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# L-Citrulline supplementation attenuates blood pressure, wave reflection and arterial stiffness responses to metaboreflex and cold stress in overweight men

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## Abstract

Combined isometric exercise or metaboreflex activation (post-exercise muscle ischaemia (PEMI)) and cold pressor test (CPT) increase cardiac afterload, which may lead to adverse cardiovascular events. L-Citrulline supplementation (L-CIT) reduces systemic arterial stiffness (brachial-ankle pulse wave velocity (baPWV)) at rest and aortic haemodynamic responses to CPT. The aim of this study was to determine the effect of L-CIT on aortic haemodynamic and baPWV responses to PEMI + CPT. In all, sixteen healthy, overweight/obese males (age 24 (SEM 6) years; BMI 29·3 (SEM 4·0) kg/m$^2$) were randomly assigned to placebo or L-CIT (6 g/d) for 14 d in a cross-over design. Brachial and aortic systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP), aortic augmented pressure (AP), augmentation index (AIx), baPWV, reflection timing (Tr) and heart rate (HR) were evaluated at rest and during isometric handgrip exercise (IHG), PEMI and PEMI + CPT at baseline and after 14 d. No significant effects were evident after L-CIT at rest. L-CIT attenuated the increases in aortic SBP and wave reflection (AP and AIx) during IHG, aortic DBP, MAP and AIx during PEMI, and aortic SBP, DBP, MAP, AP, AIx and baPWV during PEMI + CPT compared with placebo. HR and Tr were unaffected by L-CIT in all conditions. Our findings demonstrate that L-CIT attenuates aortic blood pressure and wave reflection responses to exercise-related metabolites. Moreover, L-CIT attenuates the exaggerated arterial stiffness response to combined metaboreflex activation and cold exposure, suggesting a protective effect against increased cardiac afterload during physical stress.

**Key words: Metaboreflex activation: Cold pressor test: Isometric exercise: Arterial stiffness: Wave reflection: Aortic blood pressure: L-Citrulline supplementation**

Excessive systolic blood pressure (SBP) reactivity to the cold pressor test (CPT), through increased sympathetic activity, may predict the development of hypertension in young, healthy adults[1,2]. A greater sympathetic reactivity to the CPT has been demonstrated in overweight than in lean adults despite similar brachial blood pressure (BP) responses[2]. However, brachial BP has lower sensitivity to the CPT than aortic BP[3]. In young, normotensive adults, CPT increases aortic BP, wave reflection and arterial stiffness (pulse wave velocity (PWV))[4,5]. An increased BP reactivity to CPT may predict future increases in PWV[6], which precedes the development of hypertension in obesity[7].

Isometric handgrip exercise (IHG) also elicits a sympathetic-mediated increase in BP[8] known as the exercise pressor response. The BP response to IHG is determined by neural signals from the brain (central command) and active muscles (mechanoreceptors and metaboreceptors)[8,9]. Vascular responses to muscle metaboreflex activation can be isolated from central command and mechanoreceptor influences by trapping metabolites in the previously exercised muscle via arterial occlusion (post-exercise muscle ischaemia (PEMI))[8–10]. Exaggerated BP and vasoconstrictor responses to PEMI have been shown in young, obese adults with pre-hypertension[11].

Acute increases in aortic haemodynamics and PWV are exaggerated during concurrent IHG and CPT (cold-pain stimulus) in young, healthy adults[5,12]. Similarly, we recently reported that PEMI with concurrent CPT (PEMI + CPT) caused greater increases in aortic haemodynamics and PWV than PEMI alone in young, healthy, overweight/obese men[13]. As the combined stimuli of exercise and cold may increase the risk for adverse cardiovascular events in susceptible individuals by elevating myocardial work[14], therapies targeted to attenuate cardiac afterload during stress would be cardioprotective.

L-Arginine is the substrate for endothelial nitric oxide (NO) production, a potent vasodilator. L-Citrulline supplementation

**Abbreviations:** AIx, augmentation index; AP, augmented pressure; baPWV, brachial-ankle pulse wave velocity; BP, blood pressure; CPT, cold pressor test; DBP, diastolic blood pressure; IHG, isometric handgrip exercise; L-CIT, L-citrulline supplementation; MAP, mean arterial pressure; NO, nitric oxide; PEMI, post-exercise muscle ischaemia; PWV, pulse wave velocity; SBP, systolic blood pressure; Tr, transit time of the reflected wave.

