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ORIGINAL

Acute citrulline oral supplementation induces greater post-exercise hypotension response in hypertensive than normotensive individuals

Suplementação oral aguda de citrulina induz maior resposta hipotensiva pós-exercício em indivíduos hipertensos do que normotensos

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ABSTRACT

Objective

To investigate whether acute citrulline supplementation might influence post-exercise hypotension in normotensive and hypertensive individuals.

Methods

Following a randomized double-blind design, twenty normotensive (28±7 years, 74±17kg, 1.7±0.09m) and 20 hypertensive individuals (55±12 years, 76±15kg, 1.59±0.09m) were randomly assigned to one of the four

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experimental groups (Normotensive-Placebo; Normotensive-Citrulline; Hypertensive-Placebo; Hypertensive-Citrulline). The placebo groups ingested 6g of corn starch and the citrulline groups ingested 6g of citrulline dissolved in water. The participants performed 40 minutes of walking/running on a treadmill at 60-70% heart rate reserve. Blood pressure was measured immediately after a 60-min exercise session using an oscillometric device and 24-h ambulatory monitoring.

Results

The post-exercise hypotension was more pronounced in hypertensives and the Hypertensive-Citrulline group showed a consistent systolic blood pressure reduction during the laboratorial phase, which can be seen by looking at the mean of 60 minutes (-15.01mmHg *vs* -3.14mmHg [*P*=0.005]; -4.16mmHg [*P*=0.009]; -6.30mmHg [*P*=0.033] in comparison with the Normotensive-Placebo, Normotensive-Citrulline, and Hypertensive-Placebo groups, respectively). During ambulatory blood pressure monitoring, the Hypertensive-Citrulline group showed a significant reduction in systolic blood pressure (-21.05mmHg) in the awake period compared with the Normotensive-Citrulline group (-3.17mmHg [*P*=0.010]).

Conclusion

Acute citrulline oral supplementation can induce greater post-exercise hypotension response in hypertensive than normotensive individuals.

Keywords: Blood pressure. Citrulline. Dietary supplements. Exercise. Hypertension.

RESUMO

Objetivo

Investigar se a suplementação aguda de citrulina pode influenciar a hipotensão pós-exercício em indivíduos normotensos e hipertensos.

Métodos

Seguindo delineamento duplo-cego randomizado, vinte indivíduos normotensos (28±7 anos, 74±17kg, 1,7±0,09m) e 20 hipertensos (55±12 anos, 76±15kg, 1,59±0,09m) foram randomizados e distribuídos em um dos quatro grupos experimentais (Normotenso-Placebo; Normotenso-Citrulina; Hipertenso-Placebo; Hipertenso-Citrulina). Os grupos placebo ingeriram 6g de amido de milho e os grupos citrulina 6g de citrulina, dissolvidos em água. Os participantes realizaram 40 minutos de caminhada/corrida em esteira a uma intensidade entre 60-70% da frequência cardíaca de reserva. A pressão arterial foi aferida imediatamente após a sessão de exercício por 60 minutos usando um equipamento oscilométrico, e durante 24 horas por monitorização ambulatorial.

Resultados

A hipotensão pós-exercício foi mais pronunciada nos hipertensos e o grupo Hipertenso-Citrulina mostrou uma redução consistente da pressão arterial sistólica durante a fase laboratorial, o que pode ser visto pela média de 60 minutos (-5,01mmHg vs -3,14mmHg [P=0,005]; -4,16mmHg [P=0,009]; -6,30mmHg [P=0,033] em comparação com o Normotenso-Placebo, Normotenso-Citrulina e Hipertenso-Placebo, respectivamente). Durante a monitorização ambulatorial da pressão arterial, o Hipertenso-Citrulina demonstrou uma redução significativa na pressão arterial sistólica (-21,05mmHg) durante a vigília em comparação com o Normotenso-Citrulina (-3,17mmHg [P=0,010]).

Conclusão

A suplementação oral aguda de citrulina induz maior resposta hipotensiva pós-exercício em indivíduos hipertensos do que normotensos.

