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Combined effects of aerobic exercise and L-arginine ingestion on blood pressure in normotensive postmenopausal women: A crossover study

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ABSTRACT

After menopause the incidence of cardiovascular diseases increases in women. A decrease in nitric oxide (NO) bioavailability has been pointed out to play a major role in this phenomenon. Since it is believed that L-arginine administration could improve NO bioavailability, the aim of this study was to examine the effects of acute L-arginine administration associated with aerobic exercise on blood pressure (BP), redox state and inflammatory biomarkers in normotensive postmenopausal women (NPW). Sixteen volunteers (57 ± 6 yr) were subjected to four experimental sessions (crossover design): arginine + exercise (A-E); arginine (ARG); exercise + placebo (EXE); control (CON). Each session was initiated with either 9 g of L-arginine ingestion (ARG or A-E days), placebo (EXE day), or nothing (CON day). The participants performed 30 min of aerobic exercise (A-E and EXE days) or sitting rest (CON and ARG days). Blood samples were collected before each session and 45 min after the intervention. Office BP and ambulatory blood pressure monitoring (ABPM) were evaluated. NO/cGMP pathway, redox state and inflammatory biomarkers were measured. Systolic BP decreased during the 24-hour in A-E and EXE sessions. However, diastolic BP reduced only in A-E session. No changes were found in the biomarkers concentrations. In conclusion, the association was effective in lowering diastolic BP in NPW. Additionally, physical exercise alone promoted a long lasting effect on systolic BP measured by ABPM in this population, although this beneficial effect was not associated with changes in the cardio-inflammatory biomarkers. Possibly, other factors such as neural influences could be mediating this effect.

1. Introduction

The period after menopause is associated with increased prevalence of metabolic syndrome and cardiovascular diseases in women [1,2]. Evidence suggests that estrogen deficiency is the primary cause of the increased cardio metabolic diseases in this population as well as a significant decline in regular physical exercise practice [1,3]. Data from experimental models of menopause have shown that estrogen deficiency increases the activity of the renin-angiotensin-aldosterone system, oxidative stress, production of inflammatory mediators, and endothelial dysfunction [4]. Indeed, it is believed that estrogenic deficiency could lead to a decrease in nitric oxide (NO) production or its bioavailability to the cells which, in turn, could explain the high prevalence of cardiovascular diseases in women after menopause [5].

In vascular endothelial cells NO is synthesized from the amino acid L-arginine by the enzyme endothelial nitric oxide synthase (eNOS). NO diffuses to the vascular smooth muscle cells and promotes relaxing responses regulating blood flow by stimulation of the cytosolic enzyme, soluble guanylate cyclase, which catalyses the production of cyclic 3′5′-guanosine monophosphate (cGMP). This lead to an increased extrusion of Ca^{2+} from cytosol in vascular smooth muscle, and consequently an inhibition of the contractile machinery as well as preventing cytokine formation and platelet aggregation [6].

In addition, it has been reported that administration of exogenous L-arginine restores NO biovailability [7]; however, it is not clear whether L-arginine administration improves endothelial function [8]. Moreover, experimental studies have demonstrated the phenomenon named L-arginine paradox. L-Arginine is a semi-essential amino acid, but increased concentration of L-arginine by exogenous administration is required to elicit maximal NO release from cells [9].

It is well known that subjects physically active present lower prevalence of cardiovascular diseases and the practice of regular physical exercise might delay the developing of cardiovascular events in subjects with cardio metabolic disorders [10,11]. The beneficial effects

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of physical exercise are related to the activation of several physiological systems improving sympatho-vagal balance [12,13], lipid profile, and increased NO production or its bioavailability [10,14]. Particularly in endothelial cells, physical exercise is a powerful stimulus to promote vascular shear stress activating mechanosensors. These mechanosensors are coupled to complex biochemical signal pathways, such as Ras/MEK/ERK, c-Src, G proteins, ion channel, VE-cadherin, and PI3K/Akt, which in turn regulate NO/cGMP pathway [15].

Given that the incidence of cardiovascular diseases in women increases after menopause, pharmacological and non-pharmacological approaches to control blood pressure or to prevent its complication are clinically relevant to this population. Therefore, the aim of this study is to examine the effects of L-arginine administration associated with a single bout of aerobic physical exercise on blood pressure in normotensive postmenopausal women. To further evaluate the insight mechanisms of the association, we measured cardio-inflammatory biomarkers in plasma.

2. Methods

2.1. Participants

This study was approved by the Ethical Committee of Institute of Bioscience of the University of São Paulo State (UNESP, protocol number 6817). Sixteen normotensive postmenopausal women were enrolled in this study. The inclusion criteria for the participants in this study were: normotensive; body mass index ≤30 kg/m^2; physically inactive (<150 min of moderate physical activity per week or <60 min of vigorous physical activity per week). The exclusion criteria were diabetes, smoking, menopausal hormone therapy (MHT) use, presence of cardiovascular or renal diseases or any orthopedic muscular problems affecting exercise on the treadmill. Postmenopausal status was determined as the absence of menses for at least 1 year. The volunteers were informed about the procedures and risks of the study before accepting to participate and signed a consent form in accordance with the Ethical Committee of UNESP.

