RBFEx
ISSN Online: 2675-1372

Revista Brasileira de Fisiologia do Exercício

Original article

Glucose threshold: concurrent validity with lactate threshold and concordance with heart rate variability threshold

Limiar glicêmico: validade com limiar de lactato e concordância com a variabilidade da frequência cardíaca

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ABSTRACT

Introduction: At lactate threshold (LT2) intensity, there is an increase in the activity of the sympathetic nervous system, an increase in plasma catecholamines, and an increase in blood glucose, which represent the glucose threshold (GT) and the heart rate variability threshold (HRVT2). These thresholds may concord and allow exercise prescription through more accessible means. **Aim:** To analyze the concurrent validity of GT with LT2 and assess whether there is a concordance between GT and HRVT2. **Methods:** 31 healthy and active men (22 ± 2 years) underwent two days of cardiopulmonary exercise testing (CPET). On the second day, the test aimed to identify GT, LT2, and HRVT2. The intraclass correlation coefficient (ICC), typical error (TE), coefficient of variation (CV), and Bland-Altman tested their reliability and concordance. **Results:** The HR showed good reliability (ICC = 0.80) and good precision (TE = 4.7% and CV = 6.6%) for GT with LT2. For GT and HRVT2, the HR showed moderate reliability (ICC = 0.60) and good precision (TE = 5.9% and CV = 8.4%). **Conclusion:** GT and LT2 presented concurrent validity for identifying the second anaerobic threshold. GT concordance with LT2 is reinforced by the mechanisms that link them.

Keywords: anaerobic threshold; exercise intensity; blood glucose; heart rate.

RESUMO

Introdução: Na intensidade do limiar de lactato (LL2) ocorre um aumento da atividade do sistema nervoso simpático, aumento das catecolaminas plasmáticas e da glicemia, que representam o limiar glicêmico (LG) e o segundo limiar de variabilidade da frequência cardíaca (LVFC2). Esses limiares podem apresentar concordância e permitir a prescrição do exercício por meios mais acessíveis. **Objetivo:** O objetivo do estudo foi analisar a validade concorrente do limiar glicêmico com o limiar de lactato; e sua concordância como o limiar de variabilidade da frequência cardíaca para identificação do segundo limiar anaeróbio. **Métodos:** 31 homens saudáveis e ativos (22 ± 2 anos) foram submetidos a um protocolo escalonado de Teste Cardiopulmonar de Exercício (TCPE), monitorados por medidas de glicemia, lactacemia e variabilidade da frequência cardíaca para a identificação do LG, LL2 e LVFC2. O coeficiente de correlação intraclasse (CCI), erro típico (ET), coeficiente de variação (CV) e Bland-Altman testaram a confiabilidade e concordância. **Resultados:** A HR apresentou boa confiabilidade (ICC = 0,80) e boa exatidão (ET = 4,7% e CV = 6,6%) para o LG com LL2. Para o LG e LVFC2, a FC apresentou confiabilidade moderada (ICC = 0,60) e boa precisão (ET = 5,9% e CV = 8,4%). **Conclusão:** O LG e o LL2 apresentaram validade concorrente para identificação do segundo limiar anaeróbio. A concordância entre o LG e o LVFC2 é reforçada pelos eventos fisiológicos que os relacionam.

Palavras-chave: limiar anaeróbio; intensidade do exercício; glicemia; frequência cardíaca.

Received: September 10, 2022; Accepted: November 23, 2022.

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Introduction

The definition of more precise intensities for the practice of physical exercises identifying the metabolic thresholds favors the determination of individualized training zones and more efficient improvements in the parameters of physical fitness related to health [1] or performance [2]. Metabolic thresholds consist of submaximal physiological index measured during an incremental exercise test.

The anaerobic threshold (AT) is widely used in the science of sports [3,4] being a physiological phenomenon that can be identified using different parameters, for example, ventilatory threshold (LV), lactate threshold (LT), heart rate variability threshold (HRVT1 and 2), glucose threshold (GT), among others [3,5]. Traditionally, LT and VT have been used for assessing this phenomenon [6,7]. The LT can be identified at two distinct moments: aerobic and anaerobic threshold (Lactate Threshold 2 – LT2) [8]. It is known that LT2 is characterized by the exponential increase of lactate in the blood and that, when considered fixed values, it is approximately 4 mmol·L-1 [8].