Palavras-chave: *Pressão arterial. Citrulina. Suplementos alimentares. Exercício. Hipertensão.*

INTRODUCTION

Post-Exercise Hypotension (PEH) is defined as a sustained reduction in Blood Pressure (BP) after a single bout of exercise [1] and this transient reduction can last up to 24h after completion of an exercise session [2]. PEH is an important physiological event in both hypertensive [3] and pre-hypertensive individuals [4] due to its association with the

chronic reduction in resting BP provided by physical training. In this sense, subjects who exhibit a greater drop in BP after an exercise session (acute effect) can present a greater reduction in resting BP after training (chronic effect). Thus, PEH suggests the possibility of predicting responders and non-responders to BP adjustments after exercise training [5].

PEH is related to several peripheral hemodynamic changes, including reductions in total peripheral resistance and cardiac output [6]. Nevertheless, reports appear to vary from normotensive to hypertensive individuals. Previous studies reported that hypertensive individuals present cardiac output reduction [7,8] and normotensives present total peripheral resistance reduction [7,9,10]. A large body of evidence has demonstrated a critical role of Nitric Oxide (NO) in blood pressure regulation. Released from endothelial cells, NO increases 3'5'-Cyclic-Guanosine Monophosphate (cGMP) production and subsequent cGMP-dependent protein kinase activation in vascular smooth muscle cells, resulting in vasodilation [11,12].

Related to this point, several studies have persuasively demonstrated that citrulline (a nonessential amino acid) has a key role in the arginine-nitric oxide system, increasing NO bioavailability [13], and that chronic citrulline supplementation decreased total peripheral resistance, implying in a reduction in mean arterial pressure and diastolic pressure [14]. Thus, it is possible that PEH could be more pronounced after citrulline supplementation.

Additionally, blood pressure responses may be different as PEH can be caused by different mechanisms between normotensive and hypertensive individuals. Therefore, we could expect that single dose of citrulline oral supplementation may acutely contribute to improving the peripheral vasodilation mechanisms in hypertensive individuals, resulting in greater PEH magnitude and/or duration.

Accordingly, the aim of the present study was to investigate whether acute citrulline supplementation might influence PEH in normotensive and hypertensive individuals. We hypothesized that the PEH would be more pronounced in hypertensive individuals after citrulline supplementation.

METHODS

Participants

After sample size calculation (see statistical analysis section), 40 individuals (20 normotensives and 20 hypertensives) who were sedentary (less than 150 minutes per week of moderate physical activities) participated in the study. Volunteers were adult women or men without osteoarticular disabilities and medical clearance to exercise. Participants were recruited from a community-based outreach exercise program offered by the university that provided stretching and functional exercise sessions to the outside community. The study followed the Declaration of Helsinki and was approved by the Research Ethics Committee of the university (78697617.4.0000.0108). All participants were informed about the methods before giving written informed consent.

Study design

This was an acute, randomized, double-blind, placebo-controlled study to evaluate the effects of citrulline supplementation on PEH in normotensive and hypertensive individuals. The participants were randomly allocated (using a random number table - https://www.random.org/) into four different experimental groups (Normotensive-Placebo [NP]; Normotensive-Citrulline [NC]; Hypertensive-Placebo [HP]; Hypertensive-Citrulline [HC]). Supplementation was performed in a double-blind design. The participants ingested a sachet containing citrulline (6 grams) or placebo (6 grams of cornstarch) dissolved in 120mL of water.

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The substances were ingested 120 minutes before the experimental session. Anthropometric measures were taken before the rest period. The experimental session consisted of a 5-min warm up (50% of 65% HR reserve) and 40 minutes of running/walking at 60-70% HR reserve on a treadmill. This was followed by a progressive speed reduction until the subject was walking, which lasted 5 minutes (cooldown).

After the exercise sessions, the blood pressure was measured every 10 minutes over a course of 60 minutes. The participants were then allowed 15 minutes to take a shower and change their clothes before the ambulatory blood pressure device was attached to their arm. The ambulatory blood pressure was recorded for 24 hours. The participants were asked to return to the laboratory the following day to remove the device.

The schematic representation of the experimental design is shown in Figure 1. Data collection occurred between January and July 2018. The study protocol was registered in ClinicalTrials.gov (NCT03378596).

Anthropometry

Weight was measured using a digital anthropometric scale (Urano, OS180A, *Canoas*, Brazil), with an accuracy of 0.1kg and height was measured using a stadiometer with an accuracy of 0.1cm, according to the procedures described by Gordon *et al*. [15]. The Body Mass Index (BMI) was established by dividing body weight (kg) by squared height. The waist circumference (midpoint between the iliac crest and last rib) was determined using an inextensible tape (Sanny®, *São Paulo*, Brazil) with an accuracy of 0.001m.