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(L-CIT) increases plasma L-arginine and NO levels more efficiently than L-arginine supplementation[15,16]. At rest, L-CIT has reduced BP, wave reflection and PWV in middle-aged adults with increased BP and/or arterial stiffness[17,18], but not in young, healthy men[4]. L-CIT, either synthetic or from watermelon, has attenuated aortic haemodynamic responses to CPT in young, normotensive[4] and older, hypertensive adults[19]. Yet, the possible benefits of L-CIT on vascular reactivity during metaboreflex activation remain unknown.

The aim of this study was to determine the effects of L-CIT on aortic haemodynamics and PWV responses to stress in healthy, young men. We hypothesised that L-CIT would attenuate vascular reactivity to PEMI with and without CPT, although the benefits would be more apparent during combined stress.

## Methods

### Study population

In total, sixteen overweight/obese (BMI >25 and <40 kg/m$^2$) healthy males (18–35 years) participated in this study. Participants were non-smokers, sedentary (<120 min/week of exercise) and free of overt chronic diseases. Exclusion criteria included brachial systolic BP (SBP) ≥140 mmHg and use of medications and/or any supplements that may affect outcome variables. Participants were asked to maintain their ordinary diet and exercise habits. The study was approved by the Institutional Human Subjects Committee, and participants gave their written informed consent before participation.

### Study design

This was a randomised, double-blind, placebo-controlled study. Participants were familiarised with the study protocols. Cardiovascular parameters were evaluated in a quiet, temperature-controlled room (22–24°C) after an overnight fast and abstinence from beverages containing caffeine or alcohol for 12 h and from intense or prolonged physical activity 24 h before testing.

After 15 min of supine rest, cardiovascular parameters were measured in duplicate and averaged at rest and during the last minute of IHG and PEMI without CPT. Following 20 min of recovery, cardiovascular parameter measurements were repeated at rest, IHG and PEMI + CPT. The trial order was randomised. After baseline measurements, participants were randomly assigned to placebo (maltodextrin) or L-CIT for 14 d, followed by a 14-d washout period, and were then crossed over to the other supplementation. Measurements were repeated 14 d after both periods.

### Measurements

*Anthropometrics*. Body weight and height were measured using a Seca Scale and stadiometer (Sunbeam Products) to the nearest 0·1 kg and 0·01 m, respectively. Waist circumference was measured using a tape measure to the nearest 0·5 cm at the superior border of the iliac crest.

*Haemodynamics*. An automated oscillometric device (HEM-705CP; Omron Healthcare) was used to record brachial BP. These values were used to calibrate radial pressure waveforms obtained from a 10 s epoch with a high-fidelity tonometer (SPT-301B; Millar Instruments). Aortic waveforms were derived from a generalised transfer function (SphygmoCor, AtCor Medical) and composed of forward (P1) and reflected (P2) waves. Augmented pressure (AP = P2–P1) expressed as a percentage of the aortic pulse pressure gives the augmentation index (AIx). Transit time of the reflected wave (Tr) indicates the round-trip travel of the forward wave to the peripheral reflecting sites and return to the aorta. Tr and AIx have been used as markers of aortic stiffness and wave reflection, respectively. The intraclass correlation coefficient for aortic haemodynamics in our laboratory calculated on two separate days was ≥0·94.

*Arterial stiffness*. Following a 15-min rest period, PWV was evaluated using a device (VP-2000; Omron Healthcare) composed of BP cuffs positioned around the arms (brachial artery) and ankles (posterior tibial artery). Waveforms were measured simultaneously by cuff sensors, whereas the transient time was calculated automatically by relating the foot of the forward wave to the R-wave of the electrocardiogram. The distance between sampling points was calculated automatically according to the participant's height. Brachial-ankle pulse wave velocity (baPWV) was measured as the time it takes for the pulse wave to travel from the arm sensor to the ankle sensor, divided by the distance between the sensors (m/s). Heart rate (HR) was measured from the electrocardiogram. baPWV was not measured during IHG because of the difficulty in collecting pulse waveforms in the exercising arm.

