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ORIGINAL ARTICLE

Exercise intensity modulates nitric oxide and blood pressure responses in hypertensive older women

Hugo A. P. Santana · Sérgio R. Moreira · Ricardo Y. Asano · Marcelo M. Sales · Cláudio Córdova · Carmen S. G. Campbell · Foued S. Espindola · Andrei C. Sposito · Otávio T. Nóbrega · Herbert G. Simões

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Abstract

Background and aims Whether intensity or other characteristics of physical activity can better promote the release of nitric oxide (NO) and reduction of blood pressure in hypertensive older-adults is still unknown. In this study, the post-exercise blood pressure (BP) response and NO release after different intensities of aerobic exercise in elderly women were analyzed.

Methods Blood pressure response and NO were analyzed in 23 elderly mildly hypertensive women. Participants underwent (1) high-intensity incremental exercise (IT); (2) moderate-intensity 20 min exercise at 90 % of the anaerobic threshold (AT), and (3) control (CONT) session. BP was measured before and after interventions; volunteers remained seated for 1 h. NO estimates were made through NO_2^- analyses.

Results After CONT session, both diastolic BP and mean arterial pressure (MAP) were significantly higher than during pre-exercise resting. Post-exercise hypotension (PEH) was observed after exercise at IT and 90 % of AT. Although exercise in both sessions lowered SBP and MAP compared with CONT, exercise at the highest intensity (IT) was more effective on lowering systolic BP after exercise. In comparison with pre-exercise resting, NO_2^- increased significantly only after IT, but both exercise sessions caused NO_2^- to increase compared with CONT.

Conclusion Exercise intensity and NO release may exert a role in eliciting PEH in mildly hypertensive elderly women.

Keywords Aged · Atherosclerosis · Exercise test · Nitric oxide · Post-exercise hypotension

H. A. P. Santana (✉) · S. R. Moreira · R. Y. Asano · M. M. Sales · C. Córdova · C. S. G. Campbell · H. G. Simões (✉)
Graduate Program in Physical Education and Health, Catholic University of Brasília, Q.S. 07, lote 01, EPCT, Águas Claras, Taguatinga, Brasília, DF CEP 71966-700, Brazil
e-mail: hsantana85@gmail.com

H. G. Simões
e-mail: hgsimoes@gmail.com

F. S. Espindola
Institute of Genetics and Biochemistry, Federal University of Uberlândia, Uberlândia, MG, Brazil

A. C. Sposito
Faculty of Medical Sciences, State University of Campinas, Campinas, SP, Brazil

O. T. Nóbrega
Graduate Program in Medical Sciences, University of Brasília, Brasília, DF, Brazil

Introduction

During aging, the functional and structural deterioration of the arterial wall advances so slowly that it often goes unnoticed. Over time, aging reduces the functional reserve and gradually increases the susceptibility of the arterial wall to insults such as smoking, physical inactivity, diabetes, and other cardiovascular risk factors. Thus, the prevalence of hypertension increases progressively throughout life, reaching over 50 % in individuals aged between 60 and 69 years and about 75 % in those aged of 70 years or more [1]. In this scenario, genetic inheritance and its interaction with harmful or protective factors influence the rate of deterioration of the artery wall function through various mechanisms as such, for example, modulation of nitric oxide (NO) production [2].

Among these modulating factors, estrogen is particularly noteworthy for its intense and beneficial effect on endothelial function by increasing production and decreasing degradation of NO [3]. In fact, significant impairment of endothelial-dependent arterial dilation becomes apparent at the age of menopause [4], after which a rapid loss of endothelial function occurs, contributing to a rise in the prevalence of hypertension to levels equal to or even higher than those found in men [5].

A decrease in resting blood pressure after a single bout of exercise, i.e., post-exercise hypotension (PEH), has been described as an indicator of decreased cardiac output, total peripheral resistance, or both. The decrease in peripheral resistance induced by physical activity mainly results from increased bioavailability of NO produced by endothelial cells in response to autonomic, hemodynamic and humoral stimuli [6]. In subjects without heart disease, NO production is directly related to the intensity of physical activity [7].