Office Blood Pressure (laboratorial phase)

Office blood pressure was measured using an oscillometric monitor (Omron MX3 Plus, Bannockburn, United States) previously validated for clinical measures in adults [16]. First the participants remained seated (rest period) in a calm, quiet, and thermoneutral (22°-24°C) environment for 20min. The blood pressure was measured three times during the rest period (at 10min, 15min, and 20min). The mean value of the three blood pressure measurements was used as the resting blood pressure value. Immediately following the sessions (exercise or control), the blood pressure was measured

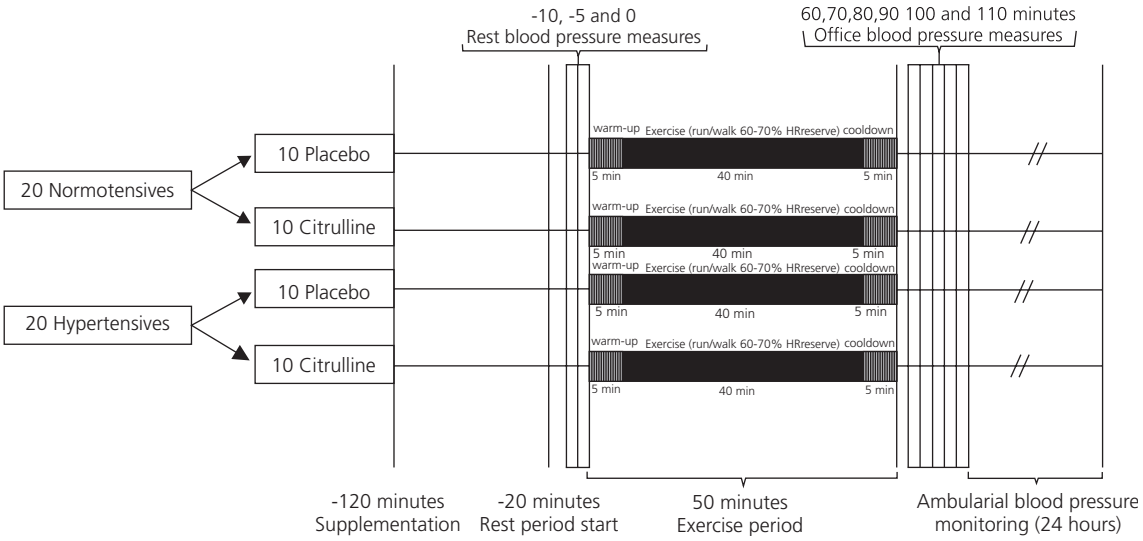

Figure 1. Schematic representation of experimental design. *Londrina* (PR), Brazil, 2018.

in a quiet environment for 60 minutes. The blood pressure measurements were taken in accordance with the American Heart Association recommendations [17].

Ambulatory blood pressure monitoring (ambulatory phase)

The ambulatory blood pressure was measured using an oscillometric device (Dyna-MAPA, *São Paulo*, Brazil) attached to the left arm, always by the same investigator, in accordance with the procedures described by the American Heart Association [17]. The participants received instructions to hold their arm outstretched during the measurements. The device was calibrated by direct comparison with a mercury sphygmomanometer by a trained technical person, in agreement with recommendations [17].

The monitor was set to record the systolic and diastolic blood pressure and heart rate every 20 minutes, except during the nighttime (23h00min to 08h00min) when the readings were taken every 30 minutes to minimize sleep disturbance. The device screen was blinded to the subject to avoid feedback. All participants were instructed to record and report their sleep time in a diary the following day.

The data were stored in the device memory and then transferred to a computer using a specific software (Aplicação Dyna Mapa, version 5.0.382.12, *São Paulo*, Brazil) for analysis. The average of valid readings was above 90% for all participants. The delta between the rest period and mean arterial blood pressure monitoring (awake, asleep, and 24 hours) was calculated for analysis.

Statistical analysis

A total of 7 subjects per group would be needed to detect a minimum difference of 7mmHg [18] with 80% power and an alpha of 5%, assuming a Standard Deviation (SD) of 5mmHg for the systolic blood pressure.

