Cardiorespiratory Optimal Point in Professional Soccer Players: A Novel Submaximal Variable During Exercise

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Abstract

Background: Maximal oxygen consumption (VO₂max) and ventilatory threshold (VT) obtained during a cardiopulmonary exercise test (CPX) are used in the evaluation of athletes. However, the identification of these variables may sometimes be unreliable, which limits their use. In contrast, the cardiorespiratory optimal point (COP) is a submaximal variable derived from CPX with objective measurement and prognostic significance. However, its behavior in athletes is unknown.

Objective: To describe the behavior of COP in professional soccer players and its association with VO₂max and VT.

Methods: VO₂max, VT and COP were obtained retrospectively from 198 soccer players undergoing maximal treadmill CPX using ramp protocol. COP was defined as the lowest value of the ventilation/oxygen consumption ratio in a given minute of the CPX. The soccer players were stratified according to their field position: goalkeeper, center-defender, left/right-back, midfielder and forwarder. Continuous variables were compared using unpaired Student t test or ANOVA, or Mann-Whitney test or Kruskal-Wallis test depending on their distribution, and categorical variables were compared using chi-square test. Pearson correlation was used to test the association between COP and other ventilatory variables. A level of 5% was used for statistical significance.

Results: COP (mean ± SD) was 18.2 ± 2.1 and was achieved at a speed 4.3 ± 1.4 km.h⁻¹ lower than that achieved at the VT. While VO₂max (62.1 ± 6.2 mL.kg⁻¹.min⁻¹) tended to be lower in goalkeepers (p < 0.05), the COP did not vary according to field position (p = 0.41). No significant association was observed between COP and VO₂max (r = 0.032, p = 0.65) or between COP and VT (r = -0.003, p = 0.96).

Conclusion: COP can be easily determined during submaximal exercise performed with incremental speed in soccer players and does not vary according to the athlete’s field position. The absence of association with VO₂max and VT indicates that COP provides distinct and complementary information to these variables. Future studies are needed to determine the practical implications of COP in assessing athletes. (Int J Cardiovasc Sci. 2018;31(4)323-332)

Keywords: Exercise; Football / trends; Spirometry / methods; Bronchospirometry / methods; Athletic Performance.

Introduction

The cardiopulmonary exercise test (CPX) is a functional and noninvasive procedure used to assess the integration of the cardiovascular, respiratory and musculoskeletal systems based on the analysis of submaximal and maximal responses to exercise.¹ The information obtained from CPX is important to the prognostic assessment of healthy and unhealthy individuals,²,³ and the measures of maximal aerobic power, represented by maximal oxygen consumption (VO₂max), and of ventilatory threshold (VT) are often used to assess and monitor athletes'
training. For example, soccer players with higher VO\textsubscript{2}\text{max} are known to cover longer distances during a match, and their mean exercise intensity during the match is approximately 75% of their VO\textsubscript{2}\text{max}, similarly to the VT level of those players.

However, limitations such as low reproducibility, different techniques and criteria for identification of both VO\textsubscript{2}\text{max} and VT hinder their routine use. In addition, mistakes in such measurements can jeopardize the planning of individualized trainings, impairing the athlete’s performance improvement.

In 2012, Ramos et al. showed the minimum value of the ventilatory equivalent for oxygen (minimum VE/VO\textsubscript{2}) during a CPX – the cardiorespiratory optimal point (COP) – and described its behavior, which, theoretically, represents the point of the best association or integration between the respiratory and cardiovascular systems. Based on the assessment of more than 600 healthy and non-athletes individuals aged between 23 and 73 years, those authors showed that COP tends to be higher in women and increases with age. In addition, studies conducted by that same group have shown that COP measurement is easy, objective and stable in CPX performed in adults, supporting its potential use in physiological research and in clinical practice. Similarly to VO\textsubscript{2}\text{max} and VT, COP proved to be an excellent predictor of all-cause mortality in healthy and unhealthy individuals aged between 40 and 85 years.

So far, the behavior of COP in athletes is unknown. Thus, our objectives are: a) to describe the behavior of COP in professional soccer players; and b) to assess its association with VO\textsubscript{2}\text{max} and VT.

