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Original article

Hypotension after high-intensity interval exercise with elastic resistance: a pilot study

Hipotensão pós-exercício intervalado de alta intensidade com resistência elástica: um estudo piloto

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ABSTRACT

Introduction: It is established that continuous aerobic training reduces post-exercise blood pressure, known as hypotension post-exercise. Traditional ergometers such as treadmills and cycle ergometers are commonly used as resources for training. However, they are expensive and this limits access to the general population. An alternative is to use elastic resistance running. However, it is not known whether the application of a high-intensity interval training session, with elastic resistance, presents favorable post- -exercise acute hemodynamic responses. **Objective:** To describe and compare the hemodynamic responses in the recovery of an interval training session performed on the treadmill and with elastic resistance performed at the same intensity. **Methods:** Four healthy adults (24.25 ± 2.75 years old) performed a session of high-intensity interval training on a treadmill and another session with elastic resistance at 85% of VO_{2max} , with an interval of one week between them and a control situation. Before and after the sessions, hemodynamic variables were monitored for 60 minutes: systolic blood pressure, diastolic blood pressure, heart rate, stroke volume, cardiac output, and peripheral vascular resistance monitored by infrared photoplethysmography (Finometer). **Results:** Interval training with elastic resistance decreased systolic blood pressure and peripheral vascular resistance concerning the control day. Comparing the protocols, only elastic resistance caused post-exercise hypotension (p < 0.05), for up to 40 minutes. **Conclusion:** The protocol with elastic resistance promoted post-exercise hypotension for SBP and reduced peripheral vascular resistance..

Keywords: high intensity interval training; post-exercise hypotension; hemodynamic monitoring.

RESUMO

Introdução: Está estabelecido que o treinamento aeróbio contínuo reduz a PA no pós-esforço, conhecido como hipotensão pós-exercício. Ergômetros tradicionais como esteiras e cicloergômetros são comumente utilizados como meio de treinamento. Eles são caros e isso limita o acesso à população em geral. Uma alternativa é o uso da corrida com resistência elástica. Entretanto não se sabe se a aplicação de uma sessão de treinamento intervalado de alta intensidade, com resistência elástica, apresenta respostas hemodinâmicas agudas favoráveis pós-exercício. **Objetivo:** Descrever e comparar as respostas hemodinâmicas na recuperação de uma sessão de treinamento intervalado realizada na esteira e a com resistência elástica realizadas na mesma intensidade. **Métodos:** Quatro adultos (24,25 ± 2,75 anos) saudáveis executaram uma sessão de treinamento intervalado de alta intensidade na esteira ergométrica e outra sessão com resistência elástica a 85% do VO_{2máx}, com intervalo entre eles de uma semana e uma situação controle. Antes e após as sessões, foram monitoradas as variáveis hemodinâmicas por 60 minutos: pressão arterial sistólica, pressão arterial diastólica, frequência cardíaca, volume sistólico, débito cardíaco e resistência vascular periférica monitorados por fotopletismografia por infravermelho (Finometer). **Resultados:** O treinamento intervalado com resistência elástica apresentou resposta hemodinâmica aguda significativa com a queda da pressão arterial sistólica e da resistência vascular periférica em relação ao dia controle. Comparando os protocolos, apenas a resistência elástica causou hipotensão pós-exercício (p < 0,05), por até 40 minutos. **Conclusão:** O protocolo com resistência elástica promoveu hipotensão pós-exercício com queda da pressão arterial sistólica acompanhada de redução da resistência vascular periférica.

Palavras-chave: treinamento intervalado de alta intensidade; hipotensão pós-exercício; monitoramento hemodinâmico.

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Introduction

The regular practice of physical exercises exerts positive effects on the cardiovascular, neuromuscular, and respiratory systems [1]. For patients in clinical conditions, they are even more effective or act synergistically with drug treatment, minimizing the negative clinical effects of some diseases [2]. High-intensity interval training (HIIT) is a modality that has gained prominence, as it presents evidence that it is a time-efficient therapy to improve the cardiometabolic health profile of individuals with or without risk of metabolic disease, even in the absence of weight loss [3]. HIIT is characterized by repeated "near maximal" efforts (e.g., \geq 90% HR $_{max}$ or \geq 85% VO_{2peak}) and periods of active or passive recovery [4]. Different HIIT protocols are presented in the literature and advocated to promote benefits in physical fitness [5] and health [6,7]. However, these studies are limited to the ergometers use, such as treadmills and bicycles, which are equipped with limited access to the general population, and this can reduce adherence.