The data are reported as mean and standard error. One-way Analysis of Variance (ANOVA) was used to compare the characteristics of participants.

To compare the absolute values between experimental groups, Mauchly's test of sphericity was applied first followed by the Greenhouse-Geisser correction if necessary. Next, these data were compared with a one-factor repeated measures General Linear Model (GLM). Fisher's multiple comparison test was used to examine differences between pairs of trials.

To compare the delta values for each period between the groups, Levene's test for equality of variances was applied and corrected if necessary. Next, the data were compared with an independent samples *t*-test.

Effect size from the paired *t*-test was calculated (d=mean/SD). Statistical significance was defined as $P<0.05$. The statistical analysis was generated using Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, United States) version 20, for Windows.

RESULTS

Descriptive statistics for age, weight, height, body mass index, waist circumference, and resting systolic and diastolic blood pressure values for participants in each group and an overview of the antihypertensive drug profile of each of the hypertensive experimental groups are shown in Table 1. The hypertensive individuals presented higher values for age, body mass index, waist circumference, and resting systolic and diastolic blood pressure when compared with both normotensive groups.

Intragroup post-exercise hypotension (absolute values)

The HC presented systematic reductions (absolute value comparison – Table 2[A]) in

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Table 1. General characteristics of participants. *Londrina* (PR), Brazil, 2018.

Genereal characteristics	NP (n=10) [M4-F6]		NC (n=10) [M6-F4]		HP (n=10) [M4-F6]		HC (n=10) [M2-F8]		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*
Age (years)	27.70	2.4	29.90	2.5	52.00	4.8*†	58.60	2.7*†	<0.001
Weight (kg)	70.90	6.2	76.40	4.6	79.60	5.5	72.50	4.1	0.629
Height (m)	1.69	0.03	1.71	0.03	1.61	0.03†	1.58	0.03*†	0.008
BMI (kg/m^2)	24.40	1.5	25.90	1.1	30.80	2.1*†	29.20	1.8*†	0.038
WC (cm)	79.30	4.3	84.40	3.5	98.50	4.7*†	99.10	3.5*†	0.002
SBP (mmHg)	116.00	5.0	120.00	4.0	137.00	4.0*†	142.00	6.0*†	0.001
DBP (mmHg)	71.00	3.0	74.00	2.0	86.00	3.0*†	86.00	3.0*†	0.002
Antihypertensive drugs					n		n		
Beta-blocker					5		3		
Angiotensin-converting-enzyme inhibitor					4		6		
Diuretic					-		1		
All drugs					1		-		

Note: *P<0.05 *vs* NP; †: P<0.05 *vs* NC.
NP: Normotensive-Placebo group; NC: Normotensive-Citrulline group; HP: Hypertensive-Placebo group; HC: Hypertensive-Citrulline group; M: Male; F: Female; SE: Standard Error; BMI: Body Mass Index; WC: Waist Circumference; SBP: resting Systolic Blood Pressure; DBP: resting Diastolic Blood Pressure.

relation to pre-exercise values (-11%[P=0.006]; -12%[P=0.001]; -1%[P=0.003]; -9%[P=0.007]; -11%[P=0.001] and -10%[P=0.001] at 10, 20, 30, 40, 50, and 60 minutes post-exercise, respectively). Therefore, the HC presented systolic blood pressure reductions in mean office (-11%[P<0.001]) [laboratorial phase] and 24-hour blood pressure (-12%[P=0.020]) [ambulatory phase].

The NC also presented systolic blood pressure reductions (-6%[P=0.024]; -6%[P=0.049]; -6%[P=0.004], at 40, 50, and 60 minutes post-exercise, respectively). Likewise, the NC presented systolic blood pressure reductions in mean office (-3%[P=0.031]) [laboratorial phase] and 24-hour blood pressure (-6%[P=0.048]) [ambulatory phase]. There were no statistically significant differences in the NP and HP.

Considering diastolic blood pressure, the HC demonstrated reductions (-17%[P<0.001]) in the mean 24 hours.

Table 2[B] presents the effect sizes from the paired *t* test (mean rest *vs* mean 60 min, mean awake, mean asleep, and mean 24 hours) for each experimental group. Considering systolic BP, the NP showed a significant effect for the asleep period, the NC showed a significant effect for the 60 min and asleep period, and the HC showed a significant effect for all periods (60 min, awake, asleep, and 24 hours). Additionally, considering diastolic BP, the NP and NC presented a significant effect for the asleep period, the HP and HC presented a significant effect for the awake, asleep and 24-hour periods. The HP presented greater effect sizes (above 2) for the awake, asleep and 24-hour periods.

