of 750 mg capsules corresponding to 4 capsules (3 g) before breakfast and 4 capsules before sleeping. Likewise, the WBVT group consumed 4 capsules of maltodextrin before breakfast and 4 capsules before sleeping to match the number of capsules ingested daily by the WBVT + L-citrulline group. The selected dose and times of ingestion were based on previous studies with L-citrulline or watermelon supplementation that showed decreases in BP, arterial stiffness, and wave reflection magnitude (Figueroa et al., 2013b; Ochiai et al., 2012; Schwedhelm et al., 2008). The last dose of L-citrulline and placebo was ingested 48 h prior to the last visit in order to avoid any possible acute vascular effects of L-citrulline. Participants self-recorded supplementation logs on a weekly basis. Participants were required to return the logs and unused capsules after 4 and 8 weeks to verify their adherence to the supplementation. Compliance was calculated by dividing the consumed capsules by the expected number of capsules.
2.5. Body composition

Body weight and height were measured to the nearest 0.1 kg and 1 cm using a beam scale (Sunbeam Products Inc., Boca Raton, FL) and stadiometer, respectively. BMI was computed as weight in kg/height in m². Leg LM, arm LM, appendicular LM (ALM = arms LM + legs LM), leg LM index (LM / total mass), and body fat mass percentage (BF%) were evaluated using whole-body dual-energy X-ray absorptiometry (DXA) scans (GE Lunar DPX-IQ, Madison, WI). Appendicular LM index (ALMI) was calculated as ALM/height in m².

2.6. Muscle strength

The eight-repetition maximum test (8RM), defined as the maximum weight that can be moved in good form eight times through a full range of motion, was used to assess muscle strength (MedX Corp., Ocala, FL, USA). The 8RM has been shown to be safe and reliable in postmenopausal women (Figueroa et al., 2013a, 2014a; Phillips et al., 2010).

2.7. Statistical analysis

The Shapiro–Wilks test was used to examine data normal distribution. Based on previous data (Donley et al., 2014; Figueroa et al., 2014b), we calculated that 12 participants per group would provide 80% power (two-sided α = 0.05) to detect a 9% and 7.5% reduction in cfPWV and faPWV. One-way analysis of variance (ANOVA) was used to examine group differences at baseline. Changes in dependent variables were evaluated by a 3 × 2 ANOVA with repeated measures (group [WBVT, L-citrulline, and WBVT + L-citrulline] × time [before × after 8 weeks]). When a significant group-by-time interaction and/or time effect was identified, between-groups and within-group comparisons were performed using Tukey’s test and paired t-test, respectively. P < 0.05 was considered statistically significant. Pearson’s correlation coefficients were used to examine associations between changes in cfPWV and indices of muscle function (leg LM index and strength). Data analysis was performed using SPSS version 21.0 (SPSS, Chicago, IL, USA). Data are presented as mean ± SE.

Table 1: Participants’ characteristics at baseline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBVT</th>
<th>L-CIT</th>
<th>WBVT + L-CIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Age</td>
<td>58 ± 1</td>
<td>58 ± 1</td>
<td>58 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.9 ± 2.8</td>
<td>83.8 ± 2.3</td>
<td>88.3 ± 3.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.60 ± 0.01</td>
<td>1.60 ± 0.01</td>
<td>1.62 ± 0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.0 ± 0.9</td>
<td>33.0 ± 0.8</td>
<td>33.8 ± 1.1</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>141 ± 3</td>
<td>137 ± 4</td>
<td>140 ± 3</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>89 ± 2</td>
<td>77 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>Arm LM (kg)</td>
<td>5.0 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Leg LM (kg)</td>
<td>16.0 ± 0.8</td>
<td>14.8 ± 0.5</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>Leg strength (kg)</td>
<td>238 ± 19</td>
<td>227 ± 15</td>
<td>233 ± 14</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

WBVT, whole-body vibration training; L-Cit, L-citrulline supplementation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; and LM, lean mass.

3.3. Body composition and muscle strength

Body weight, BMI, arm LM, and arm strength did not change following the three interventions. WBVT + L-citrulline increased leg LM (6.0 ± 2.2%, P < 0.05) and ALMI (4.8 ± 1.8%, P < 0.05), but WBVT and L-citrulline did not affect leg LM (0.7 ± 1.1% and 0.8 ± 2.6%) and ALMI (Table 2). There were group–by–time interactions for leg LM index (P < 0.05) and leg strength (P < 0.001). Leg LM index increased (P < 0.001) following 8 weeks of WBVT + L-citrulline (Table 2 and Fig. 1a) compared to no changes after the other 2 interventions. Leg muscle strength increased (P < 0.001 for both) after both WBVT interventions compared to a no significant change after L-citrulline (Fig. 1b). BP decreased after WBVT (~2.0 ± 0.6%, P < 0.05) and WBVT + L-citrulline (~1.9 ± 0.7%, P < 0.01), but not after L-citrulline supplementation (Table 2).

