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ORIGINAL ARTICLE

Citrulline malate supplementation might potentiate post-exercise hypotension in hypertensives: A 24-hour analysis

La supplémentation en malate de citrulline pourrait potentialiser l'hypotension post-exercice chez les hypertendus

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KEYWORDS
Blood pressure;
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Ambulatory blood pressure monitoring

Summary
Objective. — The present study was designed to investigate whether citrulline malate supplementation might influence the acute post-exercise blood pressure response in hypertensives.
Methods. — Forty adults, hypertensive and sedentary, were randomly assigned to one of four experimental groups (control-placebo, control-citrulline, exercise-placebo, and exercise-citrulline). The volunteers ingested a sachet with placebo (6 grams) or citrulline malate (6 grams). During the exercise session, individuals performed 40 min of walking/running on a treadmill at 60—70% of reserve heart rate. For the control's session, they remained seated at rest for 40 min. Office blood pressure was taken every 10 min until completing 60 min after the experimental session. The ambulatory blood pressure device was programmed to take the readings every 20 min (awake time) and every 30 min (sleep time) over the course of 24 h of monitoring. Furthermore, heart rate variability was measured.

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Results. — Considering systolic blood pressure, the aerobic exercise combined with citrulline malate supplementation promoted an additional blood pressure reduction when compared to isolated exercise during 60 min after the experimental session (−15.01 ± 2.57 mmHg vs. −6.30 ± 3.60 mmHg, $P = 0.03$). Additionally, considering diastolic blood pressure, exercise combined with citrulline malate showed a significant effect for the ''awake'' period (−13.93 ± 1.96 mmHg vs. −6.85 ± 2.57 mmHg, $P = 0.027$) and over the course of 24 h (−13.83 ± 1.93 mmHg vs. −7.64 ± 2.58 mmHg, $P = 0.047$).
Conclusion. — Acute citrulline malate supplementation might potentiate the post-exercise hypotension effects in hypertensive individuals.

MOTS CLÉS
Tension artérielle ;
Malate de citrulline ;
Exercice ;
Hypertension ;
Compléments alimentaires ;
Surveillance ambulatoire de la pression artérielle

Résumé
Objectif. — La présente étude visait à déterminer si une supplémentation en malate de citrulline pouvait influer sur la réponse aiguë de la pression artérielle après l'exercice chez les hypertendus.
Méthodes. — Quarante adultes hypertendus et sédentaires ont été répartis au hasard dans l'un des quatre groupes expérimentaux (placebo témoin, citrulline témoin, placebo d'exercice et citrulline d'exercice). Les volontaires ont ingéré un sachet contenant un placebo (6 grammes) ou du malate de citrulline (6 grammes). Au cours de la séance d'exercice, les participants ont effectué 40 minutes de marche/course sur un tapis roulant à 60—70 % de la fréquence cardiaque en réserve. Pour la séance de contrôle, ils sont restés assis au repos pendant 40 minutes. La tension artérielle au bureau a été prise toutes les 10 minutes jusqu'à l'achèvement de 60 minutes après la session expérimentale. Le tensiomètre ambulatoire a été programmé pour prendre les lectures toutes les 20 minutes (heure de veille) et toutes les 30 minutes (heure de sommeil) au cours des 24 heures de surveillance. De plus, la variabilité de la fréquence cardiaque a été mesurée.
Résultats. — Compte tenu de la pression artérielle systolique, l'exercice aérobie associé à une supplémentation en malate de citrulline a entraîné une réduction supplémentaire de la pression artérielle par rapport à un exercice isolé 60 minutes après la séance expérimentale (−15,01 ± 2,57 mmHg vs −6,30 ± 3,60 mmHg, $p = 0,03$). En outre, compte tenu de la pression artérielle diastolique, l'exercice associé au malate de citrulline a eu un effet significatif pendant la période « éveillée » (−13,93 ± 1,96 mmHg vs −6,85 ± 2,57 mmHg, $p = 0,027$) et pendant 24 h (−13,83 ± 1,93 mmHg vs −7,64 ± 2,58 mmHg, $p = 0,047$).
Conclusion. — Une supplémentation aiguë en malate de citrulline pourrait potentialiser les effets hypotenseurs post-exercice chez les individus hypertendus.