Citrulline effects in normotensives and hypertensives

Figure 2 shows the citrulline effects on PEH for normotensive and hypertensive participants through the differences (delta) in relation to rest. No significant differences were observed for normotensive participants

Table 2. Absolute value comparisons in relation to the pre-exercise moment (A) and Effect size from Paired *t*-test (versus rest [d=mean/SD]) (B). *Londrina* (PR), Brazil, 2018.

A								
Blood pressure monitoring	NP		NC		HP		HC	
	mean	SE	mean	SE	mean	SE	mean	SE
Systolic BP								
Pre	116	5	120	4	137	4	142	6
10 min	117	4	123	3	129	3	127	3*
20 min	114	4	116	4	129	3	125	4*
30 min	113	4	115	3	130	3	127	6*
40 min	112	4	113	4*	133	6	129	6*
50 min	114	5	113	3*	131	4	126	6*
60 min	111	5	113	4*	130	4	128	6*
Mean 60 min	113	4	116	3*	131	3	127	5*
Mean 24h	110	2	113	3*	127	3	125	4*
ANOVA (*P*)	0.303		0.028		0.490		0.019	
Diastolic BP								
Pre	72	3	74	3	86	3	86	3
10 min	75	3	83	4	84	3	86	3
20 min	76	2	76	3	86	3	83	4
30 min	72	3	75	3	86	3	88	4
40 min	72	2	75	2	93	7	89	5
50 min	73	3	75	3	86	5	84	3
60 min	69	3	77	2	85	3	86	3
Mean 60 min	73	2	77	3	87	3	86	3
Mean 24h	65	2	69	3	78	2	71	3*
ANOVA (*P*)	0.047		0.050		0.101		0.007	

B								
Blood pressure monitoring	NP		NC		HP		HC	
	ES	*P*	ES	*P*	ES	*P*	ES	*P*
Systolic BP								
Mean 60 min	0.31	0.356	0.81	0.031	0.53	0.127	1.81	<0.001
Mean awake	0.18	0.578	0.30	0.361	0.54	0.120	1.25	0.003
Mean asleep	1.00	0.011	1.27	0.003	0.48	0.161	1.24	0.004
Mean 24h	0.42	0.220	0.69	0.057	0.58	0.102	0.89	0.020
Diastolic BP								
Mean 60 min	-0.28	0.403	-0.38	0.256	0.12	0.707	-0.01	0.968
Mean awake	0.51	0.139	0.06	0.846	0.81	0.030	2.06	<0.001
Mean asleep	0.89	0.020	1.77	<0.001	0.90	0.019	2.16	<0.001
Mean 24h	0.70	0.054	0.48	0.161	0.88	0.021	2.28	<0.001

Note: *$P<0.05$ *vs* Pre.

NP: Normotensive-Placebo group; NC: Normotensive-Sitrulline group; HP: Hypertensive-Placebo group; HC: Hypertensive-Citrulline group; SE: Standard Error; BP: Blood Pressure; ES: Effect Size.

(panel A). Hypertensives presented a significant citrulline effect on diastolic blood pressure for the awake period (-13.93mmHg *vs* -6.85mmHg [$P=0.042$], panel B).