Apart from estrogen deficiency, aging may idiopathically affect the production of NO [8]. For this group of individuals, it remains unknown whether intensity and/or characteristics of physical activity can better promote the release of NO and reduction of peripheral resistance [9–11]. By inference, it is reasonable to question whether physical activity can improve BP control in older women, and the aim of this study was to verify the effects of different regimes of aerobic exercise on BP responses and NO pool in elderly women. Together with the reasoning above, older women make up the fastest growing segment of the Brazilian population [12], which justifies attention towards this age group.

Materials and methods

Participants

Women aged 65 years or more, who had had hypertension treated in the previous 6 months by monotherapy with hydrochlorothiazide, were selected from medical records among outpatients of the Geriatrics Service of the Medical Department, Catholic University of Brasília, from February to July 2009. This sample was devoid of patients on hormone replacement therapy, with autoimmune disease, neoplastic disease, chronic obstructive pulmonary disease, severe chronic kidney disease (creatinine clearance $<$25 mL/min/1.73 m^2), thyroid disease, liver disease or left ventricle ejection fraction $<$50 %. Subjects with uncontrolled hypertension (SBP $\geq$140 mmHg and/or DBP $\geq$90 mmHg) at baseline in any session were rescheduled to a maximum time-span of 1 week later, and if symptoms persisted, excluded from the study. Only elderly women were investigated to prevent biases related to gender. In addition, all participants underwent exercise testing to exclude ischemic heart disease prior to enrollment. The study was approved by the institutional research ethics committee, and all participants signed their informed consent before enrollment.

Experimental design

All participants underwent three experimental procedures on different days at intervals of 48 h, in determined randomly order before each procedure. For randomization, only schemes in which the incremental test (IT) preceded exercises performed at 90 % of AT were considered. Immediately before each procedure, subjects remained seated for 20 min and had their BP measured once every 5 min; the mean value of these measures was considered the baseline rest BP for each procedure.

The experimental procedures were: (1) 20-min constant load exercise test, at an intensity corresponding to 90 % of the anaerobic threshold (AT); (2) maximal IT; and (3) control session without exercise. After all sessions, participants remained in the laboratory for 1 h to recover, and BP was measured every 15 min, so that the average value of these measures was considered the post-procedure BP.

Heart rate, rate of perceived exertion (RPE) and blood pressure

During all sessions, heart rate (HR) (Polar s810i®, Finland), RPE on a 15-point RPE scale [13], and BP (Microlife BP3AC1-1, Switzerland) were measured. All BP measurements were made according to the JNC 7 [1].

Incremental test and anaerobic threshold determination

Volunteers performed a maximal IT, which consisted of a 1-min warm-up at 0 Watts, followed by a linear incremental gradient of 15 Watts every 3 min (equivalent to one stage) until volitional exhaustion, incapacity to maintain 60 rpm, or appearance of any cardiovascular-related risk. At the end of each 3-min stage, 25 μL of blood was withdrawn from the earlobe in heparinized microcapillary tubes. Samples were stored in Eppendorf microtubes containing 50 μL of sodium fluoride (1 %), and blood lactate concentrations ([Lac]) were determined electroenzymatically (2700 S YSI, Yellow Springs, USA). Gas analysis (Cortex Metamax, Metalyzer, Germany) was performed breath by breath to evaluate ventilation (VE), oxygen uptake (VO_2) and carbon dioxide production (VCO_2) during the last 20 s of each incremental stage. The RPE was also assessed.

The incremental approach by cycle ergometry (Excalibur, Lode, Netherlands) was used to identify the anaerobic threshold (AT). The AT level was determined by assessing the ventilatory threshold [disproportional increase in the ventilatory equivalent for oxygen (VE/VO_2) in comparison with the ventilatory equivalent for carbon dioxide (VE/VCO_2)] and the blood lactate threshold (workload corresponding to the deflection point at which blood lactate concentration increased disproportionally). The AT was considered the mean workload value (Watts) between ventilatory and lactate thresholds.

Sub-maximal constant load exercise test

Participants underwent a 20-min constant load exercise test at an intensity corresponding to 90 % of the AT. Expired gases, [Lac] and RPE were measured in the last 20 s of the 20th minute, following the same procedures as described for the IT. The intensity of 90 % of the AT was chosen, because it has been used elsewhere with documented benefits for blood pressure and cognitive performance in elderly individuals [11, 14].