A presented proposal by our group was the interval exercise practiced in the form of brisk walking or running with elastic resistance by older women [8]. In this study, favorable acute effects of post-exercise hypotension (PEH) and glycemic control were observed in recovery for up to 60 minutes (min).

Investigating immediate responses to exercise recovery is important, as this information can describe a state of vulnerability [9] when there is no recovery of hemodynamic variables at a normal pace, as is the case of heart rate (HR) recovery [10], or a substantial drop in diastolic blood pressure (DBP) that can reduce myocardial perfusion [11]. On the other hand, recovery is a phase of opportunity to enhance the stimuli imposed by physical effort, culminating in autonomic adjustments that favor the reduction of peripheral vascular resistance (PVR) and systolic blood pressure (SBP) [9]. HIIT also seems to be more favorable, in terms of hypotensive response, than continuous moderate-intensity training, as it has a higher post-exercise excessive oxygen consumption (EPOC) and a higher rate of accumulated heat [12]. This response may favor the reduction of SBP and, consequently, the systolic volume (SV) for thermal regulation [12]. Furthermore, it is a type of effort that promotes greater vascular shear stress during exercise that is associated with better vascular adaptations, increasing arterial compliance, for example [13-15].

HIIT has been a highly acclaimed method to promote physiological adaptations beneficial to health, but incremental tests that used elastic resistance indicate a change in the characteristic of muscle contraction, as it requires a strength component and even a more exacerbated eccentric phase of contraction [16]. This more intense request of the muscular system by the elastic resistance can interfere with the hemodynamic responses to the exercise [17], causing a more exacerbated PEH by adding the mechanical and metabolic effects of the exercise [18]. Therefore, it is necessary to clarify whether the elastic resistance application combined with cardiorespiratory work, as in high-intensity interval training with elastic resistance (EL-HIIT),

presents a more exacerbated acute hypotensive response than HIIT on a treadmill.

Therefore, the objective was to describe the pressure and hemodynamic response after a single session of EL-HIIT compared to traditional HIIT performed on a treadmill.

Methods

This is a cross-sectional study, supplementary to the OBHIIT project (Obesity Research in High-Intensity Interval Training), registered in PRPPG nº 93906/2019. The project was approved by the Ethics Committee for Research with Human Beings of the Federal University of Espírito Santo (CAAE nº 09109319.2.0000.5542). All participants were informed of the risks and benefits of the research and consented to participate by signing the informed consent form (TCLE).

Sample

The characteristics of the participants are presented in Table I. There were four participants (3 men and 1 woman), healthy young people (BMI \geq 18 and \leq 25 kg·m⁻²), aged between 18 and 35 years, physically active (\geq 150 min/ week of physical exercise), free of cardiometabolic diseases and without declared use of dietary supplements or anabolic steroids. Participants were selected through invitations and disclosures on social networks. All were assessed by a physician before inclusion in the survey. In addition, they answered a questionnaire to assess signs and symptoms of disease, use of medication, family history of chronic non-communicable diseases, and characterization of the level of physical activity (IPAQ, short version).

Data presented as mean ± Standard Deviation. BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure. *Values referring to the control

Anthropometric Assessment and Body Composition

Body mass and height were measured using a digital anthropometric scale and a one-millimeter precision stadiometer (Marte Científica, L200, São Paulo) with a maximum capacity of 210 kg and sensitivity of 50 g. All procedures were performed by the same evaluator.

Experimental procedures

Each participant visited the Exercise Physiology laboratory (LAFEX/NUPEM/ UFES) on 4 occasions with intervals of at least 1 week (Figure 1). The visits consisted of Cardiopulmonary Exercise Test (CPET) and medical anamnesis (day 1), Cardiopulmonary Exercise Test with Elastic Resistance (CPET) (day 2), and HIIT or EL-HIIT session (days 3 and 4) organized in a randomized way. Before and after the HIIT and EL-HIIT sessions, the hemodynamic variables SBP, DBP, HR, SV, PVR, and Cardiac Output (CO) were collected. Before the procedures of the first or second week, the BP was measured in the Finometer® for 60 minutes to have the reference of the control day.