1. Introduction

Post-exercise hypotension (PEH) is defined as a sustained reduction in blood pressure (BP) after a single bout of exercise [1] and this reduction can last up to 24 h after completion of an exercise session [2]. PEH is now considered to be an important physiological phenomenon [3], which can play a major role in BP management, since previous studies have reported that the magnitude of PEH following a single bout of exercise was correlated with chronic changes in resting BP after a period of exercise training [4—8]. This fact can be explained as some peripheral [4—8] and central [4—8] physiological mechanisms associated with acute and chronic BP response are the same. For this reason, subjects who are responsive to PEH demonstrate a greater chance of presenting chronic BP reduction [9]. In this sense, the magnitude of PEH could be a promising candidate for the individual prediction of BP-related training efficacy [4].

Although it is well established in the literature that aerobic [10] and resistance [11] exercise are efficient for PEH, especially in hypertensives, there is still a lack of information regarding the extrinsic factors to exercise which may affect PEH. Considering that reduced vascular resistance is the main cause of acute and chronic BP-lowering effects of exercise, at least in formerly untrained subjects [12], it is expected that a vasodilator substance may potentiate PEH, such as citrulline malate (CM). To our knowledge, the first paper that investigated the CM effects in humans showed that citrulline malate stimulates hepatic ureogenesis and favorizes the renal reabsorption of bicarbonates. These metabolic actions had a protective effect against acidosis and ammonia poisoning and explain the anti-fatigue properties of citrulline malate in man [13]. From there to here, CM supplementation has been mainly used as an ergogenic aid.

The CM is formed by a combination of L-citrulline (a non-essential amino acid that has a key role in the arginine-nitric oxide system, increasing nitric oxide [NO] biodisponibility [14]) and malate (or acid malic) — a salt primarily found in apples. Although low concentrations of CM can be provided by nutritional sources in regular food, citrulline availability is mainly produced endogenously through two different pathways:

- NO co-product (secondary amount) and;
- ornithine carbamylation (principal amount) by metabolites (glutamine, proline, and arginine) in only two cell types (enterocytes and hepatocytes) [15].

The citrulline produced in the liver is all channeled to the urea cycle, thus, small or negligible amounts of citrulline are directed to the circulation [16]. On the other hand, the citrulline produced by enterocytes enters the circulation system, bypasses the liver, and enters the kidneys (and other tissues) for arginine synthesis [17,18]. For this reason, it is suggested that CM supplementation could be an efficient strategy to increase extracellular arginine levels, which is recognized as the NO synthesis precursor [19]. In this line, some studies have indicated that CM supplementation increases plasmatic NO metabolite concentration [20,21], an important peripheral dilate mediator.

Therefore, it is possible that CM supplementation improves PEH effects (duration and/or magnitude). To our knowledge, there are no other studies investigating the effects of acute CM supplementation on BP after aerobic exercise. Thus, assessment of this hypothesis could help to improve non-pharmacological intervention strategies focusing on the prevention and treatment of hypertension.

Accordingly, the aim of the present study was to investigate whether CM supplementation might influence acute post-exercise BP response in hypertensive subjects. We hypothesized that acute PEH would be more pronounced after CM supplementation.

2. Materials and methods

2.1. Participants

After sample size calculation (see statistical analysis session), 40 hypertensive individuals, not physically active (<60 min of aerobic exercise per week and no resistance training during the previous 6 months), and nonsmokers participated in the study (Table 1). All volunteers (both sexes) were adults without osteoarticular disabilities and had medical authorization for physical exercise practice. Participants were recruited from an exercise program project linked to the university that offered stretching and functional exercise sessions to the external community. The study followed the Declaration of Helsinki and the Institutional Ethics Committee approved all experimental procedures and protocols. Each participant was fully informed of all potential risks and experimental procedures, after which, informed written consent was obtained. The study protocol was registered in ClinicalTrials.gov (NCT03378596).

2.2. Study design

Following a double-blind, placebo-controlled, parallel-groups clinical trial (Fig. 1), the participants were randomly allocated (using a random number table — https://www.random.org/) into four experimental groups (exercise/citrulline [EC]; exercise/placebo [EP]; control/citrulline [CC]; control/placebo [CP]). The participants ingested a sachet, which contained 6 grams of CM or placebo (corn starch) dissolved in water (100 mL). The selected dose of CM was based on previous studies [22,23].