Control session

Volunteers remained seated for 20 min instead of exercising. However, all measurements were the same as in the exercise sessions. Gas analysis, blood lactate and RPE measurements were carried out as described for the 90 % AT session.

Nitrite measurement

The NO pool was inferred from the measurement of saliva nitrite (NO_2^-) [23–25]. For this analysis, saliva was collected on a cotton swab (Salivette Sarstedt®), chewed for 1 min on three different moments: during rest before exercise (or control) in all testing days (PRE); immediately after exercise (IAE); and concomitantly with BP measurements throughout the recovery period.

Each cotton swab was centrifuged and supernatants were stored at −20 °C for later analysis by the Griess' colorimetric method [15] according to manufacturer's instructions. Briefly, N-(1-naphthyl)-ethylenediamine (NED) (Sigma®) was prepared at 0.1 % and sulfanilamide (Sigma®) at 1 %, both with phosphoric acid at 2.5 % as diluent. Saliva (50 μL) and the Griess reagent (50 μL) were mixed and placed in microplates. Absorbance was measured at 450 nm in a VersaMax tunable® (Molecular Devices, USA), and sodium nitrite ($NaNO_2^-$) was used as standard. Data were analyzed by Microplate® software.

To minimize the influence of ingestion on the NO pool, each volunteer was instructed to reproduce, in all testing sessions, the same food intake done before the first testing day.

Statistical analyses

After exploratory data analysis, the Kolmogorov–Smirnov test was used to check for the hypothesis of normality of data; then descriptive statistics were performed. Data are presented as means ± standard deviations (SD) and NO_2^- and absolute variations (delta) from rest to post-procedure values are expressed as mean ± standard error (SE). In addition, contrasts in repeated measures were carried out by main effect comparisons. To verify any significant linear trend in delta variations of variables with increasing exercise loads, polynomial contrasts were used. The level of significance adopted was $p \leq 0.05$. The assumption of sphericity in data distribution was checked by Mauchly's test and, whenever the test was violated, technical corrections were made by applying the Greenhouse-Geisser test. All calculations were performed with the Statistical Package for Social Sciences (SPSS) for Windows, version 15.0.

Results

Twenty-three women (70.5 ± 6.0 years old, 60.4 ± 8.5 kg, 153.3 ± 6.3 cm, and 25.7 ± 3.0 kg/m^2) fulfilled all criteria for the study protocol. Power output, oxygen consumption, heart rate, metabolic variables and RPE results corresponding to studied exercise intensities, are listed in Table 1. These results are compatible with participants exhibiting an overall state of low aerobic fitness, and evidenced higher cardiovascular and metabolic stress at the higher intensity (IT) exercise session, in comparison with the session at 90 % of AT.

Comparisons between the resting and post-exercise moments for systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR), both within and across experimental sessions, are listed on Table 2. As a main result, mean SBP was significantly lower than pre-exercise during post-procedure

Table 1 Means ± standard deviations for exercise variables assessed at peak of incremental test (IT) and at 90 % of anaerobic threshold (90 % of AT) in the elderly women ($n = 23$)

	IT (peak)	90 % of AT
Load (Watts)	63.3 ± 21.7*	36.5 ± 13.7
VO_2(mL kg^{-1} min^{-1})	20.9 ± 4.0*	15.2 ± 2.6
HR (bpm)	150.1 ± 20.5*	120.3 ± 15.4
Lactate (mM)	4.8 ± 1.9*	3.0 ± 1.1
RPE	17.7 ± 1.4*	13.2 ± 1.9
Time (min)	11.6 ± 4.0	20.0

VO_2 oxygen consumption, *RPE* rate of perceived exertion, *HR* heart rate

* $p < 0.05$ compared with group exercising at 90 % AT

Table 2 Comparison of blood pressure and heart rate data pre (rest) and post-procedure ($n = 23$)