Figure 1 – Experimental study procedures

Pre-assessment care and guidance

Participants were instructed not to perform physical activities the day before and on the day of the test, to eat two hours before the stress tests, to present themselves with appropriate clothing for the tests to be performed, not to drink any energy drink (coffee or others) on the day of the assessments and not to show up for the assessments with symptoms that may be associated with COVID-19.

Cardiopulmonary Exercise Test (CPET)

In a silent, air-conditioned room (21 and 24ºC), blood pressure (BP) was measured following the recommendations of the 2020 Brazilian Guidelines on Arterial Hypertension [19]. The participants were instructed on the procedures for carrying out the test, which included: remaining breathing normally; not using the handrail of the treadmill as a support during the test, unless necessary; requesting interruption of the test, in case of chest pain or any other discomfort that prevents the continuation of the test. The test was performed on a motorized treadmill (*Inbra Sport Super ATL, Porto Alegre, Brazi*l) following an individualized ramp protocol, aiming at a duration between 10 and 12 min. The speed was gradually increased until the individual reached exhaustion. Participants started at a 4 km/h warm-up (3 min) with increments of 1 km/h every minute, with a 1% incline.

The ventilatory variables were measured using a metabolic gas analyzer (*Cortex Metalyzer 3B model, Germany*), with breath-by-breath collection, and then 20-second averages were calculated and analyzed using the Metasoft program. The Cortex unit was calibrated by the closed-loop method, using calibration gas (original 16% O_2 and 5% CO $_2$ cylinder, supplied by the manufacturer). To identify the test as maximum, we analyzed the following criteria: a) voluntary exhaustion; b) Maximum HR attained of at least 90% of that predicted by age (220-age); c) respiratory exchange ratio equal to or above 1.05 [20]. The VO_{2max} was extracted between the highest values of the final 30 seconds of the test.

Elastic Resistance Cardiopulmonary Exercise Test (EL- CPET)

The EL-CPET environment was the same as previously described for CPET, as well as the procedures for measuring BP. All participants were instructed about the test procedures. The EL-CPET was performed on a rubberized mat marked with 11 lines of 5 cm thick and separated by spaces of 30 cm between one and the other. The rug measures 4.50 m high x 1.00 m wide. Lines painted in different colors (white and black) comprise stages 1 to 10 (Figure 2). Two meters of silver elastic tubing (*®Thera- -band Tubing, Malaysia*) was used, wrapped in an eyelet, and fixed by a steel cable to a stainless steel support bar.

The protocol was explained and familiarized before running the session. The belt was adjusted at the height of the iliac crest, allowing the participant to run forward and backward, changing stages with constant feedback from the researchers. After a 3 min warm-up, the test consisted of gradual increments every 1 min. During the test, participants were encouraged to follow the rhythm emitted by a metronome (*Cellphone application – ®Cifraclub*). They were encouraged to maintain a rhythm of 180 bpm during warm-up and 200 bpm in the following stages. If the individual reached the last stage and did not become exhausted, 10 bpm were added until exhaustion. Ventilatory variables and oxygen consumption (VO₂) were measured as described in CPET. This test was proposed by Gasparini-Neto *et al*.[16].

WU = Warm Up; S = stage. Warming up (0) and eight gains (E1 to E8) - 60 cm between steps, interspersed with black and white colors. Rubberized mat with a length of 4.50 meters, marked with 11 lines (0-10) – 30 cm between lines.

Figure 2 – Schematic model of a mat for incremental testing with elastic resistance

HIIT session on treadmill

Initially, the participant remained at rest in the lying position (dorsal decubitus) for 10 min and 60 min post-exercise for each measurement of the hemodynamic variables. Soon after, the mask was adjusted for ventilatory measures. Ten series of exercises lasting one minute were performed at 85% of the $VO_{2\text{max}}$ determined in CPET, with a passive interval of one minute between series, protocol 10 x 60s: 60s – adapted from Little *et al*. [5]. All were verbally encouraged during the effort.