2.2. Study design

This study was conducted as a double-blinded, randomized, crossover design. Participants were instructed to maintain their regular diet and daily activities during the study period. After that, the volunteers were familiarized with exercise on the treadmill and the aerobic fitness and exercise intensity were determined using the maximal lactate steady state (MLSS) test. The exercise intensity corresponding to MLSS was used during exercise trials, and it was chosen because it has a better correlation with performance in endurance exercise. It represents the highest exercise intensity with a steady state in several physiological parameters such as lactate, oxygen consumption, carbon oxide output and respiratory exchange ratio [16]. Briefly, postmenopausal women performed two to five tests with fixed duration (30 min) and walking speed (5.5 km/h) on a treadmill (Movement RT 250 PRO). The grade of the ergometer ranged from 1 to 15%, and it was used to control the intensity, which was adjusted in each test according to the aerobic capacity of the participant. Measurement of the blood lactate concentration was performed at rest, after 10 and 30 min. MLSS was determined when the difference of blood lactate concentration between 10th and 30th minutes did not exceed 1 mM [16].

To check the effectiveness of L-arginine administration and/or aerobic exercise, all participants completed four acute experimental trials separated by at least 72 h, with no more than 7 days apart, and in at random. Participants were instructed to avoid vigorous exercise, caffeine and alcohol consumption at least for 24 h before the trials.

2.3. Experimental trials

Each experimental trial was performed in the morning, and lasted about 3.5 h. The experimental trials consisted of: acute L-arginine administration and aerobic exercise (A-E); acute L-arginine administration alone (ARG); aerobic exercise without L-arginine administration (EXE); and no exercise or L-arginine administration (CON).

The trials started at 7:00 am and the volunteers were instructed to arrive after eating their regular breakfast. The breakfast was recorded and did not differ among the four sessions of each volunteer. After 20 min of resting in a seated position, blood pressure and heart rate were measured, and blood samples were collected (baseline). In A-E day, 9 g of L-arginine base (acid (2S)-2-amino-5-guanidopentanoic — Ajinomoto, Japan) was administrated orally, and 45 min after, the participants performed 30 min of exercise on a treadmill at the MLSS intensity. Given that the bioavailability of L-arginine reaches its peaks within 1 h, lasting for several hours, both dose and time were chosen based on pharmacokinetic properties obtained in previous studies [17–20]. Blood venous samples were collected at baseline, before interventions and 45 min after the end of the exercise/resting intervention. Following the exercise, blood pressure was measured every 15 min, over a 90-minute period. Participants also performed further three different trials in random order: EXE day — (exercise performance associated with placebo pill intake); ARG day — no exercise performance and only L-arginine ingestion; and CON day — no L-arginine administrated nor exercise performed. Office blood pressure measurements and blood samples were obtained at the same time points in all experimental trials. Ambulatory blood pressure started 90 min after exercise/resting intervention had finished (10:15 am). Systolic and diastolic blood pressures were recorded for 24 h as described in Fig. 1.

2.4. Office blood pressure and ambulatory blood pressure monitoring

The volunteers were instructed not to exercise outside the laboratory before cardiovascular measurements. After 15 min sitting rested position, office blood pressure was measured by auscultation using aneroid sphygmomanometer (Tycos, Raleigh, NC). All measurements were performed three times and the average values were used to determine changes in blood pressure. The delta of both systolic and diastolic blood pressures was calculated subtracting the blood pressure in each time point from that measured at baseline. The incremental areas under the curve (AUC) of blood pressure over time were also calculated using the trapezoidal method, and compared in experimental trials.

Ambulatory blood pressure monitoring was performed using a non-invasive automatic device (DYNAMAPA+ Cardius, SP, Brazil) with the cuff on the non-dominant arm fitted during the period of 24 consecutive hours. The ambulatory blood pressure (ABPM) from all volunteers starting 90 min after exercise (EXE and A-E experimental trials) or resting (CON and ARG experimental trials). Measurements were taken every 15 min during awake time and every 30 min during asleep time. Awake and asleep times period were done based in their daily activities reporting. The volunteers were instructed to maintain their normal daily activities and to avoid vigorous muscular activity performance during the monitoring period. Volunteers who had an error >20% on the measurements were reevaluated in the entire experiment.

2.5. Blood samples

Fasting blood samples were collected after 12 h of overnight fast. Venous blood samples were collected at baseline, before interventions and 45 min after the end of the exercise/rest intervention. Briefly, 12 mL of venous blood samples were collected using two vacuum tubes (BD vacutainer tubes®): One for plasma (EDTA K3) and another for serum (clot activator and gel for serum separation). Plasma tubes were immediately centrifuged at 3000 rpm, 12 min at 4 °C. Serum tubes sat for 30 min at room temperature and then centrifuged. After