*Exercise and metaboreflex activation*. The highest of three maximal compressions using a handgrip dynamometer (Lafayette Instrument Co.) was considered the maximal voluntary contraction (MVC). IHG was performed with the dominant arm at 30 % of MVC for 2 min using a handgrip dynamometer. Visual feedback and verbal encouragement were provided to maintain the targeted grip force; 10 s before completion of the IHG, a cuff positioned proximally on the exercising forearm was inflated to suprasystolic levels (200 mmHg or at least 50 mmHg above the brachial SBP during IHG) for 3 min with an automated pneumatic device (Hokanson E20; Hokanson, Inc.).

*Cold pressor test*. The left foot of participants was passively immersed into ice-cold water (4°C) for 3 min during the PEMI + CPT trial. Participants were instructed to maintain consistent breathing rate/depth throughout the test.

*L-Citrulline supplementation*. Participants were instructed to ingest four capsules of 750 mg L-citrulline or placebo (NOW Foods) twice a day (before breakfast and sleep) for 14 d. To avoid acute vascular effects of L-CIT, the last capsule was ingested 48 h before the 2-week testing. Participants returned unused capsules and records to assess their compliance.

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*Statistical analyses*. On the basis of previous data[4], it was calculated that sixteen participants would provide 85 % power (two-sided $\alpha = 0{\cdot}05$) to detect a 7·5 % decrease in brachial SBP during PEMI + CPT after L-CIT. Statistical analyses were conducted using SPSS 21.0. Normality was confirmed by the Shapiro–Wilk test for all measurements. An independent *t* test was used to assess possible between-group differences at baseline. Separate two-way repeated-measures ANOVA was performed to determine differences within and between interventions in cardiovascular parameters in the four conditions: rest, IHG, PEMI and PEMI + CPT. When a significant group-by-time interaction was identified, paired *t* tests were used to assess within-group differences between baseline and 2 weeks. Data are presented as mean values with their standard errors. Statistical significance was set at $P < 0{\cdot}05$.

## Results

Participant characteristics are presented in Table 1. Weight, BMI and waist circumference did not change significantly after both supplementations. Cardiovascular parameters before and after the supplementations are presented in Tables 2 and 3. There were no differences between supplementations at baseline and no significant changes were detected after 2 weeks.

During IHG, L-CIT decreased brachial SBP ($P < 0{\cdot}05$), diastolic blood pressure (DBP) ($P < 0{\cdot}05$) and mean arterial pressure (MAP) ($P < 0{\cdot}05$) as well as aortic SBP ($P < 0{\cdot}01$), AP ($P < 0{\cdot}001$) and AIx ($P < 0{\cdot}001$) compared with the placebo group (group-by-time interactions $P < 0{\cdot}05$). L-CIT had no effect on HR, aortic DBP, aortic MAP and Tr.

During isolated PEMI, L-CIT reduced brachial DBP ($P < 0{\cdot}05$) and aortic DBP ($P < 0{\cdot}01$), MAP ($P < 0{\cdot}05$) and AIx ($P < 0{\cdot}001$) compared with placebo (group-by-time interaction $P < 0{\cdot}05$). HR, brachial SBP and MAP, aortic SBP, AP, Tr and baPWV (Fig. 1) were not affected by L-CIT.

During PEMI + CPT, L-CIT decreased brachial SBP ($P < 0{\cdot}01$), DBP ($P < 0{\cdot}001$) and MAP ($P < 0{\cdot}001$) as well as aortic SBP ($P < 0{\cdot}001$), DBP ($P < 0{\cdot}001$), AP ($P < 0{\cdot}001$), AIx ($P < 0{\cdot}001$) and baPWV (Fig. 1, $P < 0{\cdot}05$) compared with placebo (group-by-time interaction $P < 0{\cdot}05$). L-CIT did not affect HR and Tr.

## Discussion

The main findings of our study are that L-CIT attenuated (1) aortic BP and AIx responses to IHG, PEMI and PEMI + CPT and (2) baPWV response to PEMI + CPT but not to PEMI alone in young, healthy, overweight/obese men. These findings suggest that L-CIT effectively buffered exercise- and cold-induced vascular reactivity.