EL-HIIT session

Initially, the volunteer remained at rest in the lying position (dorsal decubitus) for 10 min and 60 min post-exercise for each measurement of the hemodynamic variables. Soon after, the mask was adjusted for ventilatory measures. Ten series of exercises lasting one minute were performed, prescribed at 85% of the VO_{2max} determined by the EL-CPET (according to the stage of the mat corresponding to 85% of the VO_{2max}), and one minute of passive interval between the series, protocols 10 x 60s: 60s – adapted from Little *et al*. [5].

Hemodynamic analysis

In a quiet room with low lighting, participants lay on a stretcher. After a 5-min rest in the supine position, BP monitoring was performed using infrared photoplethysmography with a Finometer® monitor (*Finapres Medical System, BV Netherlands*) on a control day, and on two more days, before and after the training session (10 min at rest and 1 hour after exercise, lying down). BP was measured on the right arm due to the positioning of the room and equipment, certified to be positioned at heart level, as recommended by the equipment itself. For the acquisition of pressure curves, the cuff was placed on the middle finger, which takes into account the age, body mass, height, and gender of the participant. To calculate the post-exercise hemodynamic differences between the protocols, the net effect of exercise was calculated as the difference between the responses in the exercise and the control situation $[(post-exercise BP - pre-exercise BP) - (post-control BP - BP pre-control)] [21]. For$ the analyses, the variables presented in the software (*Beat Scope® software*) were considered: Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart Rate (HR), Systolic Volume (SV), Cardiac Output (CO) and Peripheral Vascular Resistance (PVR).

Statistical analysis

Data are presented as mean \pm standard deviation and tested for normality using the Shapiro-Wilk test. All data were tabulated and double-checked by independent researchers. For effect size (ES), Cohen's d was used [22], classified as: ≤ 0.49 small effect; 0.50 – 0.79 moderate effect; 0.80 – 1.29 large effect; > 1.30 very large effect [22]. To analyze the differences in hemodynamic parameters, a two-way ANOVA of repeated measures with post hoc Bonferroni correction (time points vs. protocols) was used. The software used for static analysis was IBM/SPSS v. 20.0. p < 0.05 was considered.

Results

Table II presents the CPET and EL-CPET parameters applied before the protocols. The load corresponding to the percentage of 85% of HR_{max} was used for the prescription of training sessions.

Variables	CPET	EL-CPET
$(m1 \cdot kg^{-1} \cdot min^{-1})$ VO _{2max}	46.50 ± 7.14	42.88 ± 5.84
(bpm) HR_{max}	203.25 ± 7.72	200.00 ± 4.55
RER	1.06 ± 0.04	1.05 ± 0.02

Table II – Maximum parameters of cardiopulmonary tests

Data presented as mean ± standard deviation. $\rm{HR_{max}}$ = Maximum heart rate; RER = Respiratory exchange ratio; CPET = Cardiopulmonary treadmill exercise test; EL- CPET = Cardiopulmonary resistance exercise test; VO $_{\rm 2max}$ = Maximum oxygen consumption

Hemodynamic response: comparison with the control group

The values obtained during the control compared to the post-exercise ones are presented in Table III. After the EL-HIIT session, the SBP presented a decrease in relation to the control at 20 min (12.7%), 30 min (11.8%), and 40 min (7.1%), with very large ES (1.70, 1.72, and 0.82, respectively). DBP was lower only at 30 min (5.2%) of EL- -HIIT with large ES (0.81). HR showed higher values at 20 min (35.7%), 30 min (29.4%), 40 min (19.1%), 50 min (21.2%), 60 min (16.4%), and 70 min (13.4%) with large and very large ES (1.17, 2.00, 1.28, 1.71, 1.43, and 1.09, respectively). SV exhibited higher values at 20 min (6.3%), 30 min (4.8%), and 50 min (4.2%) post-exercise, with large ES (1.13, 0.88, and 0. 88, respectively). CO values were higher at 20 min (22%), 60 min (11.3%), and 70 min (13.2%) with large ES (1.11, 0.94, and 1.21, respectively), and in the 30th (11.6%), 40th (9.3%), and 50th (11.6%) minutes, the CO tended to increase in relation to the control, with a moderate ES (0.64, 0.57, and 0 .75, respectively). PVR decreased from 20 min (24.7%) to 30 min (13.6%), and at 50 min (9.6%), 60 min (8.3%), and 70 min (7.9%) with ES from very large to large (1.46, 0.87, 0.90, 1.02, and 1.16, respectively); and at 40 min (10.4%) with moderate ES.