This phenomenon occurs at exercise intensities with increased plasma catecholamines [9]. The plasma catecholamine increase promotes the bioavailability of blood glucose through glycogenolysis and gluconeogenesis stimulation [10]. The scientific literature indicates a concordance between the glycemic and second ventilatory methods for anaerobic threshold determination in patients with type 2 diabetes [11].

A growing body of evidence indicated the AT determination by glucose levels, called Individual Glucose Threshold (IGT) [12]. The authors observed that blood glucose levels reduced while blood lactate increased (LT) [12]. The lowest glucose value defined the IGT before the abrupt increase associated with LT2 in runners who performed a track test [12].

In addition, a physiological relationship is expected between glycemic response during exercise and heart rate variability due to the sympathetic nervous system of the effect on glucose metabolism [10]. The literature suggests using HRV to estimate physiological thresholds, such as the ventilatory or lactate threshold, in an incremental test [13]. It is believed that HRVT2 can identify the intensity of effort similar to that of GT because, in exercises with higher intensities, the sympathetic nervous system is predominant [14], and factors that prove the increase in blood glucose are also observed [10].

Therefore, it is necessary to analyze the concurrent validity of the glucose threshold with the lactate threshold and their concordance on the heart rate variability threshold to identify the anaerobic threshold in a more accessible way. We hypothesized that GT would have a good concordance with LT2 and HRVT2.

Methods

Sample

The sample was composed of 31 university students, healthy, male, age 22 ± 2 years old. The participants were informed about the procedures. Inclusion criteria: to perform a physical exercise (evaluated by the usual level of physical activity ≥ 150 min·week^{-1}) and between 18 and 30 years old. Exclusion criteria were: medication use, locomotor limitations or diagnosed diseases, smoking story, and alcohol. The research ethics committee approved the study of the Federal University of Espirito Santo. (CAAE 76607717.5.0000.5542).

Procedures

The procedures were performed as shown in Figure 1. The health and personal information survey was done through a self-completed questionnaire. First, the participants were eligible, respecting the inclusion and exclusion criteria. Subsequently, there was a visit to the laboratory in two days, with an interval of 48 hours. On the first day, anamnesis, anthropometric assessment, and cardiopulmonary test were performed with blood collection from the earlobe, rest, and recovery. In addition, cardiopulmonary testing was applied for medical screening and familiarization. On the second day, the second cardiopulmonary test (step protocol) was performed due to the stability necessary for a good collection of heart rate variability and blood collection at rest, during the test, and during recovery. Finally, glucose, lactate, and heart rate variability thresholds were identified. Three participants were excluded due to the difficulty in identifying the glycemic threshold, as described in the section on glycemic threshold collection and identification. (Figure 1).

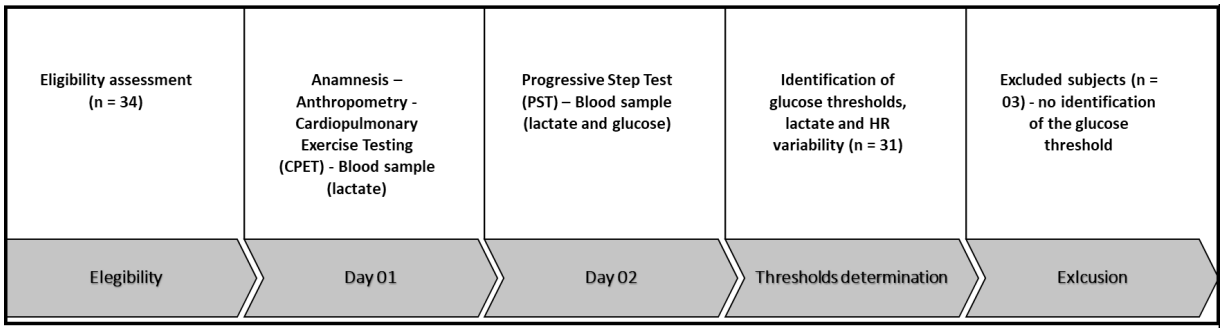


Figure 1 – Study design

Anthropometry

Body mass and height were measured using a digital anthropometric scale with a 1mm precision stadiometer (Marte Cientifica, L200, São Paulo), with a maximum capacity of 201 kg and a sensitivity of 50 g to calculate the body mass index (BMI). The body composition was measured using a scientific skinfolds caliper with 0.1 mm sensitivity and 85mm reading amplitude (Mitutoyo/Cescorf, RS), including seven skinfolds (tricipital, subscapular, pectoral, mid-axillary, suprailiac, abdominal

and thigh). Finally, the fat percentage was calculated using the formula proposed by Jackson and Pollock [15].

