Effect of isometric contraction duration on hemodynamic responses in hypertensive women

Efeito da duração da contração isométrica nas respostas hemodinâmicas de mulheres hipertensas

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ABSTRACT
Introduction: Isometric exercise (IE) is one of the non-pharmacological strategies for reducing post-exercise blood pressure measurements. Objective: To compare the acute effect of two protocols with different configurations of isometric exercise prescription on hemodynamic parameters in hypertensive women. Methods: Ten hypertensive women performed 2 sessions of IE at 30% of 1RM on the horizontal bench press with a guided bar. The protocols were: 4 sets of 2 minutes of isometric contraction with 2 minutes of rest (4x2x2); and 16 sets of 30 seconds of isometric contraction with 24 seconds of rest (16x30x24), with a 7-day interval between sessions. Blood pressure was measured using a digital sphygmomanometer, pre-exercise, immediately after exercise, and during the 60-minute recovery period. Results: Both protocols showed an increase in heart rate and double product (4x2x2, p = 0.002 and p < 0.001; 16x30x24, p = 0.001 and p = 0.002, respectively). Protocol 4x2x2 increased systolic blood pressure and mean arterial pressure (p = 0.014 and p = 0.034, respectively), while 16x30x24 increased pulse pressure (p < 0.001) compared to rest immediately after exercise. Normalization of the evaluated values was observed from 10 minutes of recovery in both protocols. There was no interaction effect between the protocols. Conclusion: Isometric exercise for the upper limbs with different configurations (4x2x2 and 16x30x24), when equalized by work-to-rest ratio, promotes similar acute hemodynamic responses in controlled hypertensive women.

Keywords: isometric exercise; blood pressure; hypertension.

RESUMO
Introdução: O exercício isométrico (EI) constitui uma das estratégias não farmacológicas para redução de medidas da pressão arterial após o exercício. Objetivo: Comparar o efeito agudo de dois protocolos com diferentes configurações de prescrição do exercício isométrico sobre parâmetros hemodinâmicos de mulheres hipertensas. Métodos: 10 mulheres hipertensas realizaram 2 sessões de EI a 30% de 1RM no aparelho supino horizontal com barra guiada. Os protocolos foram: 4 séries de 2 minutos de contração isométrica com 2 minutos de descanso (4x2x2); e 16 séries de 30 segundos de contração isométrica com 24 segundos de descanso (16x30x24), com 7 dias de intervalo entre as sessões. A pressão arterial foi medida por esfigmomanômetro digital, pré, imediatamente após o exercício e durante os 60 minutos de recuperação. Resultados: Ambos os protocolos apresentaram elevação dos valores de frequência cardíaca e duplo produto (4x2x2, p = 0.002 e p < 0.001; 16x30x24, p = 0.001 e p = 0.002, respectivamente). O protocolo 4x2x2 aumentou a pressão arterial sistólica e a pressão arterial média (p = 0.014 e p = 0.034, respectivamente), e o 16x30x24 aumentou a pressão de pulso (p < 0.001), quando comparado ao repouso imediatamente após o exercício. A normalização dos valores avaliados foi observada a partir dos 10 min de recuperação em ambos os protocolos. Não houve efeito de interação entre os protocolos. Conclusão: O exercício isométrico para membros superiores com diferentes configurações (4x2x2 e 16x30x24), quando equalizados pela relação esforço:pausa, promove respostas hemodinâmicas agudas semelhantes em mulheres hipertensas controladas.

Palavras-chave: exercício isométrico; pressão arterial; hipertensão.
Introduction

Systemic Arterial Hypertension (SAH) is characterized by a persistent elevation of blood pressure (BP) at rest [1], with values above 130 mmHg of systolic blood pressure (SBP) and 80 mmHg of diastolic blood pressure (DBP) [2]. Hence, SAH is recognized as one of the most prevalent chronic non-communicable diseases, developing progressively due to the stiffening of blood vessels [3]. It is also regarded as a significant risk factor for coronary heart disease and stroke, contributing significantly to the high global mortality rate associated with cardiovascular diseases [4].