Table III – Acute physiological responses after sessions

Data presented as mean ± standard deviation. CO = Cardiac Output; HR = Heart Rate; DBP = Diastolic Blood Pressure; SBP = Systolic Blood Pressure; PVR = Peripheral Vascular Resistance; Effect size compared to control day, where ^s = small ≤ 0.49; ^M = moderate ≥ 0.50 – 0.79; ^L = large ≥ 0.80 – 1.29; ^{VL} = very large > 1.30; SV = Systolic Volume

In HIIT, SBP showed a reduction of (3.4%) and (5.5%) at moments 30 and 70 min after exercise with moderate ES (0.57 and 0.64, respectively) (Table III). DBP remained higher at minutes 50 (7.1%) and 70 (6.7%) with large ES (1.00 and 0.80, respectively); and at 20 min (6%), 60 min (5.1%) and 80 min (6.7%) with moderate ES (0.59; 0.10 and 0.10, respectively). HR remained elevated at minutes 20 (21.4%), 30 (17.6%), and 40 (10.3%), with ES ranging from large to very large (1.45; 1.17 and 0.83, respectively); and at minutes 50 (6%) and 70 (9%) moderate ES was observed (0.53 and 0.68, respectively). The SV showed higher values at 20 min (7.7%), 30 min (5.4%), 50 min (5%), and 70 min (3.9%), a large ES (1.20; 0. 92; 1.06 and 0.81, respectively). CO and PVR did not differ compared to the control moment.

Post-exercise hemodynamic differences between EL-HIIT and HIIT

SBP (Fig. 3 – A) was significantly lower ($p = < 0.05$) in EL-HIIT at 20 min ($p =$ 0.021), 30 min ($p = 0.012$), and 40 min ($p = 0.035$), showing differences in mmHg of 11.43, 10.93, and 4.15, respectively. For DBP (Fig. 3 - B), there was no statistical difference between the protocols. CO values (Fig. 3 - C) were lower in EL-HIIT at 60 min with a difference of 0.56 L/min ($p = 0.022$) and a downward trend at 70 min ($p = 0.054$). HR (Fig. 3 - D) tended to remain higher in EL-HIIT at 30 min ($p = 0.059$), 40 min ($p = 1$) 0.053), 50 min (p = 0.055), 60 min (p = 0.059), and 80 min (p = 0.053). For PVR (Fig. 3 -E), the EL-HIIT modality was significantly different from HIIT ($p = 0.009$), showing differences (dyne-s-cm5) with reductions of 212.75 in 20 min ($p = 0.005$), 70.62 at 30 min ($p = 0.003$), and 18.13 at 50 min ($p = 0.008$).

Differences *(p = < 0.05) of hemodynamic variables in post-exercise. **A**) Systolic Blood Pressure; **B**) Diastolic Blood Pressure; **C**) Cardiac Output; **D**) Heart Rate; **E**) Peripheral Vascular Resistance; and **F**) Systolic Volume

Figure 3 – Post-exercise hemodynamic differences between EL-HIIT vs. HIIT protocols

Discussion

This study aimed to describe and compare post-exercise hemodynamic responses in normotensive healthy young subjects submitted to two different HIIT protocols. We noticed that EL-HIIT presented a more evident acute hemodynamic response, with a drop in SBP and PVR and maintenance of high HR and CO, compared to the control situation in effect size. While for HIIT, only high HR and SV were observed in relation to the control.

Comparing the post-exercise hemodynamic response between the protocols, the main findings were that a single session of EL-HIIT caused a reduction in SBP during 40 min post-exercise, as well as for CO and PVR, compared to HIIT, with no effect on DBP, HR, and SV. This is the first study that observed PEH using EL-HIIT as a means of exercising, including the gold standard method usage of blood pressure analysis by infrared photoplethysmography.