**Table 1.** Study participant characteristics
(Mean values with their standard errors; *n* 16)

| Variable | Mean | SEM |
|---|---|---|
| Age (years) | 24·0 | 2·0 |
| Height (m) | 1·7 | 0·0 |
| Body weight (kg) | 86·8 | 3·7 |
| BMI (kg/m$^2$) | 29·3 | 1·1 |
| Waist circumference (cm) | 98·0 | 3·0 |

L-CIT failed to reduce resting cardiovascular parameters in healthy, overweight/obese, young men. These findings are consistent with previous data showing unapparent effects of L-CIT on resting BP and wave reflection in young, normotensive, lean men due to normal BP and arterial function[4]. In contrast, Bailey *et al.*[15] recently reported a decrease in resting brachial BP in young, normotensive men after 1 week of L-CIT. It is possible that these results could reflect an acute vascular effect as L-citrulline was ingested 90 min before the BP measurement. Cardiovascular parameters were measured 48 h after the last L-citrulline dose to assess the chronic rather than the acute vascular effects. We have reported unapparent vascular effects of L-CIT in normotensive or pre-hypertensive individuals[4,20]. In contrast, L-CIT and watermelon supplementation have reduced resting BP in pre-hypertensive[20] and hypertensive adults[18,19] as well as resting baPWV in middle-aged and older adults[17,18].

L-CIT attenuated brachial BP and wave reflection responses to IHG. Previous studies have reported that treatment with nebivolol, which exerts a vasodilator effect via endothelial NO production, reduced MAP responses to IHG in hypertensives[21]. These findings suggest that improved peripheral vasodilation attenuates the exercise pressor response. During PEMI following IHG, sympathetic-mediated peripheral vasoconstriction maintains SBP and MAP higher than at rest but similar to that during IHG[8,9]. In healthy, young men, the BP response to PEMI is attributed to increases in peripheral resistance[9] and stroke volume[22,23]. In the present study, IHG and PEMI elevated brachial SBP to 145 and 135 mmHg, respectively, at baseline. We found that L-CIT did not reduce SBP during PEMI, suggesting that L-CIT is less effective in reducing a small SBP response to PEMI in young, healthy men. Importantly, L-CIT reduced aortic DBP and wave reflection (AP and AIx) during PEMI, which may indicate a decreased vasomotor tone. Previous studies have demonstrated that acute NO-donor or antihypertensive therapy acutely decreases resting DBP and wave reflection magnitude independently of reflection timing (Tr) via reduction in vascular tone in the small arteries but not in the aorta[24,25]. Therefore, despite persistent elevation of sympathetic activation during PEMI[8], L-CIT may counteract the increase in vasomotor tone during metaboreflex activation in young, healthy men.

Young, normotensive, overweight men have an exaggerated sympathetic reactivity to CPT[2], which may be a mechanism for cold-induced hypertension[14]. The CPT evokes a sympathoexcitation-mediated hypertensive response through skin cooling and pain sensation[14]. Previous studies have shown that haemodynamic responses are greater during combined stimulation by IHG or PEMI with cold exposure compared with IHG or PEMI alone in young, healthy adults[5,12]. Similarly, we found greater haemodynamic response to PEMI + CPT compared with isolated PEMI in young men[13].

We noted attenuation of haemodynamic responses to PEMI + CPT after L-CIT. Consistent with these findings, we previously reported reductions in aortic SBP and AIx responses to CPT or whole-body cold exposure following 2–4 weeks of

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**Table 2.** Haemodynamic parameters before and after supplementation (Mean values with their standard errors; *n* 16)