In women, the incidence of SAH increases [5] after menopause due to a gradual reduction in circulating levels of sex hormones (estrogen and progesterone) [6]. This reduction and a gradual increase in adiposity lead to elevated circulating pro-inflammatory markers, and these markers contribute to the onset and worsening of insulin resistance, renin-angiotensin system alterations, sympathetic nervous system hyperactivity, and endothelial dysfunction [6,7]. Moreover, persistent elevations in BP have been increasingly associated with cardiovascular autonomic dysfunction. The autonomic nervous system (ANS) regulates sympathetic modulation of the myocardium and parasympathetic modulation of the sinus node, atrioventricular node, and atrial myocardium [8]. Interventions are needed to assist this demographic in mitigating the impact of multiple hypertension risk factors, leading to a consequent reduction in the health complications associated with this condition. While SAH is multifactorial, it often correlates with environmental factors such as diet, physical inactivity, and alcohol consumption [2,4].

Regarding the impact of physical activity, the effects of exercise on controlling and regulating hypertension have been well-documented since the 1960s [9]. However, recent studies have linked isometric exercise (IE) with enhanced BP control in individuals with hypertension [1,10]. These exercises are characterized by their simplicity and versatility, allowing for effortless performance with low resistance and different prescription configurations (number of sets, contraction duration, and rest intervals). Importantly, they maintain consistent joint angles and typically result in minimal or no changes to muscle length [1]. These attributes highlight IE as a methodological training alternative and an accessible strategy for treating hypertension [10-12].

Some effects of IE on BP have already been established [1,11-14]. A commonly used configuration in studies focusing on hypertension treatment involves four sets of 2-minute isometric contractions at 30% of maximum voluntary force [15]. However, there is limited research on the various prescription configurations of IE and their effects on controlling SAH and its application alongside commonly practiced gym exercises. Studies analyzing BP responses to different configurations of these exercises have predominantly involved normotensive individuals [16-19]. It is conceivable that altering the configuration of these exercises by manipulating variables such as the duration of IE could affect one or more training responses. Understand-
ding these effects can aid practitioners in selecting a protocol that aligns with their preferences and goals.

This study aimed to compare the acute effect of two protocols with different IE prescription configurations on hemodynamic parameters in hypertensive women. To do this, we sought to test the hypothesis that exercise with a short duration of isometric contraction can promote similar hemodynamic responses to that performed with a long duration when equalized by the work-to-rest ratio.

**Methods**

**Participants**

The study is a cross-sectional, quasi-experimental study comprising ten hypertensive women undergoing regular drug treatment for this condition (Table 1) with medical clearance to engage in exercise.

This study’s target population was participants in a multidisciplinary institutional program for people aged 55 and over, which was aimed at the community living around the university. The sample was selected based on convenience, and all volunteers received prior information about the study’s aims and detailed explanations of the procedures involved. Subsequently, they provided informed consent by signing the consent form.

The procedures in this study adhered to the guidelines outlined in Resolution No. 466/12 of the National Health Council of the Ministry of Health. They were conducted only after obtaining approval from the Research Ethics Committee of the Federal University of Maranhão (UFMA) (No.43181015.6.0000.5087).

**Experimental design**

The study was conducted at the Strength Physiology Laboratory of the UFMA Sports Center, where the selected volunteers were already participating in other ongoing programs.

The procedures were conducted over eight sessions on different days, following this sequence: Session 1 - Introduction of the project, collection of informed consent signatures, medical history review, and completion of the IPAQ (International et al.) [20] and PAR-Q (Physical Activity Readiness Questionnaire); Session 2 - Anthropometric and body composition assessments, BP and heart rate (HR) measurements; Sessions 3 and 4 - Familiarization sessions on the horizontal bench press exercise; Sessions 5 and 6 - Test and retest to determine maximum voluntary strength, using the 1-repetition maximum (1RM) test, separated by a 48-hour interval; Sessions 7 and 8 - Implementation of the selected exercise protocol for the respective days, 48 hours after the 1RM test and retest. The second protocol was performed seven days later.
Figure 1 - Experimental study design. The figure represents the moment of application of the questionnaires, anamnesis, signing of the informed consent form, moments of application of the intervention protocol and their intervals

**Body assessment**

Height was measured using a portable stadiometer (Sanny®, model ES2060, São Paulo, Brazil) and body mass using a digital scale (Omron®, model HN-289, Omron Co., Osaka, Japan). Hip, waist, and abdominal circumference measurements were taken using an anthropometric tape measure (Sanny®, model TR-410, São Paulo, Brazil).