Cardiopulmonary Exercise Test – (CPET)

The individuals underwent cardiological assessment of rest and exertion to assess their health conditions and define ventilatory threshold 1 (VT1) intensity. The velocity corresponding to VT1 was the parameter adopted to determine the start speed of the progressive step test.

Ventilatory variables were measured using the metabolic gas analyzer Cortex Metalyzer 3b (Leipzig, Germany) with breath-by-breath collection and analyzed using the MetasoftTM software. The equipment was calibrated using a known gas mixture (11.97% O_2 and 4.95% CO_2 original, certified by the manufacturer). The system was calibrated again at each new test using the ambient air reference. The volume sensor was calibrated using a 3L syringe (Hans Rudolph, Oklahoma, USA). Collecting masks of varying sizes were used according to the facial dimensions of each subject. The test was monitored by a cardiologist and a physical education professional. There was verbal encouragement in the final phase of the CPET, aiming at reaching maximum effort. At least three of the following criteria were used to identify the test as the maximum: a) voluntary exhaustion reported immediately after the test; b) heart rate of at least 90% of predicted for the age (220-age); c) respiratory exchange ratio (RER) equal to or above 1.05; d) maximum oxygen consumption, observed by the plateau or peak concept [16].

Progressive Step Test – (PST)

At least 48 hours after the first visit, the participants performed a new exertion test. The protocol used was a continuous load increment, 3 min per stage, with an increment of 1 km·h^{-1} every 3 min. Before the test, the participant remained at rest, being 5min supine and 5min standing. The protocol's initial speed was 4 km·h^{-1} below the VT1 identified by the first CPET and continued up to the maximum effort, not exceeding 10 exercise stages.

Glucose Threshold (GT) and Lactate Threshold (LT)

During the rest period, 25 µL of arterialized blood were collected from the earlobe, without hyperemia, to determine the blood glucose and blood lactate concentration. Blood was immediately transferred to 1.5 ml capped polyethylene microtubules containing 5 µL sodium fluoride (1%) and packaged for further analysis.

During exercise, a blood sample was collected from the earlobe (25 µL) between each stage. At the end of each stage, the participant jumped off the treadmill, and soon after collection, he returned. They were later analyzed for blood glucose, and blood lactate values (mmol·L-1) were collected [6]. The YSI 2300 STAT Plus Glucose and Lactate Analyzer (Ohio, USA) was used. For joint analysis, blood samples were stored under refrigeration (-80°C).

The criterion to determine at which stage the glucose threshold occurred was the lowest glucose value before an abrupt increase [17]. Lactate Threshold 2 was identified by the second linearity breakpoint and exponential lactate accumulation [18,19].

Heart Rate Variability Threshold

A Polar H7 Heart Sensor connected via Bluetooth with a smartphone was used to collect the heart rate variability (HRV) and beat-to-beat (R-R intervals). Each stage described in the step test was recorded in the Elite HRV app (Elite HRV, Asheville-NC) [20] and edited in the Kubios HRV Standard 3.0 software [21]. Data at rest and immediately after the end of the exercise test were used for analysis.

R-R intervals were grouped into three-minute sequences for analysis of HRV. Data filtering was performed in the Kubios software when there were demonstrations of interference in the data. The first 90 seconds of physical activity at each stage were excluded from the analysis due to the adjustment time for HR and HRV.

This study chose the Root Mean Square of the Successive Differences (RMSSD) and Poincaré plot indexes (SD1 and SD2) as the HRV indexes (time-domain and non-linear HRV parameters). To analyze the HRVT2, the variables RMSSD and SD1 were used, defined as RMSSD – analysis of heart rate variability in the time domain, calculated by the root mean square of the successive differences between adjacent R-R intervals and SD1 – analysis of heart rate variability by the geometric method (Poincaré plot). It represents the short-term analysis of the variability of the R-R interval.