The participants were asked to refrain from caffeine and alcohol for 24 h before the experimental session and advised not to make changes to their regular lifestyles other than the assigned interventions. The substances were ingested 120 min before the experimental or control session. Anthropometric measures were taken before the rest period. The exercise session was conducted on a treadmill and consisted of: a 5 min warm up (50—65% $HR_{reserve}$); 40 min of running/walking at 60—70% $HR_{reserve}$; and a 5 min progressive cooldown. In the control session, the participants remained seated in a quiet room for 40 min.

After the exercise/control sessions, the BP was measured every 10 min over the course of 60 min and heart rate variability was recorded continuously for 60 min (laboratorial phase). Next, 15 min was allowed for the participants to take a shower and change their clothes before the ambulatory BP device was attached on their arm. The ambulatory BP monitoring (ABPM) was recorded over 24 h. The next day, the participants were asked to return to the laboratory to remove the ABPM device. Testing was conducted in the morning at the same time of day 9:00 am (±1 h) in a quiet, temperature-controlled room (23 °C ± 1 °C).

2.3. Anthropometry

Weight was measured using a digital anthropometric scale (Urano, OS 180A, Canoas, Brazil), with an accuracy of 0.1 kg and height was measured by a stadiometer with an accuracy of 0.1 cm, in accordance with the procedures described by Gordon et al. [24]. The body mass index (BMI) was defined as the body mass (kg) divided by the square of the body height.

2.4. Office blood pressure (laboratorial phase)

The office BP measurements were taken with an oscillometric device (Omron MX3 Plus, Bannockburn, USA) previously validated for clinical measures in adults [25]. Firstly, the participants remained seated (rest period) in a calm, quiet, and thermoneutral (22°—24 °C) environment for 20 min. The BP was measured three times during the rest period (at 10 min, 15 min, and 20 min). The resting BP value was considered as the average of these three measurements. Immediately following the sessions (exercise or control), the BP was measured in a quiet environment for 60 min. The BP measurements were taken according to the American Heart Association recommendations [26].

Table 1 Characteristics of participants and antihypertensive medications.

	Control-Placebo		Control-CM		Exercise-placebo		Exercise-CM		F	P
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Age, (years)	62.3	5.9	60.6	5.3	52.0	4.8	58.6	2.7	0.864	0.469
Weight, (kg)	77.2	5.2	76.5	2.9	79.6	5.4	72.5	4.1	0.419	0.740
Height, (m)	1.66	0.04	1.59	0.03	1.61	0.03	1.58	0.029	1.309	0.286
BMI	27.9	1.3	30.6	1.4	30.8	2.0	29.1	1.8	0.625	0.603
WC, (cm)	98.2	3.1	101.4	2.9	98.5	4.7	99.1	3.5	0.159	0.923
SBP	140	6.0	132	4.8	137	3.7	142	6.4	0.714	0.550
DBP	82	1.6	80	2.7	86	3.5	86	3.2	0.889	0.456
	n	%	*n*	%	*n*	%	*n*	%		
BB	3	30	5	50	5	50	3	30		
ACEI	5	50	3	30	4	40	6	60		
DIUR	0	0	1	10	0	0	1	10		
BB + ACEI	2	20	1	10	0	0	0	0		
ACEI + DIUR	0	0	0	0	0	0	0	0		
BB + ACEI + DIUR	0	0	0	0	1	10	0	0		

CM: citrulline malate; SE: standard-error; BMI: body mass index; WC: waist circumference; BB: beta-blockers; ACEI: angiotensin-converting-enzyme inhibitor; DIUR: diuretics.

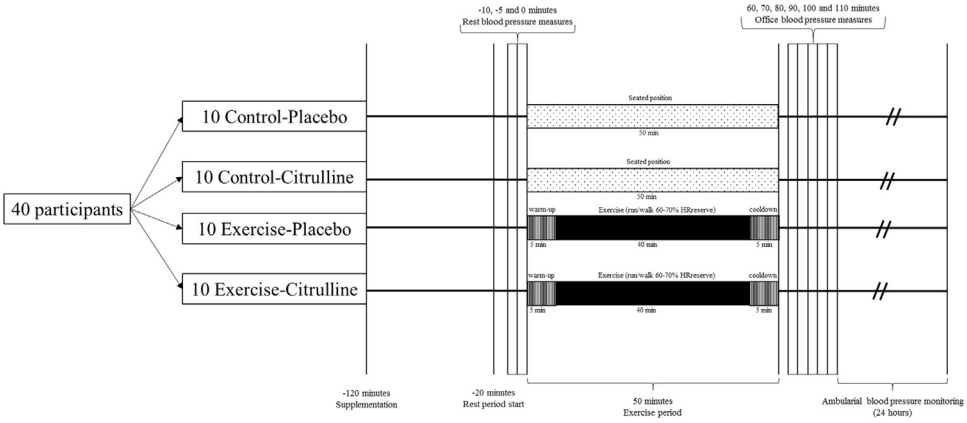

Figure 1 Study design.