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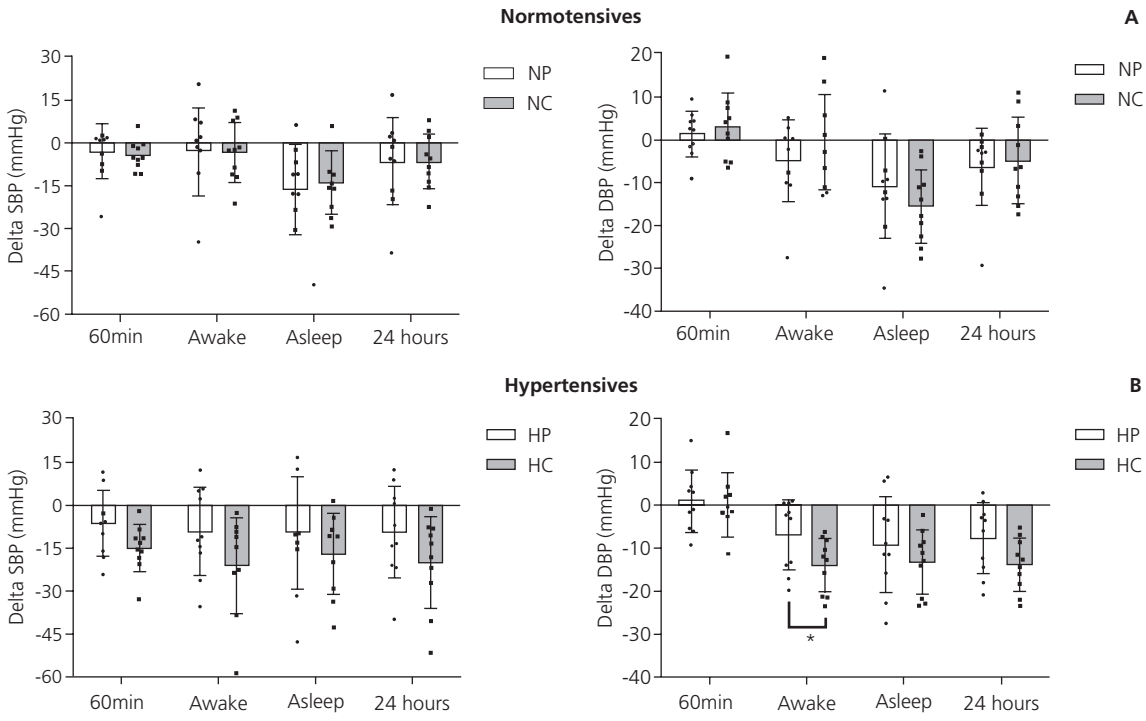

Figure 2. Citrulline effects on post-exercise hypotension for normotensives and hypertensives. *Londrina* (PR), Brazil, 2018.

NP: Normotensive-Placebo group; NC: Normotensive-Citrulline group; HP: Hypertensive-Placebo group; HC: Hypertensive-Citrulline group; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