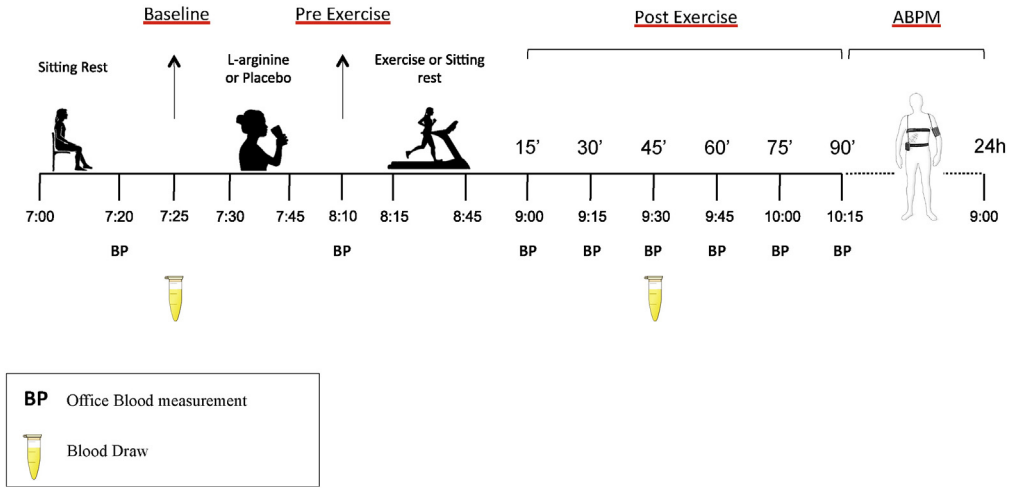

Fig. 1. Experimental design. All volunteers arrived at 7:00 am at clinical research unit, remained seated and resting for 20 min, and ingested 9 g of L-arginine or placebo, followed by a 30 min of aerobic exercise performance, 30 min of exercise or sitting rest. Blood pressures (BP) were measured at baseline (7:25 am), pre-exercise or sitting rest (8:10 am) and every 15 min during 90 min at clinical research unit, and during 24 h using ambulatory blood pressure measurement (ABPM). Blood draws were performed at baseline (7:25 am) and 45 min (9:30 am) after exercise performance.

that, the supernatant (plasma and serum) was stored in aliquots at −80 °C for future biochemical analyses.

2.6. Biochemical assays

Total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), triglycerides and blood glucose were determined using automated standard methods (Cobas Mira Plus).

Nitrite/nitrate (NO_x^-) plasma concentrations were measured by a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA) and used according to the manufacturer's instructions. Samples were ultra-filtered through micro filter (Microcon centrifugal filters units, 10 kDa, Milipore, Bedford, MA) before the assays.

Plasma cyclic guanosine monophosphate (cGMP) levels were measured by ELISA method using a commercially available kit (R&D Systems, Mineapolis, MN, USA), according to manufacturer's instructions.

Superoxide dismutase (SOD), catalase activity and malondialdehyde (MDA) levels were measured by ELISA using a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA). The SOD's assay detects superoxide radicals generated by xanthine oxidase and hypoxanthine, revealing the plasma activity of this enzyme. Catalase's assay is based on the reaction of the enzyme with methanol in an optimal H_2O_2 concentration. MDA levels were determined to evaluate lipid peroxidation according to Yagi [21].

Interleukin 6 (IL-6) and interleukin 10 (IL-10) plasma levels were measured by ELISA kits (R&D Systems, Mineapolis, MN, USA).

2.7. Statistical analysis

Data are presented as mean ± standard error. Biochemical analyses are present as mean and upper and lower 95% of confidence interval (95% CI). The Komogorov-Smirnov's test was used to test for the normality of the data. The effect of the intervention on the ambulatory blood pressure AUC between the trials was evaluated using one-way ANOVA. Post hoc comparisons were made with the Newman-Keuls test when the analysis of variance produced significant interaction. The effect of intervention over time on blood pressure, inflammatory and antioxidants makers was evaluated by two-way ANOVA (Time vs Trial) and post hoc comparison was made using the Newman-Keuls test when the analysis of variance produced a significant interaction. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using STATISTICA, version 7.0.

3. Results

3.1. Measurements at baseline

All women were overweight, and approximately 56% of the participants were not on drug therapy, 13% were on hypolipidemic therapy, 13% were on hypothyroid therapy and 19% were on anxiolytic drug therapy. The characteristics of the subjects are showed in Table 1.

In order, to examine the NO/cGMP signaling pathway, both biomarkers were evaluated. Neither NO_x^- nor cGMP levels were different in any experimental trials at baseline (Table 2). Similarly, inflammatory biomarkers and redox state were not distinct in normotensive postmenopausal women at baseline (Table 3).

Table 1
Characteristics of studied population (n = 16).

Age (yrs)	57 ± 6
Time after menopause (yrs)	8 ± 6
BMI (kg/m^2)	27 ± 3
W-C (cm)	86 ± 10
WHR	0.82 ± 0.06
Resting SBP (mm Hg)	115 ± 11
Resting DBP (mm Hg)	73 ± 8
Resting HR (bpm)	75 ± 10
Total cholesterol (mg/dL)	190.8 ± 29.8
HDL-C (mg/dL)	51.4 ± 13.8
LDL-C (mg/dL)	122.8 ± 17.5
Triglycerides (mg/dL)	97.9 ± 49.4
VLDL (mg/dL)	19.6 ± 9.9
Glucose (mg/dL)	87.0 ± 10.7
FSH (mIU/mL)	42.8 ± 21.4
LH (mIU/mL)	26.7 ± 13.7
No drug therapy	n = 9 (56%)
Anxiolytic drug therapy	n = 3 (19%)
Hypolipemiant therapy	n = 2 (13%)
Hypothyroidism drug therapy	n = 2 (13%)

BMI: body mass index; W-C: waist circumference; WHR: waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; VLDL: very low density lipoprotein; FSH: follicle-Stimulating hormone; LH: luteinizing hormone.

Table 2
Plasma NO_x (nitrite/nitrate) and cyclic guanosine monophosphate (cGMP) levels in postmenopausal women at baseline and after 45 min (PE-45′) during experimental trials: control (CON), arginine (ARG), exercise (EXE), arginine + exercise (A-E), n = 16.