2.5. Heart rate variability measures

Heart rate variability was monitored during the rest period using a cardiac monitor (Polar RS800CX, Kempele, Finland), previously validated [27]. The recorded R-R intervals were transferred to a computer using specific software (Polar Pro-Trainer software, Kempele, Finland). Fast Fourier Transformation was applied to quantify the low and high frequencies into normalized units, in accordance with the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [28]. Participants taking beta-blockers were excluded from this analysis.

The time domain analysis was obtained by SDNN (standard deviation of the NN interval), RMSSD (the square root of the mean of the sum of the squares of differences between adjacent NN intervals), and pNN50 (NN50 count divided by the total number of all NN intervals) indices. The range interval analysis was 5 min (rest, prior to exercise), and 30 min (post-exercise — laboratorial phase) using Kubios HRV, version 2.2 (Kuopio, Finland).

2.6. Ambulatory blood pressure monitoring (ambulatory phase)

The ABPM were taken with an oscillometric device (Dyna-MAPA — São Paulo, Brazil) attached on the left arm, always by the same investigator, in accordance with procedures described by the American Heart Association [26]. The participants received instructions to maintain their arm outstretched and immobile during the measures. The device was calibrated by direct comparison with a mercury sphygmomanometer, by a trained technical person, in agreement with recommendations [26].

The monitor was set to register the systolic and diastolic BP and heart rate every 20 min during ''daytime'' (08:00 am to 11:00 pm) and every 30 min during ''night-time'' (11:00

pm to 08:00 am) to reduce sleep disturbances. The device screen was electronically blinded to avoid feedback. All participants were instructed to register and report their sleep time in a diary on the following day.

The data were recorded in the device memory then sent to a computer using specific software (Aplicação Dyna-Mapa — Version 5.0.382.12) for analysis. The average of the valid readings was above 90% for all participants.

2.7. Statistical analysis

Assuming a standard deviation of 5 mmHg [29] for the systolic BP, an alpha of 5%, and a desired statistical power of 80% for detecting a minimum difference of 7 mmHg [29], 7 subjects were required in each group.

The data are presented in the text as mean and standard-error. The Mauchly's sphericity test was applied. The Greenhouse-Geisser correction was used if necessary. Next, these data were compared to a one-factor repeated measures general linear model. Fisher multiple comparisons were employed to examine differences between pairs of trials.

The ABPM was analysed as awake, sleep, and 24 h of monitoring. Statistical significance was defined as $P < 0.05$. The statistical analysis was generated using SPSS, version 20, system for windows.

3. Results

Characteristics of the participants and an overview of the antihypertensive drugs profile are shown in Table 1. The four experimental groups were not different regarding age, weight, height, body mass index, waist circumference, and systolic/diastolic resting BP. The most commonly ingested drug by the participants was angiotensin-converting-enzyme inhibitor.

3.1. Exercise effects

Fig. 2 presents the acute effects of exercise on systolic and diastolic BP. No significant differences were found for the placebo groups (Fig. 1A). On the other hand, when comparing the CM groups (Fig. 1B), a significant systolic BP reduction was identified for the EC in the first 60 min (-15.01 ± 2.57 mmHg vs. 5.43 ± 2.98 mmHg, $P < 0.001$ [mean laboratorial phase]). The same lowering effects of exercise were observed during arterial BP monitoring for the ''awake'' period (-21.05 ± 5.29 mmHg vs. -4.23 ± 3.88 mmHg, $P = 0.013$ [systolic] and -13.93 ± 1.96 mmHg vs. -4.68 ± 2.33 mmHg, $P = 0.005$ [diastolic]) and over the course of 24 h (-20.02 ± 4.99 mmHg vs. -6.09 ± 4.01 mmHg, $P = 0.036$ [systolic] and -13.83 ± 1.93 mmHg vs. -6.52 ± 2.03 mmHg, $P = 0.021$ [diastolic]).