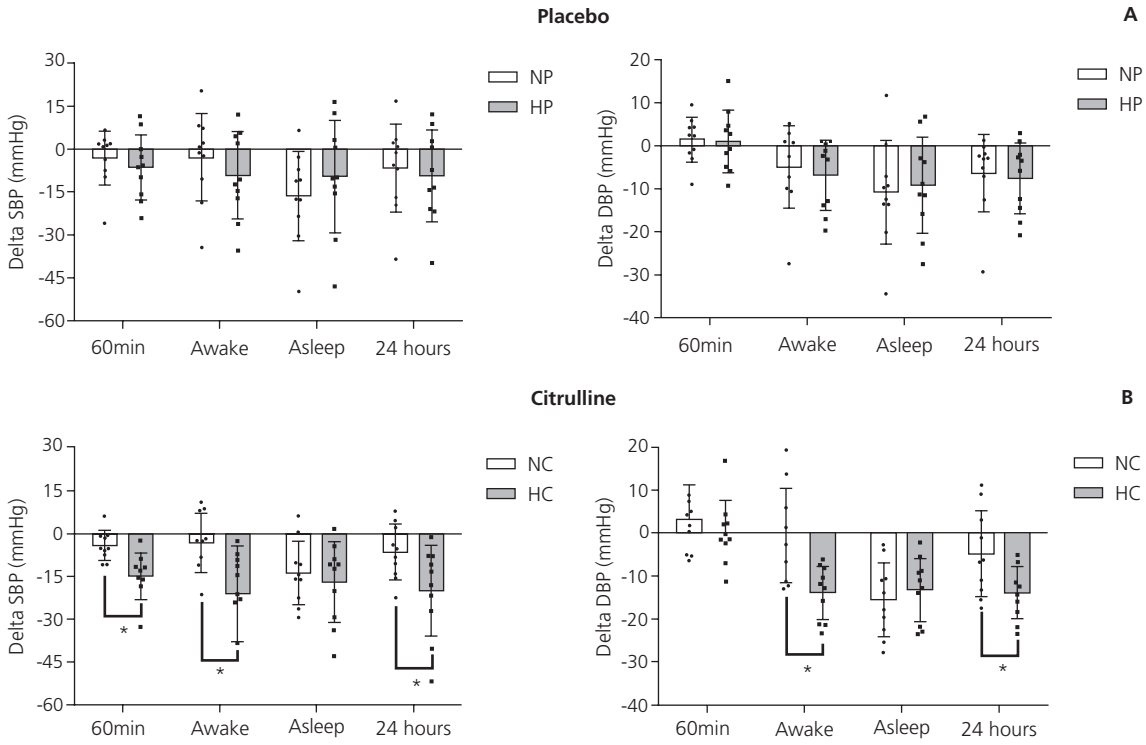

Figure 3. Citrulline effects on post-exercise hypotension between normotensives and hypertensives. *Londrina* (PR), Brazil, 2018.

NP: Normotensive-Placebo group; NC: Normotensive-Citrulline group; HP: Hypertensive-Placebo group; HC: Hypertensive-Citrulline group; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.

Citrulline effects between normotensives and hypertensives

Figure 3 shows the effects of one isolated acute citrulline supplementation on PEH between normotensives and hypertensives. No significant differences were observed between groups for placebo conditions (panel A). Considering the citrulline supplementation groups, hypertensive participants presented a higher reduction in systolic blood pressure when compared with the normotensives during the laboratorial phase (60min) (-15.01mmHg *vs* -3.99mmHg [*P*=0.002], awake (-21.05mmHg *vs* -3.17mmHg [*P*=0.01] and 24-hour periods (-20.02mmHg *vs* -6.58mmHg [*P*=0.033]. Similar responses were observed for diastolic blood pressure during the awake (-13.93mmHg *vs* -0.53mmHg [*P*=0.004] and 24-hour periods (-13.83mmHg *vs* -4.79mmHg [*P*=0.026].

DISCUSSION

The purpose of the present study was to compare the effects of oral citrulline supplementation on the PEH response in normotensive and hypertensive subjects. Studies involving citrulline oral supplementation are relatively recent, starting around the year 2000. To the best of our knowledge, the purpose of this study of combined non-pharmacological treatments (aerobic exercise and citrulline) for hypertensives and normotensives appears to be unpublished. Therefore, it is difficult to compare these findings with other studies.

Investigations have shown that citrulline oral supplementation is related to improvement of physical activity performance, since its effect on the body promotes changes in the muscle metabolism, facilitating energy production from the aerobic system [19].

Improvement in the aerobic metabolism seems to be related to the increasing plasma levels of arginine, citrulline, and consequently, NO concentration [20]. Previous studies reported that oral citrulline supplementation raises plasma arginine concentration and increases NO production through the citrulline-NO cycle [21,22]. It is exactly because of this vasodilation resulting from greater NO release that citrulline oral supplementation might potentiate the PEH response, especially in hypertensive individuals.

Some evidence suggests a blood pressure reduction after citrulline supplementation [23]. Decreases in systolic and diastolic blood pressure (~7/3mmHg) and aortic pressure (~9/3mmHg) have been observed when 6g/day-1 of citrulline was administered orally in obese, pre-hypertensive and hypertensive individuals [24].

The hypothesis was that blood pressure responses would be different among groups, as PEH can be caused by different mechanisms between normotensive and hypertensive individuals. Previous studies have shown that hypertensive individuals present a deficiency in peripheral vasodilation modulators, as a compensation mechanism, so the autonomous nervous system can work to reduce cardiac output [7,8]. On the other hand, normotensive individuals have shown a reduction in peripheral vascular resistance [7,9,10].

Additionally, hypertensive individuals can present an increase in peripheral vascular resistance [25]. Therefore, we could expect that citrulline oral supplementation may contribute to improving the peripheral vasodilation mechanisms in hypertensive individuals, resulting in a greater magnitude and/or duration of PEH.

The ages, waist circumference and BMI of patients (normotensives *vs* hypertensives) were different. These differences need to be considered. On the other hand, post-exercise hypotension can occur irrespective of age [26]. A recent meta-analytic investigation [27] showed a weak correlation between age and post-exercise hypotension in both systolic (*r*=0.21) and diastolic (*r*=0.12) blood pressure. Weak correlations were also observed between BMI and post-exercise hypotension in both systolic (*r*=0.26) and diastolic (*r*=0.09) blood pressure.