The HRVT2 was determined by the linearity breakpoint after the lowest value, with a subsequent increase in the RMSSD and SD1 confirmed by the linearity break of the SD1/SD2 variables only when necessary (determined by visual inspection) [22,23]. They were identified in Excel software (Microsoft Excel® 2022).

Statistical procedures

All data were tabulated and double-verified by independent researchers. The normality was tested using the Shapiro–Wilk test and submitted to evaluate the histogram, kurtosis, and skewness. The results were presented as mean ± standard deviation (SD). To compare the identification methods of GT with LT2 and of GT with HRVT2, the paired Student's "t" test was used. Reproducibility, reliability, and precision were comprehended by the intraclass correlation coefficient (ICC), typical error (TE), and coefficient of variation (CV) tests. For ICC, values < 0.5 indicate low reliability, 0.5 – 0.75 moderate, 0.75 – 0.90 good, and > 0.90 excellent reliability [24]. To present good reproducibility and reliability, the TE and CV values must be below the cutoff point, 10% and 20% [25], respectively. The Bland Altman technique was used to analyze the concordance between the methods. The analysis was performed using SPSS 20.0 software, and the figures were created by GraphPad Prism 6. The significance level adopted was $P < 0.05$.

Results

Table I shows the characteristics of the subjects, who present normal BMI and fat percentage according to the WHO [15]. The performance variables confirm that the maximum effort was achieved in the Cardiopulmonary Exercise Test.

Table I - Anthropometric and maximal physiological characteristics of participants at Cardiopulmonary Exercise Test. (n = 31)

	Mean ± SD
Age (years)	22.0 ± 2.0
Body mass (kg)	73.6 ± 8.8
Height (cm)	176 ± 7
Fat percentage (%)	9.0 ± 3.7
BMI ($m \cdot kg^{-2}$)	23.8 ± 2.6
Max speed ($km \cdot h^{-1}$)	13.4 ± 1.2
Measured HRmax (bpm)	194 ± 8
Predicted HRmax (%)	97.9 ± 3.9
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	47.7 ± 4.6
VO_{2max} ($L \cdot min^{-1}$)	3.5 ± 0.4
RER_{peak}	1.02 ± 0.09
[La]peak after the test ($mmol \cdot L^{-1}$)	10.54 ± 2.06

Values of mean ± SD. BMI = Body Mass Index; [La] = Lactate concentration; Max = maximal; HRmax = Maximal Heart rate; VO_{2max} = Maximal oxygen consumption; RER = Respiratory exchange ratio; RR = Resting R-R interval; SD = Standard deviation

No differences were found (Table II) between the GT vs. LT2 and GT vs. HRVT2 methods for variables: speed, VO_2, $\%VO_2$, HR, %HR, lactate, and RER ($P > 0.05$).

Table II - Comparison between identification methods

Variableas	GT	LT	HRVT2
	Mean ± SD	Mean ± SD	**Mean ± SD**
Velocidade ($km \cdot h^{-1}$)	11.15 ± 1.75	10.72 ± 1.02	10.82 ± 1.15
Lactato ($mmol \cdot L^{-1}$)	4.89 ± 2.43	4.09 ± 1.49	4.15 ± 1.28
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.59 ± 5.64	39.48 ± 3.70	39.79 ± 3.94
$VO_{2máx}$ (%)	85.27 ± 10.76	82.93 ± 5.78	83.56 ± 6.29
RER	0.94 ± 0.07	0.92 ± 0.07	0.92 ± 0.07
FC (bpm)	177 ± 16	175 ± 13	175 ± 11
FC_{max} (%)	89.82 ± 6.46	88.81 ± 3.77	89.47 ± 3.37

Values of Mean ± SD. Data were compared using paired t-test. bpm = Beats per minute; SD = Standard deviation; GT = Glucose threshold; HR = Heart rate, HRVT2 = Heart rate variability threshold; LT = Lactate Threshold; VO2 = Oxygen consumption; RER = Respiratory exchange ratio. No significant difference ($P > 0.05$).

In Table III, the GT accuracy was tested using the LT2 as a reference. The GT shows moderate reliability (ICC) for VO_2 (0.55) and good reliability for HR (0.80). In addition, there was good reproducibility for VO_2: TE = 3.8 (9.4%) and CV = 13.3%; and for HR: TE = 8.2 (4.7%) and CV = 6.6.