	Baseline	PE-45′
NO_x^- (μM)		
CON	7.6 (4.7–10.4)	6.7 (4.8–8.7)
ARG	7.8 (6.4–9.1)	6.5 (5.2–7.7)
EXE	9.0 (6.7–11.3)	8.1 (6.0–10.3)
A-E	8.6 (5.3–12.0)	8.0 (5.1–11.0)
cGMP (pmol/mL)		
CON	104.0 (77.8–130.1)	100.7 (79.0–122.4)
ARG	110.8 (87.3–134.3)	100.7 (77.9–123.5)
EXE	105.0 (82.5–127.5)	110.0 (67.3–152.6)
A-E	111.1 (75.5–146.7)	127.3 (78.6–176.1)

The numbers in parenthesis mean the 95% confidence interval.

3.2. Acute session trials

Average values for exercise intensity, blood lactate concentration, heart rate, and rate of perceived exertion at the maximal lactate steady state intensity were 6 ± 2% of treadmill grade (or treadmill inclination), 3.0 ± 1.0 mM, 157 ± 9 bpm and 13 ± 2, respectively. The intensity of exercise was used during acute experimental trials. Exercise performance and the workloads did not differ between tests within the same volunteer.

Systolic blood pressure measurements (from 15 to 90 min) showed a significant decrease in all time points in both EXE and A-E trials as compared with CON and ARG trials, approximately −5 to −9 mm Hg (Fig. 2A). In contrast, diastolic blood pressure was significantly reduced after the exercise session only in A-E trial, approximately −2 to −4 mm Hg, as compared with CON and ARG trials (Fig. 2B).

Regarding ambulatory blood pressure, the AUC of systolic blood pressure was significantly reduced in both EXE and A-E trials comparing with CON and ARG trials. No differences were observed in systolic blood pressure over time when changes were calculated using the differences between the values (Fig. 3, panels A and B). Diastolic blood pressure monitoring did not change in any of the trials, either in AUC or in the differences between the values (Fig. 3, panels C and D). L-Arginine administration failed to improve or to change ambulatory blood pressure responses after exercise performance.

Table 3
Plasma superoxide dismutase (SOD), catalase activity, malondialdehyde (MDA), interleukine-6 (IL-6) and interleukine-10 (IL-10) in postmenopausal women at baseline, and after 45 min (PE-45′) during experimental trials: control (CON), arginine (ARG), exercise (EXE), Arginine + Exercise (A-E), n = 16.

	Baseline	PE-45′
Superoxide dismutase — SOD (U/mL)		
CON	3.5 (3.2–3.8)	2.7 (2.3–3.1)
ARG	3.3 (3.0–3.7)	2.7 (2.5–3.0)
EXE	3.3 (3.0–3.6)	2.8 (2.5–3.0)
A-E	3.3 (2.9–3.7)	2.7 (2.5–2.9)
Catalase activity (nmol/min/mL)		
CON	38.5 (18.5–58.4)	41.7 (24.5–49.8)
ARG	37.1 (24.5–49.8)	35.9 (27.2–44.5)
EXE	40.3 (29.5–51.0)	36.6 (27.5–45.7)
A-E	40.4 (29.5–51.3)	32.7 (26.2–39.2)
Malondialdehyde — MDA (μM)		
CON	4.3 (3.6–5.1)	3.9 (3.2–4.6)
ARG	4.1 (3.6–4.7)	4.3 (3.6–5.0)
EXE	5.3 (3.7–6.8)	4.1 (3.4–4.7)
A-E	4.2 (3.6–4.8)	3.9 (3.3–4.5)
IL-6 (pg/mL)		
CON	28.4 (22.7–34.1)	28.2 (23.2–33.4)
ARG	29.3 (25.2–33.3)	28.8 (23.9–33.6)
EXE	30.2 (24.3–36.1)	30.0 (24.2–35.8)
A-E	31.7 (25.5–33.6)	34.0 (22.4–32.1)
IL-10 (pg/mL)		
CON	3.3 (2.0–4.7)	3.0 (1.9–4.1)
ARG	4.4 (2.7–6.2)	4.4 (2.4–6.4)
EXE	5.5 (3.4–7.5)	4.9 (3.5–6.3)
A-E	3.5 (2.3–4.6)	3.8 (2.1–5.6)

The numbers in parenthesis mean the 95% confidence interval.

3.3. Biochemical analyses

Given that evidence has shown a key role of NO on blood pressure regulation mainly after physical exercise, NO/cGMP pathway and its bioavailability were analyzed by measurement of the redox state in all experimental trials. Neither NO_x^- nor cGMP concentrations were affected by acute L-arginine administration or a bout of exercise. Table 2 summarizes these data. Similarly, no changes were found in the redox state after 45 min of L-arginine administration or a bout of exercise (PE-45′) by measuring the plasma SOD and catalase activity, as well as lipid peroxidation by measuring the plasma MDA concentrations (Table 3).

4. Discussion

This study examined whether a single dose of L-arginine associated with a bout of aerobic exercise would reduce blood pressure. We further investigated plasma biomarkers in an attempt to detect which one could be mediating the cardiovascular responses in normotensive postmenopausal women. The main finding was that L-arginine in association with aerobic exercise was effective in lowering office diastolic blood pressure during 90 min in comparison with control and no exercise day. Additionally, during 24-hour period of blood pressure monitoring, only exercise was effective in changing systolic blood pressure, no additional effect of L-arginine over time was seen. Interestingly, the reduction of office diastolic blood pressure in response to the combination of the L-arginine plus a bout of exercise was not accompanied by changes in NO/cGMP pathway, redox status or inflammatory biomarkers in normotensive postmenopausal women.