The PEH presented in our findings corroborates the results that have been previously observed in young individuals after an exercise session. Angadi *et al*. [23] monitored BP in recreationally active young people for 3 hours and observed a longer-lasting PEH (4 mmHg reduction in SBP) after long HIIT performed on a cycle ergometer close to the maximum (90-95% HR_{max}) compared to continuous exercise (75-80% HRmax). Dantas *et al*. [24] reported that a single low-volume HIIT session (10 x 1min at 100% of maximum treadmill speed interspersed with 1 min of recovery) was able to reduce SBP (3 to 5 mmHg) during the first 5 hours post-exercise in normotensive men. Thus, it is observed that there is favorable evidence in the literature for the post-exercise hypotensive response in young people who perform HIIT sessions on the treadmill or the cycle ergometer. On the other hand, our results broaden the view on exercise with elastic resistance since it seems to potentiate PEH, which is linked to a pronounced decrease in BP [25]. However, our results are only evidence to start exploring the topic since this is a pilot study, and it was not possible to reproduce the PEH observed in traditional HIIT reported in previously published studies.

The main novelty of this study was to show that EL-HIIT caused a more pronounced hypotensive response compared to HIIT in four young, healthy normotensive individuals of both sexes. Our findings also show a possible relationship with the drop in PVR, which only happened in the EL-HIIT modality. The reduction in vascular resistance seems to be one of the main hemodynamic variables that determine PEH [12,26,27]. The reduction in systemic vascular resistance can be caused by sustained post-exercise vasodilation in the lower limbs [26]. The occurrence of this phenomenon is mediated by complex interactions between local [28] and neural [27] vasodilator mechanisms, such as the re-establishment of the baroreflex, which, through sympathoinhibition, will decrease the sympathetic flow leading to vasoconstriction and activation of histamine receptors resulting in sustained vasodilation and helping to reduce vascular resistance [14]. However, there is no information about the associated mechanisms of PEH promoted by EL-HIIT. Thus, further studies are needed to clarify its post-exercise hemodynamic responses.

Another factor that may be related to the more evident hypotensive response is the mechanical demand exerted during exertion. Stavres *et al*. [18] reported that concentric exercise provided greater PEH in healthy young men and women, compared to eccentric exercise, probably due to metabolic stress combined with work. Therefore, it is speculated that the type of effort generated running back and forth performed in EL-HIIT may cause greater vascular shear stress due to increased blood flow toward the active muscle after EL-HIIT vs. HIIT, which has been associated with better vascular adaptations [13,15] and consequently strength-related benefits [16]. More studies should objectively explore the strength component after the different protocols.

The present study also expands the tool options for prescribing physical exercise in healthy young men and women. Pressure response after a single exercise session is considered a simple predictive clinical resource to manage training prescriptions [9]. Based on our findings, it is possible to speculate that in the EL-HIIT modality, individuals will be more responsive to the stimulus [26] to obtain greater assertiveness in the training prescription. However, these are still speculative findings.

Despite the promising results of this study, some limitations need to be mentioned. First, the study was carried out with only four healthy young people, which prevents extrapolations to other age groups and hypertensive and pre-hypertensive individuals. Therefore, studies with larger sample sizes, with other publics and age groups, are necessary. Second, hemodynamic variables were obtained over a short period (80 min), so further investigations should assess responses over longer periods. Finally, even though there were guidelines for individuals not to practice physical activity the day before the sessions, nor to drink energy drinks, we did not assess these factors objectively. On the other hand, our findings pave the way for new hypotheses and studies that use elastic resistance as a physical exercise tool.

Conclusion

EL-HIIT promoted PEH with a drop in SBP accompanied by PVR. These findings suggest EL-HIIT may be a new tool for exercising in healthy young men and women with beneficial effects on the cardiovascular system.

Academic affiliation

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Conflict of interests

All authors are responsible for the content of the manuscript and approved its final version. No commercial party that supports this article and has a direct financial interest in the research results confers or will confer financial benefits on the authors or any organization with which the authors are associated. The authors declare that there are no known competing financial conflicts of interest or personal relationships that may have influenced the work reported in this article.

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Authors contributions

Conception and design of the research: Carletti L, Neves LNS, Gasparini-Neto VH; **Obtaining data:** Neves LNS, Gasparini-Neto VH; **Data analysis and interpretation:** Alves RS, Neves LNS, Carletti L, Gasparini-Neto VH; **Statistical analysis:** Alves RS, Neves LNS; **Obtaining financing:** Carletti L; **Writing of the manuscript:** Alves RS, Carletti L, Neves LNS, Gasparini-Neto VH; **Critical review of the manuscript for important intellectual content:** Alves RS, Carletti L, Neves LNS, Gasparini-Neto VH.

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