| | | Placebo (*n* 16) | | | | L-CIT (*n* 16) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Before | | After | | Before | | After | | |
| Variables | Conditions | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Group differences† |
| Heart rate (beats/min) | Rest | 58 | 1 | 59 | 2 | 58 | 2 | 60 | 2 | 0·51 |
| | IHG | 70 | 3 | 76 | 3 | 74 | 3 | 72 | 3 | 0·03 |
| | PEMI | 57 | 2 | 58 | 2 | 57 | 2 | 57 | 2 | 0·75 |
| | PEMI + CPT | 67 | 3 | 65 | 2 | 66 | 3 | 67 | 2 | 0·39 |
| Brachial SBP (mmHg) | Rest | 122 | 2 | 124 | 2 | 123 | 3 | 121 | 3 | 0·20 |
| | IHG | 139 | 3 | 144 | 4 | 145 | 4 | 136* | 4 | 0·00 |
| | PEMI | 132 | 2 | 138* | 3 | 135 | 2 | 131 | 3 | 0·01 |
| | PEMI + CPT | 145 | 3 | 145 | 3 | 150 | 3 | 138** | 3 | 0·00 |
| Brachial DBP (mmHg) | Rest | 67 | 1 | 69 | 1 | 68 | 2 | 64 | 2 | 0·03 |
| | IHG | 76 | 2 | 79 | 3 | 76 | 3 | 65* | 4 | 0·00 |
| | PEMI | 72 | 2 | 74 | 2 | 72 | 2 | 68* | 2 | 0·01 |
| | PEMI + CPT | 80 | 2 | 80 | 2 | 88 | 2 | 72*** | 2 | 0·00 |
| Brachial MAP (mmHg) | Rest | 88 | 2 | 92 | 2 | 90 | 2 | 87 | 2 | 0·02 |
| | IHG | 106 | 3 | 111 | 5 | 102 | 4 | 94* | 4 | 0·01 |
| | PEMI | 98 | 2 | 100 | 2 | 98 | 2 | 95 | 2 | 0·09 |
| | PEMI + CPT | 107 | 2 | 105 | 2 | 109 | 2 | 96*** | 2 | 0·00 |
| Aortic SBP (mmHg) | Rest | 105 | 2 | 106 | 2 | 104 | 3 | 101 | 2 | 0·10 |
| | IHG | 121 | 3 | 125 | 5 | 126 | 3 | 118** | 3 | 0·00 |
| | PEMI | 117 | 3 | 121 | 4 | 120 | 3 | 116 | 4 | 0·07 |
| | PEMI + CPT | 127 | 4 | 129 | 4 | 132 | 4 | 119*** | 4 | 0·00 |
| Aortic DBP (mmHg) | Rest | 69 | 1 | 69 | 2 | 68 | 2 | 66 | 2 | 0·41 |
| | IHG | 77 | 2 | 78 | 3 | 78 | 3 | 81 | 3 | 0·80 |
| | PEMI | 75 | 2 | 75 | 2 | 77 | 2 | 71** | 2 | 0·04 |
| | PEMI + CPT | 80 | 2 | 82 | 2 | 84 | 2 | 75*** | 3 | 0·00 |
| Aortic MAP (mmHg) | Rest | 84 | 2 | 85 | 2 | 84 | 2 | 81 | 2 | 0·22 |
| | IHG | 98 | 3 | 101 | 4 | 101 | 3 | 98 | 3 | 0·13 |
| | PEMI | 94 | 3 | 95 | 2 | 96 | 2 | 89* | 3 | 0·02 |
| | PEMI + CPT | 102 | 3 | 103 | 3 | 105 | 3 | 95*** | 3 | 0·00 |

L-CIT, L-citrulline; IHG, isometric handgrip; PEMI, post-exercise muscle ischaemia; PEMI + CPT, PEMI with concurrent cold pressor test; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$ *v.* before.
† Group × time interaction from ANOVA.

**Table 3.** Wave reflection parameters before and after supplementation (Mean values with their standard errors; *n* 16)

| | | Placebo (*n* 16) | | | | L-CIT (*n* 16) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Before | | After | | Before | | After | | |
| Variables | Conditions | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Group differences† |
| AP (mmHg) | Rest | 1·6 | 0·9 | 2·3 | 0·9 | 2·4 | 0·8 | 1·7* | 1·0 | 0·01 |
| | IHG | 7·5 | 1·1 | 7·4 | 1·2 | 10·4 | 1·8 | 2·1*** | 1·0 | 0·00 |
| | PEMI | 7·9 | 1·3 | 10·6 | 2·0 | 9·4 | 2·2 | 6·4 | 2·0 | 0·04 |
| | PEMI + CPT | 8·0 | 2·2 | 8·9 | 2·1 | 11·1 | 2·0 | 3·8*** | 1·5 | 0·01 |
| AIx (%) | Rest | 5·8 | 2·3 | 6·1 | 2·7 | 7·9 | 1·7 | 7·6 | 2·0 | 0·47 |
| | IHG | 16·1 | 2·2 | 15·1 | 2·0 | 18·9 | 2·7 | 5·3*** | 2·7 | 0·00 |
| | PEMI | 17·9 | 2·2 | 21·1 | 3·2 | 18·4 | 3·6 | 9·7*** | 3·5 | 0·00 |
| | PEMI + CPT | 14·6 | 3·7 | 17·6 | 3·8 | 20·8 | 3·4 | 7·3*** | 3·0 | 0·00 |
| Tr (ms) | Rest | 164·3 | 6·4 | 171·4 | 5·6 | 170·8 | 6·4 | 170·8 | 5·3 | 0·47 |
| | IHG | 155·2 | 5·3 | 148·2 | 3·6 | 154·6 | 6·1 | 156·9 | 4·3 | 0·37 |
| | PEMI | 159·3 | 7·5 | 148·6 | 5·8 | 155·0 | 5·7 | 150·6 | 3·5 | 0·63 |
| | PEMI + CPT | 149·7 | 5·1 | 144·2 | 2·7 | 149·9 | 4·7 | 152·2 | 2·1 | 0·31 |