**Materials and Methods**

**Sample**

This study analyzed retrospectively the data of 247 soccer players of the major team of a Rio de Janeiro club of the Brazilian Soccer Championship A series, who underwent a maximal CPX at a private Exercise and Sports Medicine clinic between January 2005 and December 2016. Of those, 198 players concomitantly meeting the following inclusion criteria were selected: a) to have undergone a treadmill CPX; b) to have completed a truly maximal CPX, which was not interrupted due to clinical reasons or lack of motivation; c) to have no history of cardiorespiratory diseases. Based on the information provided by the soccer players, they were categorized according to their predominant field positions: goalkeeper, center-defender, left/right-back, midfielder and forwarder.

**Assessment protocol**

**Clinical assessment**

Included clinical history and physical examination, as well as anthropometric, spirometric and resting 12-lead electrocardiographic data.

**Resting spirometry test**

At least three maneuvers were carried out to determine the flow-volume curves using a pneumograph (SP-1 Spirometer, Schiller, Switzerland or KoKo, United States) periodically calibrated according to the protocol recommended by the North American and European guidelines.

**Maximal cardiopulmonary exercise test**

The CPX were performed on a treadmill (ATL Master, Inbramed, Brazil) in a properly climatized room. All players underwent the same ramp protocol, at an initial velocity of 8.0 km.h\textsuperscript{-1}, with progressive increase of 0.1 km.h\textsuperscript{-1} every 7.5 seconds, and without any inclination. All CPX were conducted by specialized physicians with large experience in assessing athletes, following a well-defined routine, mainly regarding the stimulus to achieve truly maximal exertion. CPX was considered maximal based on the physician’s subjective assessment and other objective variables, such as: occurrence of VT, U-pattern ventilatory equivalent, and a 10-score in the 0-10 Borg scale. During the CPX, the players were monitored continuously by use of a digital electrocardiograph (ErgoPC Elite versions 3.2.1.5 or 3.3.4.3 or 3.3.6.2, Micromed, Brazil), which measured heart rate (HR) on the electrocardiographic tracing in the CC5 or CM5 leads at the end of every minute.

**Analysis of the expired gases**

During the CPX, the expired gases were collected by use of a Prevent pneumograph (MedGraphics, United States) coupled to a mouth piece, with concomitant use of a nose clip. The expired gases were measured and analyzed with the VO2000 metabolic analyzer (MedGraphics, United States), which was calibrated with a 2L-serinje and with gases of known concentrations before the first assessment of the day, and this procedure was repeated when necessary. Pulmonary ventilation
(VE) and oxygen and carbon dioxide partial fractions were expressed every 10 seconds, and their mean values for each minute of CPX were then calculated.

**Determining maximal oxygen consumption and ventilatory threshold**

The VO\textsubscript{2}max was considered the highest value at a given minute of CPX. The VT was visually determined as the point at which an interruption in VE's curve linearity and a sustained increment in VE/VO\textsubscript{2} ratio occurred, being described as the percentage of VO\textsubscript{2}max at that velocity. In addition, the velocity and the VO\textsubscript{2} at which the VT occurred were recorded.

**Determining the cardiorespiratory optimal point**

The COP was obtained by identifying the lowest VE/VO\textsubscript{2} ratio at a given minute of CPX, being, thus, a non-dimensional value. In addition, the VO\textsubscript{2} and the running velocity in the ramp protocol at that point were recorded.

**Statistical analysis**

Data distribution was assessed by use of the Shapiro-Wilk normality test. Continuous variables with parametric distribution were expressed as mean ± standard deviation (SD), being compared by use of the unpaired Student t test or ANOVA and post-hoc Bonferroni test, when appropriate. Continuous variables with non-parametric distribution were expressed as median (interquartile range) and compared by use of Mann-Whitney test or Kruskal-Wallis test, when appropriate. Categorical variables were expressed as percentage of the frequency and compared by use of the chi-square test. The coefficients of variation of the variables COP, VT and VO\textsubscript{2}max, obtained by the ratio between standard deviation and mean, were calculated. Pearson correlation was used to test the association between COP and other ventilatory variables. The statistical calculations were performed using the Stata14® software, adopting a significance level of 5%.

**Ethical considerations**

All soccer players underwent the assessment willingly, having read and signed the specific written informed consent before the CPX, and having authorized the use of their data for scientific research. The retrospective analysis of data was previously approved by the Ethics Committee on Research of the institution.