There was a negative relationship between the changes in cfPWV and leg LM index (r = −0.63, P < 0.05). No significant relationship was found between changes in cfPWV and leg strength.

4. Discussion

The main findings of our study were that L-citrulline supplementation added to WBVT reduced cfPWV and increased leg LM index in obese postmenopausal women with prehypertension or stage-1 hypertension. Moreover, the three interventions decreased systemic and leg arterial stiffness as measured by baPWV and faPWV.

Increased cfPWV leads to systolic hypertension, preferentially in older women (Berry et al., 2004), and to higher risk for cardiovascular events and mortality (Vlachopoulos et al., 2010). The annual increase in cfPWV is ~1.47 m/s in treated hypertensive adults (Benetos et al., 2002). We observed that cfPWV was reduced by 0.91 m/s (7.6%) after 8 weeks of WBVT + L-citrulline supplementation, corresponding to ~62% of the annual increase observed in middle-aged hypertensive adults (Benetos et al., 2002). Because each 1 m/s increase in cfPWV corresponds to a 14% increase in cardiovascular events (Vlachopoulos et al., 2010), our finding is relevant for the reduction of cardiovascular risk in postmenopausal women with prehypertension and hypertension. The majority of the studies have shown that aerobic training (Monteiro et al., 2014b) and RT (Collier et al., 2008) do not reduce cfPWV in middle-aged and older adults with prehypertension or hypertension and in obese individuals (Monteiro et al., 2014a,b). In contrast, reduction of cfPWV (~1.0 m/s) has been reported following 8 weeks of aerobic training in middle-aged overweight/obese adults with high SBP (Donley et al., 2014), suggesting that cfPWV can be positively modified by short-term exercise training if the appropriate stimulus is provided to a population with high cardiovascular risk. Interestingly, some studies have shown that cfPWV was reduced (~0.4–0.6 m/s) following 9–16 weeks of aerobic training in middle-aged and older adults treated with antihypertensive drugs including vasodilators (Guimaraes et al., 2018).
mentation reduced baPWV and SBP (Figueroa et al., 2013b). Therefore, in agreement with the result of our WBVT alone, 8 to 12 weeks of RT did not reduce cfPWV in postmenopausal women (Casey et al., 2007; Figueroa et al., 2013a; Rossow et al., 2014), although some variation may exist in this response. The inability of short-term exercise training to reduce cfPWV in older adults with hypertension might be explained by a difficulty to reverse age related structural changes in the aortic wall including altered collagen and elastic fibers content (Zieman et al., 2005). It appears that cfPWV is specifically resistant to decrease in older hypertensive adults (Vogel et al., 2013) due to accelerated arterial aging (Benetos et al., 2002).

Women experience a progressive increase in baPWV after the menopause (Zaydun et al., 2006). We found that our three interventions caused similar reductions in systemic (baPWV) and leg (faPWV) PWV. Consistent with our current findings, previous studies have reported that 6–12 weeks of WBVT reduced baPWV in obese normotensive older women (−0.9 m/s) (Figueroa et al., 2012), obese prehypertensive and hypertensive postmenopausal women (−1.3 m/s) (Figueroa et al., 2014b), and non-obese normotensive older adults (−0.7 m/s) (Lai et al., 2014). Moreover, previous studies have reported decreases in baPWV (−1.1 m/s) following l-citrulline or l-citrulline-rich watermelon supplementation (Figueroa et al., 2013b; Ochiai et al., 2012). Similarly, vasodilatory drugs can reduce baPWV (Takami and Shigemasa, 2003) without affecting cfPWV in older adults with hypertension (Mackenzie et al., 2009). Since baPWV is determined by cfPWV and faPWV (Yamashina et al., 2002), and only faPWV was decreased in the present study, our findings indicate that l-citrulline supplementation and WBVT exerted their systemic vascular effects through a reduction in faPWV.

We have previously reported a reduction in faPWV after 12 weeks of WBVT in obese older women with prehypertension or hypertension (Figueroa et al., 2014b). This positive effect of WBVT on faPWV might be clinical relevant as increased leg arterial stiffness is associated with systolic hypertension (Choo et al., 2014; Wohlfahrt et al., 2013) abdominal adiposity and insulin resistance (Park et al., 2010). Of clinical concern, RT has shown to be inefficient to reduce faPWV in overweight and obese older women (Casey et al., 2007; Figueroa et al., 2013a; Rossow et al., 2014), but more importantly, it has increased faPWV and cfPWV in middle-aged adults with prehypertension and untreated stage-1 hypertension (Collier et al., 2008).