Tetrapolar electrical bioimpedance (MALTRON®, model Bf-906, United Kingdom) was employed to assess body composition. Participants were instructed to adhere to the preparation recommendations according to Heyward and Stolarczyk [21].

**Familiarization and assessment of maximum voluntary strength**

The familiarization sessions were conducted over two sessions, separated by 48 hours. Participants performed the horizontal bench press exercise during each session using a guided bar (Smith et al. G1-FW161, Matrix Fitness, United States).

Three sets of 15 repetitions were performed, with a 1-minute rest interval between sets. The initial load was set as the absolute weight of the bar, and progressions of 3 to 5% were determined based on the OMNI-RES scale for rate-perceived effort [22].

The 1-RM test was adapted following the Baechle and Earle protocol [23]. Initially, a warm-up consisting of 10 repetitions was performed using the load from the familiarization phase. After a 1-minute rest interval, the 1RM test began. Participants were allowed up to 5 attempts to determine their maximum load, with a 3 to 5-minute rest period between each attempt. The OMNI-RES scale [24] was used as a parameter to increase the loads after each attempt. Participants were instructed to remain in the room and refrain from consuming food during the protocol. After 48 hours, the same procedures were repeated (retesting) to confirm the maximum loads obtained.

**Hemodynamic parameters**

Upon arrival at the laboratory, volunteers were instructed to remain seated and refrain from exertion for 10 minutes before resting. BP and HR measurements were taken. For our analysis, BP and HR values were obtained at rest, immediately after IE protocol, and during the following 60 minutes of recovery, with 10-minute intervals between each measurement (10min, 20min, 30min, 40min, 50min, and 60min).
To obtain these measurements, an automatic pressure device was used (OMRON®, model HEM-742®, OMRON Corp., São Paulo, Brazil). Pulse pressure (PP) was calculated as the difference between SBP and diastolic blood pressure (DBP) [25].

**Experimental procedure**

This study involved two sessions of IE on the horizontal bench press, utilizing a guided bar, with one protocol employed per session. Before the first session, a random draw was conducted to determine the order in which each participant would undergo the protocols. The interventions consisted of the 4x2x2 protocol (comprising long-duration isometric contractions), involving four sets of 2 minutes of isometric contraction with 2-minute rest intervals, and the 16x30x24 protocol (comprising short-duration isometric contractions), involving 16 sets of 30 seconds of isometric contraction with 24-second rest intervals. The protocols were executed at an intensity of 30% of 1RM, maintaining a 90º angle of elbow flexion, as controlled by a goniometer (Carci®, São Paulo, Brazil). Moreover, the protocols were equalized using the work-to-rest ratio proposed by Paulo et al. [26]. The work-to-rest ratio values were determined using the following calculations: (I) total time of muscle tension/total Rest (480s:360s = 1.33), and (II) total sustained weight/total rest (480s*0.3:360s = 0.4). A seven-day interval was observed between the two exercise sessions.

**Statistical analysis**

Normality was analyzed using the Shapiro-Wilk test, and the results were presented as the standard deviation of the Mean (M±SDM). The two-way ANOVA analysis of variance for repeated measures was used to analyze possible effects of the time factor (Rest, immediately after exercise, and recovery at 10min, 20min, 30min, 40min, 50min, and 60min), the protocol factor (4x2x2; 16x30x24) and to detect possible effects of interactions between the factors, followed by the Bonferroni post hoc test. The analyses respected the assumption of sphericity assessed by the Mauchly test, and the Greenhouse-Geisser correction applied when necessary. A significance level of p ≤ 0.05 was adopted. The intraclass correlation test (ICC) was used to analyze the reproducibility of the 1RM test. The data was tabulated in the Office Excel program (Microsoft®, version 2016) and analyzed using SPSS® 25.0 (SPSS, Inc., Chicago).