Several mechanisms have been pointed out to explain the reduction in blood pressure after a single bout of exercise, such as the hyperemia derived from exercise performance [22,23], baroreceptor activity [24], reduction on sympathetic activity [13], reduction of cardiac output by lowering stroke volume [25] as well as release of vasodilatation substances, including histamine and NO [10,23]. Despite of the combination led to a reduction in diastolic blood pressure, we failed in detecting the any changing in NO/cGMP pathway or any evaluated biomarkers. Accordingly, previous studies showed no changes in plasma NO_x^- or cGMP levels in response to acute or chronic L-arginine administration even though positive effects in endothelial-derived dilation, blood flow and peripheral resistance were observed [26–30]. Additionally, no changes in SOD, catalase activity and MDA plasma concentrations were found after aerobic exercise performance or L-arginine administration alone or in combination. SOD and catalase activity as well as MDA levels which reflects lipid peroxidation and oxidative stress have been reported as important biomarkers in several diseases including diabetes, arterial hypertension and atherosclerosis [31]. However, it is not clear yet whether in postmenopausal status could alter the redox state. In addition, few studies showed positive effects of acute L-arginine supplementation in redox state and oxidative stress [32,33], as well as the findings using chronic interventions are not conclusive.

In a similar way, no changes in IL-6 and IL-10 were found after any interventions. Since diabetes was an exclusion criterion, the lack of changing in inflammatory mediators might have been due to the absence of chronic metabolic disorders in the studied population even though results from experimental models of menopause have found an increase in pro- and a decrease in anti-inflammatory mediators [34]. A decrease in pro-inflammatory cytokines such as TNF-α and an

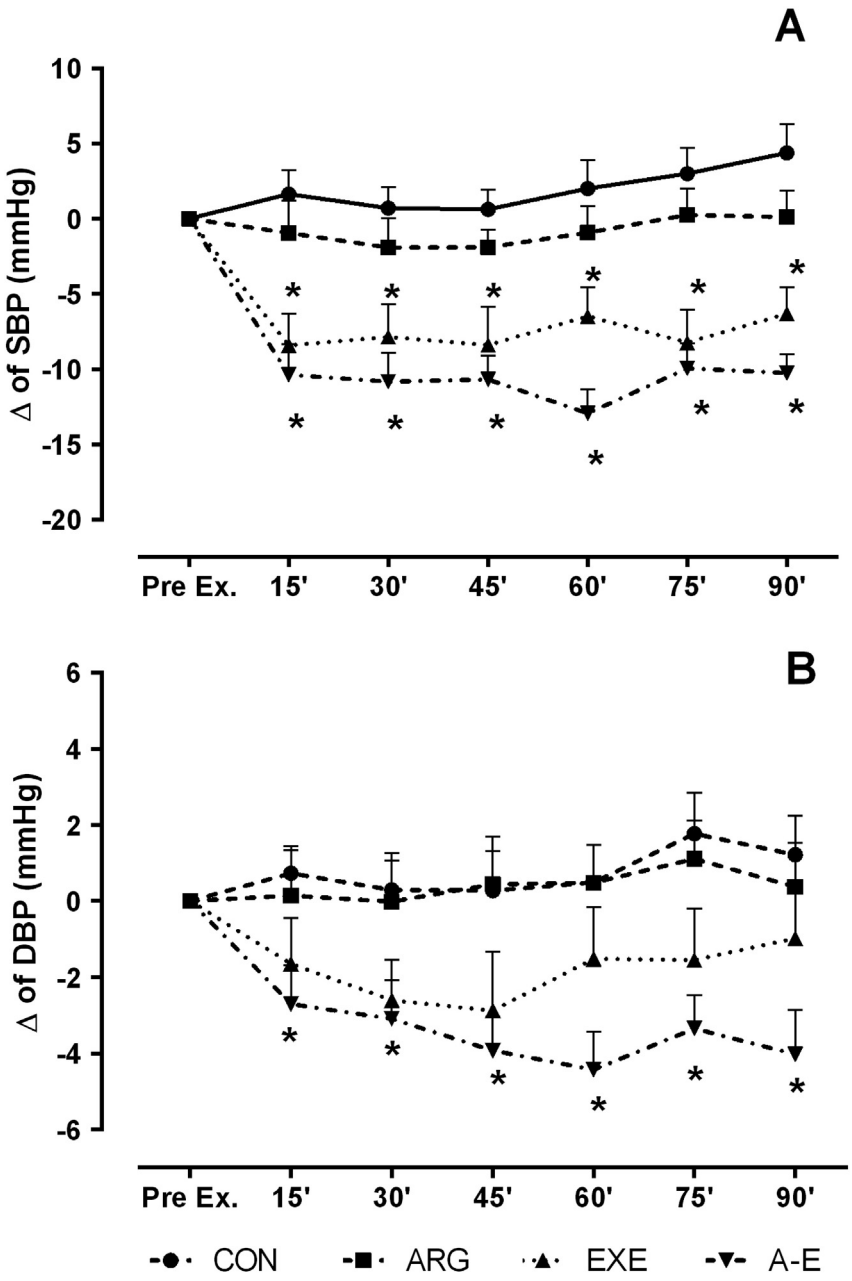

Fig. 2. Blood pressure measurements at clinical research unit. Delta of systolic blood pressure (A) and Delta of diastolic blood pressure (B) during 90 min in control (CON), arginine (ARG), exercise (EXE) and arginine + exercise (A-E) trials. * $p < 0.05$ in relation to the same time points in CON and ARG days.

increase in anti-inflammatory cytokines such as IL-10 during exercise performance were shown in previous studies [35,36]. However, these changes are detected only during exercise performance and can fall drastically down to rest levels. Another possibility for the lacking of changes in inflammatory mediators could be the type of exercise. Previous study has shown that resistance exercise is effective in changing pro- and anti-inflammatory mediators in obese postmenopausal women [37]. These findings indicate that different forms of exercise should be tested in this population.