3.2. Citrulline malate effects

Overall, isolated acute CM supplementation did not alter systolic and diastolic BP, except for an isolated difference that was observed in the CC group, presenting higher values for systolic BP compared to the CP (9.89 ± 3.74 mmHg vs. -6.16 ± 3.34 mmHg, $P = 0.004$ at 50 min, respectively) (Fig. 3).

3.3. Citrulline malate additional effects

Fig. 4 presents the CM additional effects (EP vs. EC) on systolic and diastolic BP. Considering systolic BP, the aerobic exercise combined with CM supplementation (EC) promoted an additional BP reduction when compared to isolated exercise (EP) during the laboratorial phase (-15.01 ± 2.57 mmHg vs. -6.30 ± 3.60 mmHg, $P = 0.03$). Additionally, considering diastolic BP, the EC presented a significant effect for the ''awake'' period (-13.93 ± 1.96 mmHg vs. -6.85 ± 2.57 mmHg, $P = 0.027$) and over the course of 24 h (-13.83 ± 1.93 mmHg vs. -7.64 ± 2.58 mmHg, $P = 0.047$).

3.4. Heart rate variability

No significant differences were observed between groups for heart rate variability components (Table 2).

4. Discussion

The purpose of this study was to investigate whether CM supplementation might influence the acute post-exercise BP response in hypertensive subjects. Our results showed that PEH might be more pronounced after CM supplementation. The main finding of this study was that CM supplementation might potentiate PEH in hypertensive individuals and that these effects can last 24 h. This association (exercise + CM supplementation) demonstrated expressive effects immediately after exercise, with significant reduction in systolic and diastolic blood pressures during the laboratorial phase, ''awake'' time, and over the course of 24 h of monitoring. To our knowledge, the present study was the first to investigate the acute effect of CM on post-exercise BP of hypertensive subjects.

Our results can be explained due to sustained vasodilation in the EC group caused by NO increase [30]. NO is generated through oxidation of the amino acid L-arginine (Arg) in a reaction catalyzed by NO synthase (NOS), with nitrite (NO_2^-) and nitrate (NO_3^-) being oxidation products of NO [31]. Differently from Arg, L-citrulline (*Cit*) is co-produced with NO as an end-product of NOS activity, not metabolized in the intestine or liver [32] and has been reported to increase argininemia [33] and NO synthesis [34]. NOS-derived *Cit* can be efficiently converted to Arg for subsequent NO production through the *Cit*-NO cycle [35]. It has been documented that oral *Cit* supplementation can increase the plasma Arg concentration [36] and bioavailability [37] together with increases in NOS activation [38] and NO biomarkers [39].

In this sense, concerning the mechanisms that might be involved in this response, we hypothesized that the reduction in vascular resistance due to increased NO concentration could potentiate PEH. However, we have not yet evaluated the mechanisms for this. Independent of the mechanisms involved in this response, the present study