**Results**

The study participants exhibited average BP levels indicative of well-controlled hypertension despite being overweight, as evidenced by their average BMI (Table 1). Regarding the loads employed during the protocols, the intraclass correlation coefficient (ICC) indicated good reliability between the values obtained in the 1-RM test and retest (ICC = [95% CI = 0.298 - 0.929]; F_{(9,9)} = 7.127; p = 0.004) [27].
Table I - Characteristics of the hypertensive women (N = 10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>67.53 ± 14.67</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158 ± 0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.48 ± 4.87</td>
</tr>
<tr>
<td>Waist/hip ratio (cm)</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>% Fat</td>
<td>38.26 ± 7.48</td>
</tr>
<tr>
<td>% Lean mass</td>
<td>61.74 ± 7.48</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131.5 ± 13.92</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.60 ± 9.50</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69.70 ± 6.78</td>
</tr>
<tr>
<td>Rate pressure product (mmHg-bpm)</td>
<td>9188 ± 1443</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>185.9 ± 18.16</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>49.9 ± 11.68</td>
</tr>
</tbody>
</table>

Data are presented as average and standard deviation of the Mean (M±SDM). kg = kilogram, cm = centimeters, BMI = body mass index, bpm = beats per minute, m² = square meter, % = percentage.

In response to the IE protocols, it was possible to observe an effect of the time factor on SBP measurements \([F_{(5,.7.00)} = 12.44; p < 0.001]\). In the 4x2x2 protocol, the immediate after-measurement was significantly higher than the rest \((p=0.014)\). During recovery, there was a decline in SBP at 10min, 20min, 30min, and 50min, compared to immediately after \((p = 0.015; p < 0.001; p = 0.001, and p=0.004, respectively)\). In the 16x30x24 protocol, Bonferroni post-hoc analysis revealed reductions in SBP in recovery at 10min, 20min, and 60min, compared to immediately after \((p = 0.006; p = 0.049; and p = 0.024, respectively)\) (Table II).

There was no influence of the factors time \([F_{(5,.7.00)} = 1.80; p = 0.101]\), protocol \([F_{(5,.1.00)} = 0.36; p = 0.563]\), or interaction effects between the factors \([F_{(5,.7.00)} = 0.98; p = 0.453]\) on DBP (Table II).

We also observed an effect of time on HR \([F_{(5,.2.19)} = 59.87; p < 0.001]\). In each protocol (4x2x2 or 16x30x24), HR was higher immediately after exercise compared to rest \((p = 0.002 and p = 0.001, respectively)\), and all recovery measurements showed a reduction when compared to immediately after in both protocols \((p \leq 0.001 for all moments)\) (Table II).

The mean arterial pressure (MAP) response was influenced by the time factor \([F_{(5,.7.00)} = 7.99; p < 0.001]\). In the 4x2x2 protocol, MAP increased significantly immediately after compared to rest \((p = 0.035)\). During recovery, this measurement was lower at 10min, 20min, 30min, and 50min, compared to immediately after IE \((p = 0.003; p < 0.001; p < 0.001; p = 0.013, respectively)\). On the other hand, in the 16x30x24 protocol, the only change observed was a reduction in MAP 10 minutes after recovery, compared to immediately after \((p=0.048)\) (Table II).

PP was also influenced by the time factor \([F_{(5,.7.00)} = 18.16; p < 0.001]\). In the 4x2x2 protocol, there was only a reduction in PP at 50 minutes during recovery, compared to the immediately after \((p=0.030)\). Conversely, in the 16x30x24 protocol, PP was higher immediately after exercise than rest \((p < 0.001)\). Additionally, PP was lower in
all recovery measurements than immediately after (p = 0.001; p = 0.003; p = 0.013; p = 0.011, p = 0.007; and p = 0.001, respectively) (Table II).