**Results**

Table 1 describes the major demographic characteristics, and the resting spirometry and CPX results of the soccer players. Age, weight, height and body mass index (BMI) ranged from 16 to 36 years, from 57.5 to 102.0 kg, from 163.3 to 196.3 cm, and from 19.3 to 29.6 kg.m\textsuperscript{-2}, respectively. COP, VT and VO\textsubscript{2}max showed a parametric distribution (p > 0.05), with values ranging from 13.1 to 25.3, from 61.8 to 92.7% of VO\textsubscript{2}max, and from 45.0 to 76.2 mL.kg\textsuperscript{-1}.min\textsuperscript{-1}, respectively. The coefficients of variation for COP, VT and VO\textsubscript{2}max were 16.1%, 10.7% and 10.0%, respectively. On average, COP, VT and VO\textsubscript{2}max occurred at the velocities of 10.0 ± 1.0, 14.3 ± 1.1, and 18.7 ± 0.9 km.h\textsuperscript{-1}, respectively (p < 0.01).

When stratified by their field positions during the match (Table 1), the only characteristics that differed were weight and height, with goalkeepers showing the highest values for both variables (p < 0.01). The BMI, however, was similar among the soccer players of different field positions (p = 0.86). Regarding CPX, goalkeepers achieved the lowest VO\textsubscript{2}max values relative to their body weight (mL.kg\textsuperscript{-1}.min\textsuperscript{-1}) (p = 0.01) and reached the COP at a higher HR and percentage of VO\textsubscript{2}max than the players of other field positions (p < 0.01). However, the values of COP (p = 0.41) and VT (% of VO\textsubscript{2}max) (p = 0.42) did not differ according to the soccer players’ field positions.

The coefficients of correlation between COP and VO\textsubscript{2}max (mL.kg\textsuperscript{-1}.min\textsuperscript{-1}) and between COP and VT (% of VO\textsubscript{2}max) were 0.032 (p = 0.65) and -0.003 (p = 0.96), respectively, evidencing the low association between those variables. Figure 1 shows those data.

**Discussion**

During an exercise training with progressive intensity increase up to the voluntary maximum, the relationship between VE and VO\textsubscript{2} is nonlinear, and the curve that illustrates that relation has a U shape, suggesting higher ventilatory efficiency (lower VE/VO\textsubscript{2}) at submaximal exercise levels when compared to rest and to the highest exertion intensities. Based on that, COP was described as the lowest VE/VO\textsubscript{2} value at a given minute during an incremental exercise, representing the time point with the lowest amount of ventilation per liter of oxygen to be consumed, which is the best integration of the relationship between circulation and respiration. Recent studies have shown the clinical applicability of the COP for the diagnostic and prognostic assessment.
Table 1 - Major demographic characteristics and results of maximal cardiopulmonary exercise test on treadmill of professional soccer players