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The main novel findings from this study were that a single dose of supplementation with citrulline enhanced the magnitude and duration of PEH in hypertensive individuals. These findings are important as they suggest that citrulline might be responsible for the positive effects on blood pressure and offer new insights into non-pharmacological treatment for hypertension.

Therefore, evidence has accumulated demonstrating the critical role of NO in blood pressure regulation. Once NO is released from endothelial cells, it increases 3',5'-cyclic-uanosine monophosphate (cGMP) production and subsequent cGMP-dependent Protein Kinase (PKG) activation in Vascular Smooth Muscle Cells (VSMC), resulting in vasodilation [11,12,28]. Previous studies have confirmed the essential role of NO in vasorelaxation of large human arteries [29] and evidence suggests that NO production is enhanced after citrulline supplementation [21,30,31] and citrulline can restore NO production in conditions where NO production is compromised [32,33]. Given that NO production is one of the central pathogenic mechanisms of hypertension, restoration of adequate NO in the blood vessels may serve as an important therapeutic strategy for hypertension.

On the other hand, all current treatment guidelines [34] emphasize the role of physical activity/exercise in the treatment of hypertension. To date, only one randomized clinical trial combining physical activity/exercise and citrulline supplementation in the treatment of hypertension was found [35]. The authors investigated the post-exercise blood pressure response 10 minutes after resistance exercise with citrulline supplementation (8 grams) and there was a significant reduction in diastolic BP [35]. In the present study, we observed hypotensive effects following 10 minutes post-exercise. Furthermore, in the previous investigation [35], the participants were submitted to a resistance exercise session, which is an important methodological difference that needs to be addressed, since in the present study the participants were asked to perform an aerobic treadmill exercise session. It is well established that aerobic exercise is an efficient strategy to reduce blood pressure (5-15mmHg) compared to resistance training (5mmHg) [36-39]; thus, differences between exercise types do not allow a direct comparison. Another important difference between the present study and previous investigations [35] is related to the "training status". Some data suggest that chronic resistance training can decrease PEH magnitude [40,41]. On the other hand, aerobic training apparently does not reduce PEH [27].

In the present study, PEH were confirmed only in the groups that ingested citrulline. It is suggested that oral citrulline supplementation might cause an additional effect on blood pressure reduction. This association (exercise + citrulline supplementation) showed expressive effects immediately after exercise with a significant reduction in systolic blood pressure during the office blood pressure period. During the ambulatory blood pressure monitoring, the HC showed significant reductions in diastolic blood pressure (Figure 2) in the awake period, suggesting that NO might be involved in this response and could be over stimulated by exercise and supplementation with citrulline when compared with exercise alone.

However, one of the most common questions driving the evaluation of intervention programs is "how does this effect compare with the effects of other interventions?". Therefore, previous studies have encouraged the use of "effect size" in quantitative studies [42]. Researchers are often encouraged to report effect sizes for three reasons. First, to present the magnitude of the reported effects in a standardized metric; second, effect sizes allow researchers to draw meta-analytic conclusions by comparing standardized effect sizes across studies; and third, effect sizes from previous studies can be used when planning a new study, providing an indication of the average sample size needed [43]. In the present study, we found that the effect sizes (from the paired *t* test [versus rest]) were more consistent in the HC for systolic and diastolic ambulatorial blood

pressure response, however, it is important to highlight that for diastolic BP the effects were "large" (above 2) for the "awake", "asleep", and "24-hour" periods in the HC.

Considering these interesting findings, further studies should include NO availability measurements, such as nitrite and nitrate. These measurements associated with the evaluation of important mechanisms such as peripheral vascular resistance and cardiac output might help us understand the action of citrulline. Furthermore, considering these promising results, further studies could associate citrulline with other potential vasodilatory nutrients and exercise modalities, enabling the development of adjuvant protocols to treat hypertension.

CONCLUSION

Acute citrulline oral supplementation can induce a greater PEH response in hypertensive than normotensive individuals.

CONTRIBUTORS

K GRANDOLFI, JV CAVALARI, MD POLITO and J CASONATO were responsible for the study conception and design. K GRANDOLFI, JV CAVALARI and RC GÓES performed the data collection and intervention. K GRANDOLFI, RC GÓES and J CASONATO performed the statistical analysis and interpretation of data. All authors were responsible for drafting, editing, reviewing, and approving the final version of the manuscript.

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