When analyzing rate pressure product (RPP), we also observed an effect of the time factor \( F_{(5,2.39)} = 64.99; p < 0.001 \). In the 4x2x2 protocol, there was an increase in RPP immediately after exercise compared to Rest \( (p < 0.001) \). During recovery, all measurements were significantly lower than immediately after \( (p < 0.001 \) for all moments).

### Table II - Hemodynamic responses to horizontal supine isometric exercise in hypertensive women

<table>
<thead>
<tr>
<th></th>
<th>Protocol 4x2x2</th>
<th>Protocol 16x30x24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Imm. After</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10min</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>127±14</td>
<td>147±10</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>78±8</td>
<td>79±9</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>74±8</td>
<td>101±15</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>179±19</td>
<td>200±12</td>
</tr>
<tr>
<td><strong>PP (mmHg)</strong></td>
<td>49±8</td>
<td>69±14</td>
</tr>
<tr>
<td><strong>RPP (mmHg.bpm)</strong></td>
<td>9345±1500</td>
<td>14815±1488</td>
</tr>
</tbody>
</table>

Protocolo 16x30x24

<table>
<thead>
<tr>
<th></th>
<th>Protocol 16x30x24</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>126±13</td>
<td>145±17</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>76±9</td>
<td>74±9</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>73±9</td>
<td>99±14</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>176±17</td>
<td>194±21</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>50±10</td>
<td>71±13</td>
</tr>
<tr>
<td><strong>PP (mmHg)</strong></td>
<td>9149±1297</td>
<td>8785±2998</td>
</tr>
<tr>
<td><strong>RPP (mmHg.bpm)</strong></td>
<td>8976±2998</td>
<td>893±2998</td>
</tr>
</tbody>
</table>

The data is presented as Mean±Standard Deviation of the Mean (M±SDM)

Imm. After: Immediately after; *represents the difference compared to the Rest; # represents the difference compared to immediately after

Fonte: The Authors

Similarly, after performing the 16x30x24 protocol, RPP increased immediately after compared to rest \( (p = 0.002) \). Furthermore, there was a significant reduction in RPP at all recovery times compared to immediately after \( (p = 0.001; p = 0.001; p = 0.002; p = 0.004, p = 0.002; and p = 0.003, respectively) \) (Table II).

In this study, we observed no differences in hemodynamic parameters when comparing the responses between the protocols utilized \( (p \geq 0.05) \).

### Discussion

This study aimed to compare the acute effect of two protocols with different isometric contraction durations on hemodynamic parameters in hypertensive women. Our main findings show that exercises performed on the horizontal bench press with different durations of isometric contraction but with an equated work-
-to-rest ratio promote hemodynamic responses of similar magnitude in women with controlled hypertension.

In our study, we observed that there was no difference in the hemodynamic behavior when analyzing the measures of SBP, DBP, MAP, HR, PP, and RPP among the protocols. Considering the individual analysis, in the 4x2x2 protocol, characterized by prolonged isometric muscle contraction, there was a significant decrease in SBP and MAP between the ten and 30-minute intervals during the recovery period compared to the period immediately after protocol. In the 16x30x24 protocol, characterized by short durations of isometric muscle contraction, the same behavior was in SBP for up to 20 minutes and in MAP for 10 minutes.

In this sense, previous studies comparing different types of prescriptions equalized by the work-to-rest ratio have observed similar hemodynamic responses following single sessions of IE [17-19]. For instance, Mayo et al. [19] did not observe significant reductions in BP after different protocols (5 sets of 8 repetitions with a 3min interval; 10 sets of 4 repetitions with an 80sec interval; and 40 sets of 1 repetition with an 18.5sec interval; equalized at 40reps:720sec at 10-RM) in up to 40 minutes performed on the Leg Press 45º. In another study, Mayo et al. [18] did not observe significant reductions in SBP and DBP in any of the protocols (protocol with rest between repetitions vs. protocol going to muscle failure) compared to rest in different exercises (bench press vs. free squat). Similarly, Río-Rodríguez, Iglesias-Soler, & Olmo [17] also observed no hypotensive effect in the protocols (protocol with rest between repetitions vs. protocol going to muscle failure in isometrics on the extension chair). However, studies that did not control the work-to-rest ratio showed that the amount of muscle mass involved and the total volume of training can determine whether a hypotensive response occurs [28]. One physiological mechanism that can explain the influence of volume and exercise involving large muscle groups is the increase in vasodilator substances released from the endothelial region induced by the increase in blood flow post-exertion and the reduction in peripheral vascular resistance [29].