ABPM is considered a useful tool in clinical trials providing additional information during daily activities of the patients as well as the behaviour of blood pressure during night-time [38]. The ABPM has been used in the last decades, as an important measurement to predict risks in progression of cardiovascular disease, allowing a better prognosis [39]. In agreement with office measurements of blood pressure, a significant reduction in systolic blood pressure during 24 h was found in women who performed an exercise session. Few studies have examined the effects of exercise on blood pressure over 24 h in women only. One study showed that normotensive postmenopausal women had increased systolic blood pressure values as compared with premenopausal women during daytime and nighttime [40]. Another study showed that exercise training is effective in promoting beneficial effects on daytime ambulatory BP in healthy postmenopausal women on estrogen therapy [41]. Thus, this study is the first to show that a bout of physical exercise is successful in lowering systolic blood pressure in normotensive postmenopausal women. In contrast, L-arginine alone or in combination did not affect the blood pressure behaviour. Since most of studies using L-arginine or physical exercise in human population have performed long-term interventions, but with different participants in each group, the crossover format of this study is fundamental to check the interaction of both interventions in the same participants because of the variability of human population. Besides, acute session is more feasible for examining all the measurements in

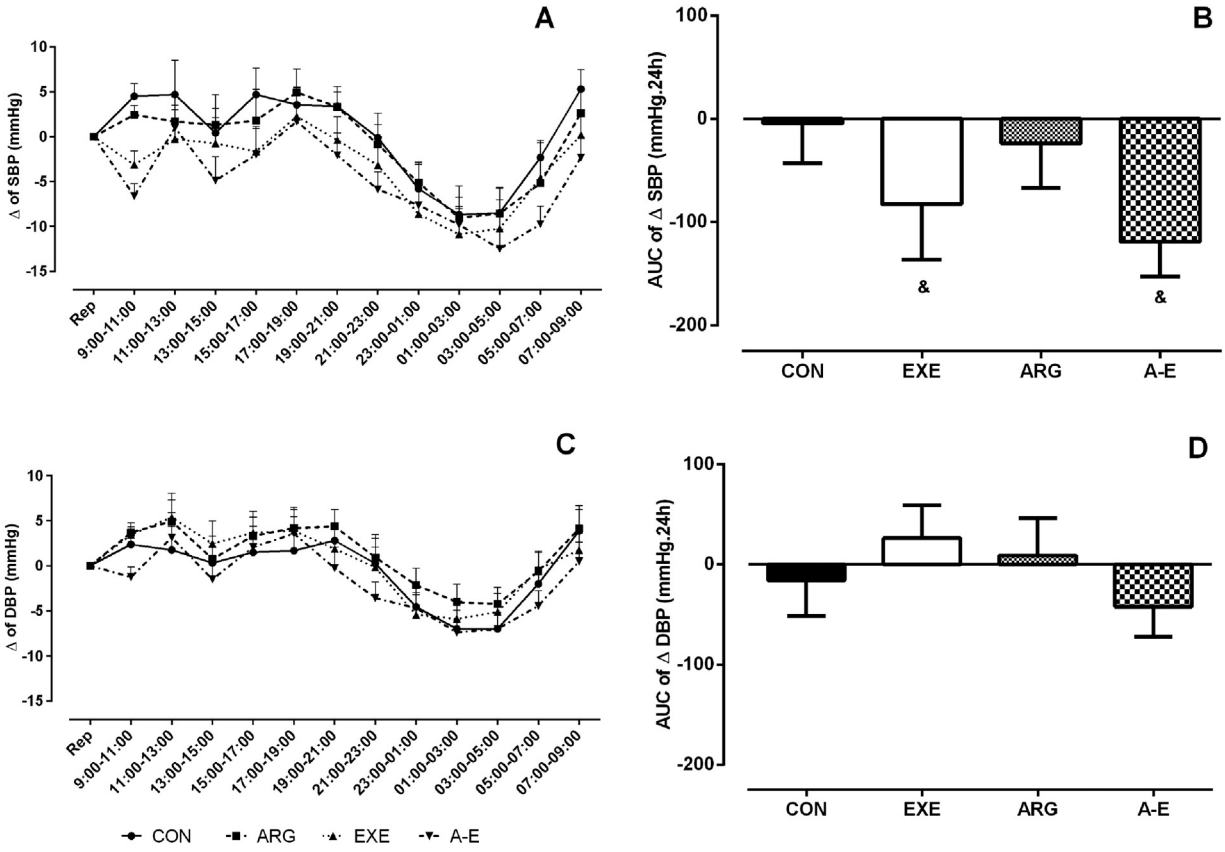

Fig. 3. Ambulatory blood pressure measurements. Delta of systolic blood pressure (A) and its incremental area under the curve — AUC (B) and Delta of diastolic blood pressure (C) and its incremental area under the curve (D) during 24 h in control (CON), arginine (ARG), exercise (EXE) and arginine + exercise (A-E) trials. & $p < 0.05$ in relation to no exercise days (CON and ARG).

the same participants even though physical training is desirable for health promotion.