| Variables* | Total (n = 198) | Goalkeeper (n = 13) | Left/right-back (n = 27) | Center-defender (n = 32) | Midfielder (n = 76) | Forwarder (n = 50) | p value |
|------------|-----------------|---------------------|--------------------------|-------------------------|---------------------|-------------------|---------|
| Characteristics |                 |                     |                          |                         |                     |                   |         |
| Age (years)  | 23 (21 - 27)    | 22 (19 - 24)        | 26 (21 - 28)             | 22 (21 - 26)            | 23 (21 - 28)        | 24 (10 - 27)      | 0.35    |
| Weight (kg)  | 76.7 ± 7.6      | 85.1 ± 4.7          | 74.3 ± 6.2               | 80.7 ± 5.4              | 74.1 ± 7.6          | 77.3 ± 7.6        | < 0.01  |
| Height (cm)  | 179.2 ± 6.4     | 187.5 ± 4.1         | 175.5 ± 4.2              | 184.3 ± 3.8             | 176.5 ± 6.0         | 180.0 ± 5.8       | < 0.01  |
| Body mass index (kg.m⁻²)| 23.9 ± 1.8 | 24.2 ± 1.1          | 24.1 ± 1.7               | 23.8 ± 1.6              | 23.8 ± 1.8           | 23.9 ± 2.1        | 0.86    |
| HR at rest (bpm) | 59 (53 - 66) | 62 (57 - 66)        | 57 (51 - 62)             | 61 (53 - 65)            | 61 (54 - 66)        | 57 (52 - 66)      | 0.15    |
| SBP at rest (mm Hg) | 130 ± 10 | 130 ± 8             | 130 ± 14                 | 129 ± 7                 | 129 ± 11             | 131 ± 10          | 0.92    |
| DBP at rest (mm Hg) | 70 ± 9 | 72 ± 7              | 71 ± 11                  | 70 ± 8                  | 70 ± 9               | 71 ± 9            | 0.95    |
| Resting spirometry |                   |                     |                          |                         |                     |                   |         |
| FEV1 (L)     | 4.31 (3.94 - 4.69) | 4.74 (4.35 - 5.04) | 4.16 (3.96 - 4.41)      | 4.44 (4.13 - 5.00)     | 4.24 (3.79 - 4.61)  | 4.41 (3.90 - 4.71) | < 0.01  |
| % of predicted FEV1 | 98.5 (90.8 - 105.6) | 101.9 (93.9 - 105.3) | 96.7 (93.5 - 104.8) | 98.5 (88.6 - 105.8) | 98.5 (91.6 - 105.0) | 100.8 (87.3 - 107.0) | 0.99    |
| FVC (L)      | 5.05 ± 0.68     | 5.70 ± 0.68         | 4.83 ± 0.50              | 5.23 ± 0.66             | 4.94 ± 0.62         | 5.05 ± 0.75       | < 0.01  |
| % of predicted FVC | 96.2 ± 10.5 | 100.5 ± 10.8        | 95.7 ± 8.4               | 94.0 ± 10.3             | 97.0 ± 10.0         | 95.6 ± 12.1       | 0.38    |
| FEV1/FVC ratio (%) | 86.0 ± 5.3 | 83.1 ± 5.6          | 87.3 ± 4.1              | 87.6 ± 5.3              | 85.4 ± 5.1          | 85.8 ± 5.9        | 0.05    |
| CPX |            |                     |                          |                         |                     |                   |         |
| Duration (min) | 13.0 (13.0 - 14.0) | 13.0 (13.0 - 14.0) | 14.0 (13.0 - 14.0) | 13.0 (13.0 - 14.0) | 14.0 (13.0 - 14.0) | 13.0 (13.0 - 14.0) | 0.09    |
| Maximal RER | 1.10 (1.06 - 1.15) | 1.09 (1.06 - 1.13) | 1.09 (1.05 - 1.15) | 1.13 (1.07 - 1.16) | 1.11 (1.06 - 1.15) | 1.10 (1.06 - 1.14) | 0.59    |
| Maximal HR (bpm) | 192 ± 9 | 194 ± 8             | 187 ± 7                  | 194 ± 10                | 192 ± 10            | 192 ± 8           | 0.01    |
| Maximal VE (L.min⁻¹) | 123.2 (113.1 - 133.2) | 129.8 (122.6 - 135.2) | 123.1 (112.8 - 133.7) | 125.4 (113.7 - 136.0) | 121.7 (111.6 - 129.9) | 122.9 (113.9 - 134.4) | 0.30    |
| Maximal velocity (km.h⁻¹) | 18.8 (18.4 - 19.2) | 18.4 (18.0 - 19.2) | 19.2 (18.4 - 19.5) | 18.5 (17.9 - 19.2) | 18.8 (18.4 - 19.2) | 18.6 (18.4 - 19.2) | 0.13    |
| COP (lowest VE/VO₂) | 18.2 ± 2.1 | 19.1 ± 2.2          | 18.7 ± 2.1               | 17.9 ± 2.5              | 18.1 ± 2.2          | 18.2 ± 1.9        | 0.41    |
| Time to reach COP (min) | 2.0 (2.0 - 3.0) | 3.0 (2.0 - 4.0) | 2.0 (2.0 - 3.0) | 2.0 (2.0 - 3.0) | 2.0 (2.0 - 2.5) | 2.0 (2.0 - 2.0) | 0.07    |
| Velocity at the COP (km.h⁻¹) | 9.6 (9.6 - 10.4) | 10.4 (9.6 - 11.2) | 9.6 (9.6 - 10.4) | 9.6 (9.6 - 10.4) | 9.6 (9.6 - 10.0) | 9.6 (9.6 - 9.6) | 0.07    |
| HR at the COP (bpm) | 132 (122 - 142) | 142 (134 - 148) | 131 (122 - 138) | 139 (128 - 152) | 131 (121 - 140) | 128 (121-137) | < 0.01  |
of the cardiorespiratory interaction of both healthy individuals and those with chronic diseases; in addition, by being a submaximal variable of CPX, the use of COP is particularly interesting for patients unable to achieve a maximal CPX because of functional limitations (eg, peripheral obstructive arterial disease, orthopedic disorders) or because of their fear of achieving peak exertion (eg, patients with panic syndrome),17,21 as well as for athletes during the competition season.22 For example, the COP bears an inverse relationship with all-cause mortality in healthy and ill individuals aged from 40 to 85 years, having, thus, prognostic value and being a new possibility for mortality risk assessment.17 Based on those observations, it is worth trying to expand the applicability of COP to other scenarios. In theory, one might assume that for athletes of modalities with high aerobic demands, such as soccer, low COP values can represent a physiological advantage, especially when occurring at relatively high velocities. Because the aerobic demands vary according to the field position during the match, the opportunity to compare a large number of elite soccer players tested in standard conditions can contribute to better understand the meaning and the potential applicability of COP in sports. The present study is an original contribution because it is the first to describe the COP behavior in athletes, in particular, high-level adult soccer players submitted to a CPX on a treadmill following the ramp protocol.