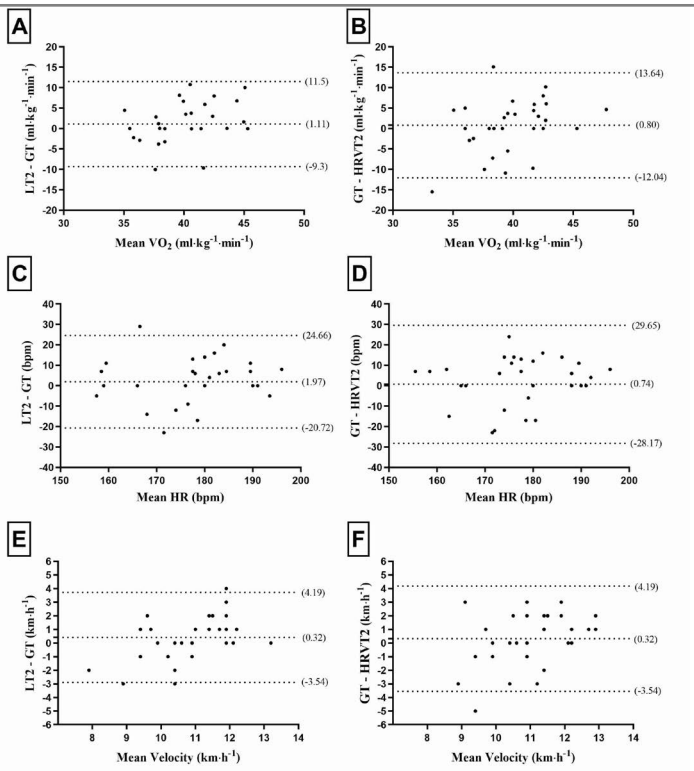
GT and HRVT2 were also compared and exhibited values of TE = 10.4 (5.9%), CV = 8.4%, and ICC = 0.60, only for the HR variable, which showed moderate reliability, good reproducibility, and precision (Table III). Speed (CV= 18.0) and VO_2 (CV=16.3) presented values within the cutoff point. However, a greater variety of data was interpreted with moderate concordance (Table III).

Table III – Values of Typical Error (TE), Coefficient of variation (CV), and Intraclass Correlation Coefficient (ICC) between the methods of GT vs. LT2 and GT vs. HRVT2

Variables	GT vs. LT2		
	TE (%)	CV (%)	ICC
Speed ($km \cdot h^{-1}$)	1.2 (10.9)	15.5	0.46
VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	3.8 (9.4)	13.3	0.55*
HR (bpm)	8.2 (4.7)	6.6	0.80**
	GT vs. LT2		
	TE(%)	CV(%)	ICC
Speed ($km \cdot h^{-1}$)	1.4 (12.7)	18.0	0.20
VO_2 ($ml.kg^{-1} \cdot min^{-1}$)	4.6 (11.5)	16.3	0.17
HR (bpm)	10.4 (5.9)	8.4	0.60**

bpm = Beats per minute; GT = Glucose Threshold; HR = Heart rate; HRVT2 = Heart rate variability threshold, LT = Lactate Threshold; VO2 = oxygen consumption. *P < 0.05; and **P < 0.01 for ICC.

Figure 2 shows the concordance values between LT2 and GT (A, C, and E) and GT and HRVT2 (B, D, and F). There was good concordance in the variables, VO_2, HR, and speed in the comparison between LT2 and GT and GT and HRVT2, which presented a mean of the differences close to zero.



Glucose Threshold (GT), Heart rate (HR), and Heart rate variability threshold (HRVT2), Lactate Threshold (LT), Relative and absolute oxygen consumption (VO_2), *P < 0.05; and **P < 0.01 for ICC

Figure 2 – Limits of concordance of the Bland Altman technique between the methods of GT vs. LT2 and GT vs. HRVT2

Discussion

The present study confirms the hypothesis that there is good concordance between GT and LT2, specifically for the HR and VO_2 parameters. This finding stands out because it enables the use of GT interchangeably with LT2 to identify the anaerobic threshold.

The lactate threshold is one of the most used methods to identify physiological thresholds. However, there is a demand for expensive equipment (laboratory lactate analyzer) to identify it. It is also possible to use portable equipment; however, the cost is higher than a portable glucometer. On the other hand, the GT method is considered a more straightforward test with a lower price and is accessible [26]. However, in this present study, the blood glucose analysis was carried out using expensive equipment. Despite this, other investigations employed measures with portable equipment showing reliable [27,11].