In conclusion, the combination of L-arginine plus a bout of physical exercise was effective in lowering diastolic blood pressure in normotensive postmenopausal women after 90 min of intervention. Additionally, physical exercise promoted, independently from L-arginine ingestion, a long lasting effect on systolic blood pressure measured by ABPM in this population. Intriguing, this beneficial effect was not associated with changes in the cardio-inflammatory biomarkers. Possibly, other factors such as neural influences could be mediating this effect.

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Disclosures

The authors declare no conflict of interest.

References

[1] L.R. Simkin-Silverman, R.R. Wing, M.A. Boraz, L.H. Kuller, Lifestyle intervention can prevent weight gain during menopause: results from a 5-year randomized clinical trial, Ann. Behav. Med. 26 (3) (2003) 212–220.
[2] D. Mozaffarian, E.J. Benjamin, A.S. Go, D.K. Arnett, M.J. Blaha, M. Cushman, S. de Ferranti, J.P. Després, H.J. Fullerton, V.J. Howard, D.M. Huffman, S.E. Judd, B.M. Kissela, D.T. Lackland, J.H. Lichtman, L.D. Lisabeth, S. Liu, R.H. Mackey, D.B. Matchar, D.K. McGuire, E.R. Mohler III, C.S. Moy, P. Muntner, M.E. Mussolino, K. Nasir, R.W. Neumar, G. Nichol, L. Palaniappan, D.K. Pandey, M.J. Reeves, C.J. Rodriguez, P.D. Sorlie, J. Stein, A. Towfighi, T.N. Turan, S.S. Virani, J.Z. Willey, D. Woo, R.W. Yeh, M.B. Turner, on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee., Heart disease and stroke statistics—2015 update: a report from the American Heart Association, Circulation (2015).
[3] M. Coylewright, J.F. Reckelhoff, P. Ouyang, Menopause and hypertension: an age-old debate, Hypertension 51 (4) (2008) 952–959.
[4] R. Maranon, J.F. Reckelhoff, Sex and gender differences in control of blood pressure, Clin. Sci. (Lond.) 125 (2013) 311–318.
[5] A.A. Knowlton, A.R. Lee, Estrogen and the cardiovascular system, Pharmacol. Ther. 135 (1) (2012) 54–70.
[6] K. Bian, F. Murad, Nitric oxide signaling in vascular biology, J. Am. Soc. Hypertens. 1 (1) (2007) 17–29.
[7] J.P.F. Chin-Dusting, L. Willems, D.M. Kaye, L-Arginine transporter in cardiovascular disease: a novel therapeutic target, Pharmacol. Ther. 116 (2007) 428–436.
[8] S.P. Schulman, L.C. Becker, D.A. Kass, et al., L-Arginine therapy in acute myocardial infarction: the vascular interaction with age in myocardial infarction (VINTAGE MI) randomized clinical trial, JAMA 295 (1) (2006) 58–64.
[9] S. Elms, F. Chen, Y. Wang, J. Qian, B. Askari, Y. Yu, D. Pandey, J. Iddings, R.B. Caldwell, D.J. Fulton, Insights into the arginine paradox: evidence against the importance of subcellular location of arginase and eNOS, Am. J. Physiol. Heart Circ. Physiol. 305 (5) (2013) H651–H666.
[10] E. Zanesco, Antunes. Effets of exercise training on the cardiovascular system: Pharmacological approaches, Pharmacol. Ther. 114 (2007) 307–317.
[11] L. Vanhees, N. Geladas, D. Hansen, et al., Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR. Part II, Eur. J. Prev. Cardiol. 19 (2012) 1005–1033.
[12] M.C. Martins-Pinge, Cardiovascular and autonomic modulation by the central nervous system after aerobic exercise training, Braz. J. Med. Biol. Res. 44 (2011) 848–854.
[13] J.R. Carter, C.A. Ray, Sympathetic neural adaptations to exercise training in humans, Auton. Neurosci. 188 (2015) 36–43.
[14] G. Schuler, V. Adams, Y. Goto, Role of exercise in the prevention of cardiovascular disease: results, mechanisms, and new perspectives, Eur. Heart J. 34 (2013) 1790–1799.
[15] J.L. Balligand, O. Feron, C. Dessy, eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues, Physiol. Rev. 89 (2) (2009) 481–534.
[16] R. Beneke, Maximal lactate steady state concentration (MLSS): experimental and modeling approaches, Eur. J. Appl. Physiol. 88 (2003) 361–369.
[17] O. Tangphao, M. Grossman, S. Chalon, B.B. Hoffman, F. Terrence, Pharmacokinetis of intravenous and oral L-arginine in normal volunteer, Br. J. Clin. Pharmacol. 47 (1999) 261–266.