Furthermore, our study observed that immediately after the protocols, SBP, MAP, HR, and RPP remained significantly elevated compared to Rest, regardless of the configuration. Conversely, Río-Rodríguez, Iglesias-Soler, and Olmo [17] observed that only HR remained high compared to the resting value in the protocol with the longest time under tension (until failure), compared to the protocol performed with rest between repetitions, both performed in isometry on the leg extension. Therefore, we posit that the persistent increase in HR after an exercise session is likely attributable to delayed vagal recovery in the heart, baroreflex withdrawal, and potential cardiovascular overload induced by exercise with a significant increase in RPP [30,31]. Consequently, analyzing sympathovagal balance would be crucial to confirm this hypothesis, necessitating further studies to deepen our understanding. Nevertheless, considering that the observed increase is transient, it does not preclude hypertensive individuals from participating in IE programs, from which they can derive chronic hypotensive effects after at least four weeks, potentially even greater in magnitude than those observed in dynamic strength or aerobic training [1].
Additionally, the 16x30x24 protocol exhibited the most significant effect on PP change, as evidenced by the higher values immediately after compared to rest and about all measurements during the 60-minute recovery. Therefore, the pronounced increase in PP immediately following stress in this protocol may be attributed to the short time interval allotted for recovery between sets. Given that this condition might necessitate Valsalva maneuvers, the rise in arterial stiffness due to elevated intrathoracic and abdominal pressure could directly impact peripheral arteries and the aorta [32].

We recognize limitations in our study, such as the small sample size consisting solely of female participants. Additionally, BP measurements relied on an indirect method, lacking 24-hour ambulatory monitoring for accuracy and protocol assessment. Furthermore, the study only examined one session per protocol, focusing solely on upper limb muscle groups.

**Conclusion**

When equalized by the work-to-rest ratio, IE for the upper limbs with different configurations (4x2x2 and 16x30x24) promotes similar acute hemodynamic responses in controlled hypertensive women.

As practical applications based on this study, we propose that IE for the upper limbs (bench press with the help of a guided bar) with a longer or shorter duration of muscle contraction (4x2x2 and 16x30x24) can be used to help control BP levels in people with SAH, without any additional cardio hemodynamic risk being observed. In addition, the protocol with a shorter duration under isometric tension can serve as an alternative for people who use this type of exercise but have complaints about the sensation of effort caused by prolonged isometric contractions, as both protocols promote equivalent hemodynamic responses. Although this condition still needs to be tested, it can serve as a guide when choosing between these protocols.

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This article represents the final coursework of Leudyenne Pacheco de Abreu, supervised by Professor Mário Alves de Siqueira-Filho at the Federal University of Maranhão.

**Conflict of interest**
The authors declare that no known competing financial conflicts of interest or personal relationships may have influenced the work reported in this article.

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**Authors’ contribution**
Conception and design of the research: Abreu LP, Leite TG, Mostarda CT, Siqueira-Filho MA, Leite RD; Data collection: Abreu LP, Leite TG, Siqueira-Filho MA; Data analysis and interpretation: Abreu LP, Reis CBF, Siqueira-Filho MA; Statistical analysis: Abreu LP, Reis CBF, Siqueira-Filho MA; Manuscript writing: Abreu LP, Reis CBF, Siqueira-Filho MA; Critical review of the manuscript for important intellectual content: Leite RD, Siqueira-Filho MA.
Referencias


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