The running speed corresponding to the GT seems to be a good predictor of LT2 and maximal lactate steady state (MLSS) for inactive men who performed the treadmill test [26] and tests performed on track with subjects physically active [28]. In addition, our findings showed statistical similarities between glucose, VO_2, and HR variables. Emphasizing mainly VO_2 and HR, which are essential parameters for exercise prescription [2].

Therefore, the present study presents the finding that the phenomenon is also observable during a CPET, which consists of a stress assessment, is more applied in clinical settings, and can be used in populations with comorbid conditions [11]. In this sense, these findings make the GT method more accessible. This test can be included as a complementary measure in clinical trials when there is interest in accessing more individualized information for exercise prescriptions. For example, one study analyzed the response in cardiorespiratory capacity in sedentary men and women, comparing an individualized training program with pre-established training zones based on percentages of HRmax. The same concluded that individualized training had a more significant effect on the response of cardiorespiratory capacity [29]. Therefore, using thresholds to determine training zones can be an excellent option to obtain more efficient results.

In this study, GT and LT2 were identified at an intensity of 89.8% and 88.8% of FCmax, respectively, demarcating very close intensities when one or the other method was used. This finding helps to reinforce the similarity between the two methods corresponding to AT. This protocol makes it possible to use the GT to identify the AT for professional use in practice.

Moreover, if you collect HR values during the GT test, it will be possible to use them to control exercise intensities. For example, studies that performed the prescription individualized by threshold with non-athlete adults observed significant improvements in ventilatory efficiency, tolerance to maximum and submaximal exercise [1], and cardiorespiratory fitness [29].

Another aim of this study was to test whether the GT concords with the HRVT2 to clarify if their relationship is observed in identifying the AT. Some physiological events may explain the proximity of GT and LT2 since there is a relationship between the increase in plasma glucose and the accumulation of lactate. At exercise intensities corresponding to the AT, plasma catecholamine concentration is high. These biomarkers act concomitantly as neurotransmitters and hormones with hyperglycemic action; they break down hepatic glycogen (glycogenolysis) and stimulate gluconeogenesis from lactate, alanine, and glycerol, increasing blood glucose levels [10,30]. In addition, high levels of catecholamines inhibit insulin release, contributing to the hyperglycemic effect [10].

In this context, the blood lactate increases induce a stimulus for the feedback of peripheral metaboreceptors to stimulate the action of the cardiovascular control center, further increasing the stimulation of the sympathetic nervous system, which affects additional increases in cardiac activity. Thus, it is possible to observe that the concordance observed in the present study between the HRVT2, which is an indicator of cardiac autonomic balance, and the GT has physiological explanations and reinforces the use of the GT for the prescription of exercise intensity

Conclusion

In conclusion, our results suggest that GT is a valid method to identify the anaerobic threshold, having LT2 as a reference. Furthermore, the concordance between the GT and the HRVT2 reinforces the method's reliability, as their mechanisms are related. Therefore, GT employing the FC for prescription offers an option to replace the traditional LT2 method in physically active young adults.

Academic affiliation
This article is the result of the Institutional Scientific Initiation Program of the Federal University of Espírito Santo (UFES), by class student Igor Ziviani Araújo, supervised by Professor Luciana Carletti, UFES.

Conflict of interest
All authors are responsible for the manuscript's content and approve 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 no known competing financial conflicts of interest or personal relationships may have influenced the work reported in this article.

Funding
The present work was carried out with the support of a scientific initiation scholarship from the Universidade Federal do Espírito Santo.

Authors' contribution
Conception and design of the research: Carletti L, Araujo IZ, Neves LNS, Gasparini Neto VH, Leite RD; **Data collection:** Araujo IZ, Neves LNS, Gasparini Neto VH; **Data analysis and interpretation:** Carletti L, Araujo IZ, Neves LNS, Gasparini Neto VH, Leite RD; **Statistical analysis:** Carletti L, Araujo IZ, Neves LNS; **Writing of the manuscript:** Carletti L, Araujo IZ, Neves LNS, Gasparini Neto VH, Leite RD; **Critical review of the manuscript for important intellectual content:** Carletti L, Araujo IZ, Neves LNS, Gasparini Neto VH, Leite RD.

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