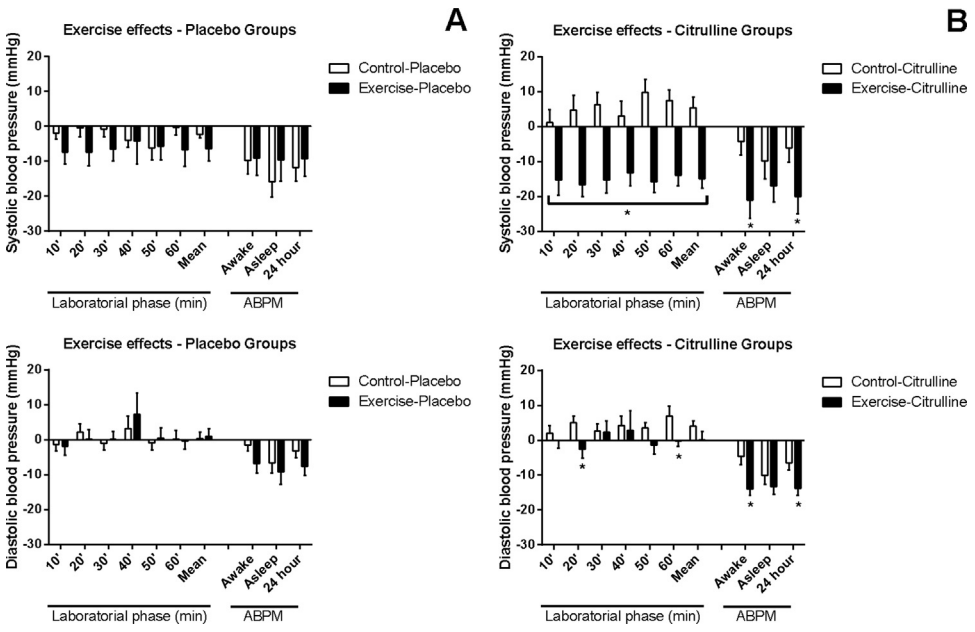

Figure 2 Acute effects of exercise on systolic and diastolic blood pressure. A. placebo groups. B. Citrulline malate groups. * = $P < 0.05$.

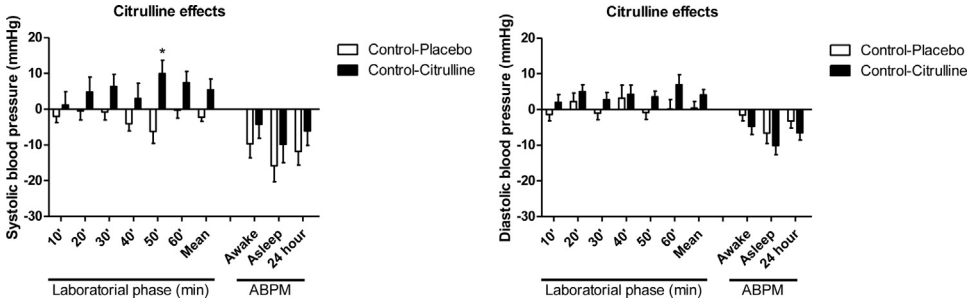

Figure 3 Effects of isolated acute citrulline malate supplementation on systolic and diastolic blood pressure. * = $P < 0.05$.

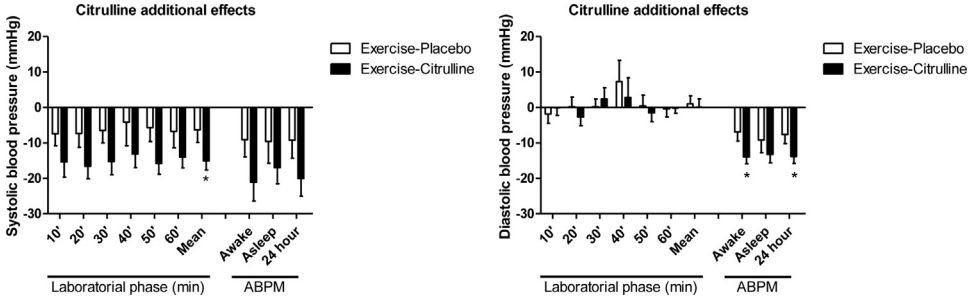

Figure 4 Citrulline malate additional effects on post-exercise hypotension. * = $P < 0.05$.

demonstrated that exercise plus CM (intermediate of NO metabolism) cause a greater hypotensive effect and this effect can last up to 24 h. This finding suggests that the NO might be involved in this response [40] and may be over stimulated by exercise plus CM compared to one of them alone.

An isolated exercise session caused significant systolic BP reduction in the first 60 min. The same lowering exercise effects were observed during arterial BP monitoring for the ''awake'' period and over the course of 24 h for systolic and diastolic BP when comparing ''CM groups''. Recently a study [41] demonstrated that aerobic exercise (treadmill for 45 min; 65—70% VO_{2max}) produces significant reductions in both diastolic and mean BP in the first hour following exercise in older patients with essential hypertension.

On the other hand, isolated acute CM supplementation did not alter systolic or diastolic BP in the present study. It is important to highlight that acute ingestion of 3 g of

Table 2 Heart rate variability component variations at P30 (mean of 0—30 min post-exercise minus rest) and P60 (mean 30—60 min post-exercise minus rest).

