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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