Increased PWV is associated with sarcopenia in the legs (Abbatecola et al., 2012; Ochi et al., 2010). We used the leg LM index (lean mass / total mass) because our WBVT exclusively targeted leg muscles. In the present study, leg LM index increased with WBVT + l-citrulline by increasing LM without changing fat mass. Because adiposity is associated with an accelerated loss of leg LM (0.02 kg/year) in older women (Koster et al., 2011), our findings are in accordance with previous reports that increased LM is associated with reduced PWV (Rossow et al., 2014). Moreover, previous studies have reported decreases in PWV by a difference of 0.9 m/s following L-citrulline supplementation (Figueroa et al., 2012), obese prehypertensive and hypertensive postmenopausal women (−0.9 m/s) (Figueroa et al., 2014b), and non-obese normotensive older adults (−0.7 m/s) (Lai et al., 2014). However, previous studies have reported decreases in PWV (−1.1 m/s) following l-citrulline or l-citrulline-rich watermelon supplementation (Figueroa et al., 2013b; Ochiai et al., 2012). Similarly, vasodilatory drugs can reduce PWV (Takami and Shigemasa, 2003) without affecting cfPWV in older adults with hypertension (Mackenzie et al., 2009). Since baPWV is determined by cfPWV and faPWV (Yamashina et al., 2002), and only faPWV was decreased in the present study, our findings indicate that l-citrulline supplementation and WBVT exerted their systemic vascular effects through a reduction in faPWV.
may reduce sarcopenia-related physical disability in obese postmeno-
pausal women. In contrast, WBVT alone had no apparent effect on leg
LM index. Indeed, this is not surprising since no muscle hypertrophy
has been reported in postmenopausal women after 10 to 24 weeks of
RT (Figueroa et al., 2013a; Phillips et al., 2010; Verschueren et al.,
2004), which is considered the most effective exercise modality for im-
provements in muscle function. It could be that 8 weeks of WBVT is too
short and/or the intensity is low (20 repetitions/set) (Tapp and
Signorile, 2014) for increasing leg LM in obese middle-aged women
(Milanese et al., 2013).

A greater decrease in leg muscle strength than LM occurs in older
women (Koster et al., 2011); thus, strength training may prevent mus-
cle weakness and disability in older women. In the present study, leg
muscle strength increased following 8 weeks of WBVT (33.4%) and WBVT + l-citrulline (41.0%). Since leg muscle loss is 2.6% per year in older women (Koster et al., 2011), the 7.6% difference be-
tween both WBVT interventions in strength gain might correspond to
the reversal of muscle strength loss that is experienced in 3 years.

These findings confirm that WBVT is an effective strengthening alterna-
tive for postmenopausal women (Figueroa et al., 2014b; Machado et al.,
2010; Tapp and Signorile, 2014; Verschueren et al., 2004). The lack of
variation between the changes in leg muscle strength and the three
PWV parameters indicates that muscle strength gain is not a determi-
nant for the improvements in PWV observed with WBVT. Nevertheless,
because cardiovascular disease risk increases by 23% in obese adults
with reduced muscle mass, but not in those with normal strength
(Stephen and Janssen, 2009), improvements in leg muscle strength
with WBVT may reduce cardiovascular risk in obese postmenopausal
women by reducing physical disability. It is known that overweight
and obese older adults with high strength levels have reduced all-
cause mortality (Stenholt et al., 2014) emphasizing the importance
of strength training. Our study demonstrated that improvements in
leg muscle strength and leg PWV coexist in postmenopausal women
after WBVT.

Aortic stiffening is determined by structural characteristics of the arte-
rial wall (Zieman et al., 2005) and impaired endothelial NO-dependent
vasodilation (Campbell et al., 2011). Both WBVT and l-citrulline supple-
mentation have shown to increase circulating NO (Humphries et al.,
2009; Ochiai et al., 2012) and reduce baPWV (Figueroa et al., 2012,
2014b; Ochiai et al., 2012). Although the exact underlying mechanism
for the increase in leg LM index is unclear, it is possible that the increased
leg muscle perfusion through NO-induced vasodilation may contribute
to muscle mass gains (Dillon et al., 2011).

Our study is limited by a relatively small sample size, although our
findings were both statistically and clinically significant. Moreover,
our participants were overweight–obese postmenopausal women
with prehypertension or stage-1 hypertension, and thus, the findings
of this study may not apply to a population with different age, sex,
and health condition. Because the increase in vascular reactivity appears
to occur earlier than the reduction in cPWV with strength training in
some postmenopausal women (Rossow et al., 2014), interventions lon-
ger than 8 weeks are needed to clarify the effect of WBVT on PWV and
lean mass.

5. Conclusion

In conclusion, 8 weeks of combined WBVT and l-citrulline supple-
mentation improved aortic stiffness and muscle function in obe-
se postmenopausal women. Although 8 weeks of l-citrulline
and WBVT alone reduced leg arterial stiffness, l-citrulline had no ef-
fect on muscle function while WBVT increased muscle strength but
not LM index. Therefore, WBVT + l-citrulline supplementation
could be an alternative intervention for counteracting the effects of
aging and obesity on arterial stiffness and leg sarcopenia in postmen-
opausal women.