L-CIT, L-citrulline; AP, augmentation pressure; IHG, isometric handgrip; PEMI, post-exercise muscle ischaemia; PEMI + CPT, PEMI with concurrent cold pressor test; AIx, augmentation index; Tr, transit time of the reflected wave.
* $P<0·05$, *** $P<0·001$ *v.* before.
† Group × time interaction from ANOVA.

L-CIT in young, healthy men[4]. Similarly, L-citrulline from watermelon attenuated CPT-induced increases in aortic BP and AP, but not in AIx, in older, obese adults with hypertension. An explanation for this discrepancy could be that AP is a better index of wave reflection at rest[26] and during PEMI[27] in older adults[26]. On the other hand, AIx is a reliable measure of

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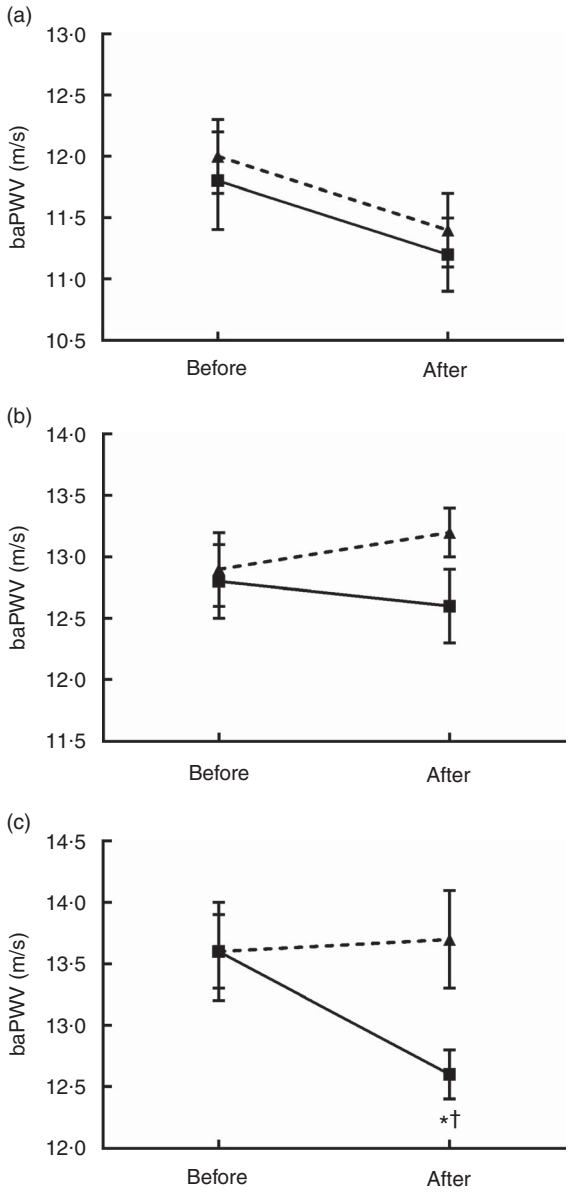


**Fig. 1.** Brachial-ankle pulse wave velocity (baPWV) at rest (a), during post-exercise muscle ischaemia (PEMI) (b) and PEMI + cold pressor test (c) before and after placebo (- -▲- -) and L-citrulline (—■—) supplementation. Values are means with their standard errors. * $P < 0{\cdot}05$ *v.* before; † $P < 0{\cdot}05$ *v.* placebo.