[18] R. Hambrecht, L. Hilbrich, S. Erbs, S. Gielen, E. Fiehn, N. Schoene, G. Schuler, Correction of endothelial dysfunction in chronic heart failure: additional effects of exercise training and oral L-arginine supplementation, J. Am. Coll. Cardiol. 35 (3) (2000) 706–713.
[19] S.C. Forbes, G. Bell, The acute effects of a low and high dose of oral L-arginine supplementation in young active males at rest, Appl. Physiol. Nutr. Metab. 36 (2011) 405–411.
[20] E.I. Closs, M.A. Ostad, A. Simon, A. Warnholtz, A. Jabs, A. Habermeier, A. Daiber, U. Förstermann, T. Münzel, Impairment of the extrusion transporter for asymmetric dimethyl-L-arginine: a novel mechanism underlying vasospastic angina, Biochem. Biophys. Res. Commun. 423 (2) (2012) 218–223.
[21] K. Yagi, Simple assay for the level of total lipid peroxides in serum or plasma, Methods Mol. Biol. 108 (1998) 101–106.
[22] J. Bangsbo, Y. Hellsten, Muscle blood flow and oxygen uptake in recovery from exercise, Acta Physiol. Scand. 162 (1998) 305–312.
[23] J. Halliwill, T.M. Buck, A.N. Lacewell, S.A. Romero, Post-exercise hypotension and sustained post-exercise vasodilation: what happens after we exercise? Exp. Physiol. 98 (1) (2013) 7–18.
[24] C.Y. Chen, A.C. Bonham, Post exercise hypotension: central mechanisms, Exerc. Sport Sci. Rev. 38 (3) (2010) 122–127.
[25] C.C. Rezk, R.C. Marrache, T. Tinucci, D. Mion Jr., C.L.M. Forjaz, Post-resistance exercise hypotension, hemodynamics, and heart rate variability: influence of exercise intensity, Eur. J. Appl. Physiol. 98 (1) (2006) 105–112.
[26] G. Theilmeier, J.R. Chan, C. Zalpour, B. Anderson, B.Y. Wang, A. Wolf, P.S. Tsao, J.P. Cooke, Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 3557–3564.
[27] C. Baylis, P. Vallance, Measurement of nitrite and nitrate levels in plasma and urine—what does this measure tell us about the activity of the endogenous nitric oxide system? Curr. Opin. Nephrol. Hypertens. 7 (1998) 59–62.
[28] L. Blum, R. Hathaway, Mincemoyer, et al., Effects of oral L-arginine on endothelium-dependent vasodilation and markers of inflammation in healthy postmenopausal women, J. Am. Coll. Cardiol. 35 (2) (2000) 271–276.
[29] R.W. Evans, J.D. Fernstrom, J. Thompson, S.M. Morris Jr., L.H. Kuller, Biochemical responses of healthy subjects during dietary supplementation with L-arginine, J. Nutr. Biochem. 15 (2004) 534–539s.
[30] T.S. Álvares, C.A. Conte Jr., V.M.F. Paschoalin, et al., Acute L-arginine supplementation increases muscle blood volume but not strength performance, Appl. Physiol. Nutr. Metab. 37 (2012) 115–126.
[31] A.P. Jarrete, I.P. Novais, H.A. Nunes, G.M. Puga, M.A. Delbin, A. Zanesco, Influence of aerobic exercise training on cardiovascular and endocrine-inflammatory biomarkers in hypertensive postmenopausal women, J. Clin. Transl. Endocrinol. 1 (3) (2014) 108–114.
[32] H. Kawano, T. Motoyama, N. Hirai, K. Kugiyama, H. Yasue, H. Ogawa, Endothelial dysfunction in hypercholesterolemia is improved by L-arginine administration: Possible role of oxidative stress, Atherosclerosis 161 (2) (2002 Apr) 375–380.
[33] Y. Lin, L.N. Wang, Y.H. Xi, H.Z. Li, F.G. Xiao, Y.J. Zhao, Y. Tian, B.F. Yang, C.Q. Xu, L-Arginine inhibits isoproterenol-induced cardiac hypertrophy through nitric oxide and polyamine pathways, Basic Clin. Pharmacol. Toxicol. 103 (2) (2008 Aug) 124–130.
[34] F.F. Conti, O. Brito Jde, N. Bernardes, S. Dias Dda, C. Malfitano, M. Morris, S.F. Llesuy, M.C. Irigoyen, K. De Angelis, Positive effect of combined exercise training in a model of metabolic syndrome and menopause: autonomic, inflammatory, and oxidative stress evaluations, Am. J. Physiol. Regul. Integr. Comp. Physiol. 309 (12) (2015 Dec 15) R1532–R1539.
[35] B.K. Pedersen, M.A. Febbraio, Muscle as an endocrine organ: focus on muscle-derived interleukin-6, Physiol. Rev. 88 (4) (2008 Oct) 1379–1406.
[36] G. Van Hall, A. Steensberg, C. Fischer, C. Keller, K. Møller, P. Moseley, B.K. Pedersen, Interleukin-6 markedly decreases skeletal muscle protein turnover and increases nonmuscle amino acid utilization in healthy individuals, J. Clin. Endocrinol. Metab. 93 (7) (2008 Jul) 2851–2858.
[37] M.D. Phillips, R.M. Patrizi, D.J. Cheek, J.S. Wooten, J.J. Barbee, J.B. Mitchell, Resistance training reduces subclinical inflammation in obese, postmenopausal women, Med. Sci. Sports Exerc. 44 (11) (2012 Nov) 2099–2110.
[38] R. Fagard, L. Vanhees, Twenty-four hour blood pressure after exercise in patients with coronary artery disease, J. Hum. Hypertens. 14 (2000) 231–234.
[39] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, Heart rate variability: standards of measurement, physiological interpretation and clinical use, Eur. Heart J. 17 (1996) (354–38).
[40] D.R. Seals, E.T. Stevenson, P.P. Jones, C.A. DeSouza, H. Tanaka, Lack of age-associated elevations in 24-h systolic and pulse pressures in women who exercise regularly, Am. J. Phys. 277 (3 Pt 2) (1999 Sep) H947–H955.
[41] C.G. Cardoso Jr., F.C. Rosas, B. Oneda, E. Labes, T. Tinucci, S.B. Abrahão, A.M. da Fonseca, D. Mion Jr., C.L. Forjaz, Aerobic training abolishes ambulatory blood pressure increase induced by estrogen therapy: a double blind randomized clinical trial, Maturitas 69 (2) (2011 Jun) 189–194.