	IT			90 % AT			CONT		
	Rest	1 h recovery	Δ	Rest	1 h recovery	Δ	Rest	1 h recovery	Δ
SBP (mmHg)	122.9 ± 12.6	119.0 ± 3.6*	−3.9 ± 6.8†	121.1 ± 11.6	118.0 ± 12.0	−3.1 ± 7.6†	120.6 ± 12.0	123.5 ± 2.8*	2.8 ± 6.7
DBP (mmHg)	75.5 ± 6.8	75.6 ± 7.6	0.1 ± 6.8	74.4 ± 5.8	74.8 ± 4.9	0.4 ± 3.8	74.0 ± 7.2	76.1 ± 7.1*	2.1 ± 2.7
MAP (mmHg)	92.0 ± 8.7	90.6 ± 9.0	−1.4 ± 5.6†	90.3 ± 7.3	89.6 ± 6.6	−0.7 ± 4.5†	90.2 ± 8.4	92.4 ± 8.5*	2.2 ± 3.3
HR (bpm)	72.1 ± 9.6	80.0 ± 11.3*	7.8 ± 7.4†	74.2 ± 9.1	79.2 ± 9.6*	5.0 ± 5.2†	70.3 ± 9.3	68.7 ± 9.0	−1.6 ± 4.1

IT Incremental test session, *90 % AT* 90 % of anaerobic threshold session, *CONT* control session, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *HR* heart rate. *1 h recovery* mean of measurements during 1 h of post-procedure recovery period

* $p < 0.05$ compared with rest within same session; † $p < 0.05$ compared with Δ of CONT session

recovery from IT, which characterizes PEH among the elderly women investigated. Conversely, mean SBP significantly increased while subjects were submitted to the CONT session. DBP and MAP variables did not vary significantly between resting and post-procedure, regardless of exercise intensity (IT and 90 % of AT), whereas these variables increased significantly in the CONT session. In contrast, HR increased significantly after exercise sessions, while the opposite was observed in the CONT session (Table 2). Also, delta variation results based on the post-procedure minus resting values were used to analyze fluctuations among sessions. The negative variation for SBP values after both exercise sessions and for MAP after IT differed significantly in terms of magnitude and inclination from the variation observed in the CONT session, which reveals more evidence toward the PEH phenomenon in the sample. Significant differences in delta levels ($p < 0.05$) were also found for HR, although with higher variations after exercise sessions when compared with CONT (Table 2).

In comparison with resting levels, blood lactate values were significantly higher ($p < 0.05$) in the 1-h recovery from the IT session (rest: 1.2 ± 0.4 mM; 1-h mean: 2.1 ± 0.7 mM), but not after 90 % of AT (rest: 1.4 ± 0.4 mM; 1-h mean: 1.3 ± 0.4 mM), and were actually lower ($p < 0.05$) after the CONT session (rest: 1.5 ± 0.6 mM; 1-h mean: 1.0 ± 0.4 mM). Besides confirming higher exercise intensity for IT, the higher [Lac] also indicated more significant metabolic stress in this session compared with the 90 % of AT session. Comparisons of mean NO_2^- values at rest and IAE are shown in Fig. 1, revealing a significant elevation after IT with respect to pre-

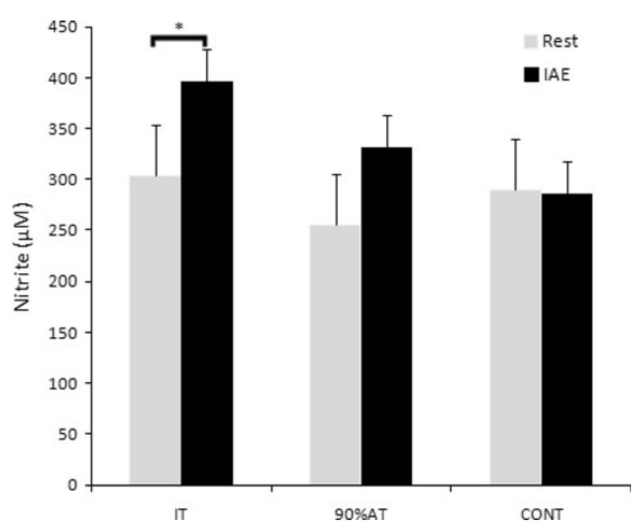

Fig. 1 Comparisons of nitrite values (mean ± SE) between resting and immediately after sessions (IAE) of incremental test (IT), at 90 % of anaerobic threshold (90 % AT) and control (CONT). *$p < 0.05$ compared with rests ($n = 22$)

exercise resting levels. Taking baseline levels as reference, the delta variations of NO_2^- immediately after both IT and 90 % of AT sessions were significantly higher than that produced in the CONT session (data not shown), but no differential magnitude was observed across these exercise intensities. No significant differences were observed in mean NO_2^- values in subsequent assessment points throughout the recovery period either, in comparison with the resting moment or among sessions (data not shown).