wave reflection in young adults[26]. Collectively, these findings indicate that L-CIT reduces cardiac afterload during cold exposure[19,28], and our present findings add to the existing knowledge that L-CIT has a cardioprotective effect during concurrent PEMI and cold exposure. Our findings may be important for the prevention of adverse cardiac events in individuals with increased cardiovascular risk, as cold stimulus combined with exercise, but not cold alone, may increase the risk of myocardial ischaemia due to excessive cardiac afterload[14].

Previous studies have shown that IHG or PEMI with concurrent CPT increases PWV by 1·2–1·8 m/s in young men[5,13]. In the present study, L-CIT attenuated the baPWV response to PEMI + CPT by 1·06 m/s, whereas L-CIT was ineffective in reducing baPWV during isolated PEMI. At baseline, baPWV levels during PEMI with and without CPT were 13·6 and 12·8 m/s, respectively. Subsequently, L-CIT reduced baPWV during PEMI + CPT to relatively the same absolute level as that evoked during isolated PEMI. To the best of our knowledge, the efficacy of L-CIT on the PWV response to cold exposure has not been previously examined. A previous investigation has reported that the anti-stiffening effect of L-CIT on resting baPWV without cold exposure was attributed to an increased NO availability[17]; 1-week L-CIT and 6-week watermelon supplementation reduced baPWV by 1·2–1·3 m/s in middle-aged adults with a baseline baPWV >14 m/s[17,18]. In the present study, only PEMI + CPT elevated baPWV close to this level, suggesting that L-CIT is able to exert a beneficial effect in conditions with increased PWV. Cold exposure[29] and vasoconstrictor drugs (angiotensin II)[24] predominantly increase peripheral (brachial) PWV. It is well known that femoral-ankle PWV (faPWV) is the largest peripheral arterial segment in baPWV[30] and is increased by PEMI[10]. As Tr, an estimate index of aortic stiffness, was not affected by L-CIT, our findings suggest that L-CIT may have reduced faPWV compared with carotid-femoral PWV (cfPWV).

The present findings could be important for individuals with hypertension and heart failure, as they have an exaggerated sympathetically mediated haemodynamic response to exercise due to metaboreflex overactivation[8,31]. In heart failure patients, PEMI-induced increase in BP may lead to reduced exercise tolerance due to increased myocardial work[31]. Evidence suggests that L-CIT improves resting BP and left ventricular function in heart failure patients[32]. Future studies are needed to examine the effects of L-CIT on haemodynamic responses to exercise and PEMI in individuals with metaboreflex overactivation.

The exact mechanism by which L-CIT counteracts vascular reactivity to physical stress is unclear. Recent evidence supports that L-citrulline is a NO precursor, as its conversion to L-arginine leads to endothelial NO synthesis[15,16]. In young men, the increase in MAP during PEMI following IHG may have been secondary to vasoconstriction and not to increase in stroke volume[9]. BP and AIx reductions by vasodilator drugs including NO donors is through a decrease in vascular smooth muscle tone of the peripheral arteries[24,25]. Although PWV is influenced by distending pressure (MAP), changes in PWV lead to changes in BP[7]. Therefore, attenuated peripheral vasoconstriction during physical stress may account for our findings.

Our study is limited by the lack of measurement of stroke volume and plasma L-arginine and NO levels. We did not measure the main components of baPWV (cfPWV and faPWV) because of technical limitations (hip was passively flexed to submerge the foot in cold water). However, baPWV is highly correlated with cfPWV[30], and increased values of both indices are similarly associated with cardiovascular risk factors[33]. Our participants were young, healthy men and the present findings may not be translated to other populations. Finally, the multiple comparisons performed in our study are a statistical concern because they may lead to false-positive significant findings.

In conclusion, our findings demonstrate that 2-week L-CIT attenuated aortic BP, wave reflection and systemic arterial stiffness responses to stress induced by exercise-related

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metabolites and local pain/cold exposure. In addition, the vascular protective effect of L-CIT during metaboreflex activation was more pronounced during cold exposure.

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None of the authors has any conflicts of interest to declare.

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