	Control-Placebo		Control-CM		Exercise-Placebo		Exercise-CM		F	*P*
	n = 5		*n* = 4		*n* = 4		*n* = 7			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
SDNN_P30	29.68	22.54	1.00	3.08	33.32	16.23	238.02	155.71	1.111	0.374
RMSSD_P30	37.08	30.20	−6.35	10.63	2.85	24.43	123.75	144.55	0.356	0.785
pNN50_P30	4.28	2.10	−5.12	7.60	−10.87	2.33	1.22	5.381	1.608	0.227
LF_P30	3.38	4.56	−14.45	13.94	13.42	4.58	3.57	14.03	0.772	0.527
HF_P30	−3.98	4.49	−30.70	31.67	−13.42	4.58	−3.57	14.03	0.562	0.648
LF/HF_P30	−1.86	2.01	−10.67	11.35	2.40	0.94	2.34	1.40	1.550	0.240
SDNN_P60	43.94	30.31	86.95	52.30	33.02	47.83	286.34	197.02	0.788	0.518
RMSSD_P60	58.10	47.13	118.72	63.73	38.85	43.66	146.40	157.76	0.184	0.906
pNN50_P60	4.50	1.82	9.75	3.83	−4.95	2.29	2.32	4.73	1.919	0.167
LF_P60	−11.18	12.35	−16.92	21.41	7.30	4.54	15.11	5.32	1.907	0.169
HF_P60	11.18	12.35	−28.22	23.97	−7.30	4.54	−15.11	5.32	1.770	0.193
LF/HF_P60	−2.56	2.04	−11.42	11.62	0.85	0.63	15.38	8.67	2.274	0.119

CM: citrulline malate; SE: standard-error; SDNN: standard deviation of the NN intervals; RMSSD: square root of the mean squared differences of successive NN intervals; pNN50: percentage of interval differences of successive NN intervals greater than 50 ms; LF: low frequency component; HF: high frequency component.

Cit increased NO synthesis; however, endothelium-mediated vasodilation was not improved in young and older adults with heart failure [42]. Evidence suggests that chronic *Cit* supplementation decreases systolic (≈7 mmHg) and diastolic BP (≈9 mmHg) after 8 weeks of *Cit* supplementation (6 g/day) in prehypertensive and hypertensive obese postmenopausal women [43]. Similarly, the hypotensive effect of 6 weeks of watermelon supplementation (*Cit* source) has been reported in individuals with prehypertension and hypertension [23,44,45]. In contrast, previous investigations have reported no significant impact of 2—4 weeks of *Cit* supplementation (6 g/day) on systolic or diastolic BP in normotensive young men [46,47].

To the best of our knowledge, this is the first report on these combined non-pharmacological treatments (aerobic exercise and CM) for hypertensives. To date, only one randomized clinical trial was found [48]. The authors investigated the post-exercise BP response 10 min after resistance exercise with CM supplementation (8 grams); however, they did not identify significant changes [48]. In the present study, we observed the hypotensive effects following 10 min post-exercise. Furthermore, in the previous investigation [48], the participants were submitted to a resistance exercise session, which is an important methodological difference that needs to be addressed, as 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 BP (5—15 mmHg) compared to resistance training (5 mmHg) [11,49—51], thus, the differences between exercises types does not allow for a direct comparison.

The literature remains controversial with regard to the duration of the effect of PEH. Previous studies [52,53] using aerobic exercise, demonstrated that the PEH does not appear to last 24 h, even in hypertensive subjects, who are exposed to higher magnitudes of pressure reduction when compared to their normotensive pairs [52,54]. On the other hand, in this clinical trial the hypotension magnitude was higher in the EC group when compared with all other groups in the ''awake'' and 24 h periods.

No differences between groups were found for heart rate variability. Therefore, we can suggest that CM supplementation may potentiate PEH and the mechanisms could be related to peripheral vasodilation (by NO release) and not to feedback of neural mechanisms.

Considering these interesting findings, it is recommended that future studies 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 to understand the action of CM. As practical applications of this study, physical exercise plus CM oral supplementation appear to contribute to the hypotensive effect in hypertensive subjects. Due to these promising results, other studies can associate CM with other potential vasodilatory nutrients and exercise modes, enabling the development of adjuvant protocols for the treatment of hypertension.

5. Conclusion

These results suggest that acute CM supplementation is able to increase the post-exercise hypotensive effects in hypertensives. Further studies involving chronic exercise and CM supplementation are needed and it might be a promising non-pharmacological therapeutic possibility for the treatment of hypertension.

Disclosure of interest

The authors declare that they have no competing interest.

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