Discussion

The main finding of our study was the occurrence of PEH following aerobic sessions on the SBP of elderly women with clinical diagnosis of hypertension (Table 2). Accordingly, individuals submitted to IT presented augmented levels of salivary NO_2^- when compared with the control group (Fig. 1). Moreover, the exercise session performed in intensity close to anaerobic threshold (90 % of AT) showed values of NO_2^- visually higher than the rest (Fig. 1), but non-significant ($p > 0.05$). These results support the role of peripheral, vascular nitric oxide on exercise-induced blood pressure lowering pathways in elderly subjects.

Increase in NO release after exercise sessions probably occurred due to shear stress in blood vessels [16, 17]. Accordingly, the observation that NO_2^- was significantly elevated only IAE may be attributed to the short half-life of NO (11–13 min) in human vasculature [18], which may have prevented the event from lasting beyond the initial assessment.

Another factor which may explain such limited contribution of peripheral NO to the PEH observed in our subjects is the fact that there are several phenotypes of vascular dysfunctions associated with the progression of aging [19, 20]. Aging per se is a significant predictor of impairment of endothelium-dependent vasodilation and impaired bioactivity of NO [21–23]. This idiopathic impairment in NO production may have occurred in our study since, although participants exhibited low aerobic fitness (Table 1), NO augmentation after the quite intense exercise performed at 90 % AT did not parallel that observed after IT. Also, despite the fact that our work was not designed to assess vascular properties in younger adults, the literature reports evidence in favor of a negative correlation between NO_2^- production and age in response to comparable exercise protocols [8, 24, 25].

The American College of Sports Medicine recommends moderately intense exercise training (40–60 % VO_2 reserve) for primary prevention, treatment and control of hypertension [10]. Evidence has also been produced with light and moderate exercise intensities eliciting PEH in hypertensive older men, reducing a magnitude detectable 5–9 h after the protocol [26]. In our conditions, exercise performed at higher intensity was able to produce PEH and lead to a detectable BP lowering effect. There is evidence that only exercise above the anaerobic threshold can induce PEH in individuals with severe endothelial impairment (e.g., type 2 diabetes [11]), and one limitation of our study is that subjects were not fully characterized for glycemic and/or lipemic variables, nor assessed for later long-term effects in blood pressure.

Another limitation of the present study was the lack of assessment of the activity of endothelial nitric oxide synthase (eNOS), which may represent the direct endothelium-dependent vasodilation phenomenon. However, some authors [27–29] have verified that saliva NO_2^- concentration predicts plasma NO_2^- concentration, which in turn is most probably the best surrogate for eNOS activity [18]. However, NO_2^- in saliva may be influenced by diet [30], all elderly participants in our study were advised to follow the same diet throughout the trial days. Hence, no differences were found for NO_2^- resting levels across experimental days.

Nevertheless, precaution should be taken when prescribing intense exercises, mainly for elderly people, since maximal tests may result in hazardous situations when not performed in controlled conditions. In the light of our results, we suggest that exercise should be prescribed in the higher secure intensity for greater PEH response, but further studies should be conducted to investigate how exercise intensities (mainly sub-maximal) and co-morbidities (mainly metabolic), other than those presented here, may contribute to exercise prescription as well as to scientific understanding of the determinants of the PEH phenomenon. Apart from these, aspects such as autonomic regulation and genetic interactions with exercise should be the focus of future research.

In conclusion, besides the possibility that higher intensities of aerobic exercise may elicit beneficial aspects of PEH in mildly hypertensive elderly women, NO release may exert a role in mediating the phenomenon.

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Conflict of interest None.

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