The COP has advantages related to its determination and measurement when compared to VO2max and VT, the two major variables of CPX used to assess the performance of athletes. Obtaining a true VO2max...
Figure 1 - Diagram of dispersion between variables: a) cardiorespiratory optimal point (COP) and maximal oxygen consumption (VO₂max); and b) COP and percentage of VO₂max reached at the ventilatory threshold (VT).
suggests the existence of a plateau in the VO\textsubscript{2} curve, which is not always possible, and it can vary according to the CPX protocol used and the gas sampling or collection interval.\textsuperscript{10,11} In addition, VO\textsubscript{2}max depends on performing a truly maximal exercise test, whose determination criteria vary in the literature, being subjective to a certain extent. On the other hand, although the VT does not require a maximal test, it requires a more intense exercise than COP does, and VT measurement is hindered by the existence of several distinct criteria for its identification and/or characterization, which, in a significant percentage of cases, cannot be obtained, limiting its use in clinical practice and sports.\textsuperscript{13} In addition, although both VO\textsubscript{2}max and VT can be detected automatically with commercial software, the methods available for that have been developed from varied definitions and algorithms, implying the need for its review by at least one experienced observer, making those measures subjective and widening the potential of high inter- and intraobserver variability.\textsuperscript{23,24} In contrast, COP is easily determined from the identification of the lowest value of the VE/VO\textsubscript{2} ratio for each minute of CPX, not depending, thus, on the interpretation and experience of the observer, and relying on a relatively small effort, because it occurs at relatively low exercise intensities, before the VT.

Regarding the COP of the soccer players assessed, some findings are worth noting: 1- as expected, COP was obtained at lower percentage of VO\textsubscript{2}max and velocity than those at the VT; 2- similarly to VT, but opposite to VO\textsubscript{2}max, COP did not differ according to the different field positions of the soccer players; 3- no significant association was observed between COP and the variables VO\textsubscript{2}max and VT; and 4- the coefficient of variation of oxygen consumption at the time of the COP was greater than that observed at the VT and VO\textsubscript{2}max. It is interesting to point out that, on average, the COP values found for the soccer players were below the 50\textsuperscript{th} percentile of the values found for healthy male non-athletes of the same age group in a previous study,\textsuperscript{15} and that only eight (4\%) soccer players had COP over 22, considered the cutoff point for optimal clinical prognosis,\textsuperscript{17} suggesting that those soccer players have a privileged circulation-respiration interaction, probably more economic at the submaximal exercise. However, it is worth noting that the COP values described for non-athletes were obtained from a CPX performed on a lower limb cycle ergometer, with an individualized ramp protocol. Thus, the description of COP in different exercise modalities and protocols should be approached in future studies, because there is evidence that the behavior of some variables obtained in CPX differ depending on the ergometer and protocol used.

The running velocity on the treadmill and the exercise intensity represented by the percentage of VO\textsubscript{2}max at which the soccer players assessed in this study reached the COP (10.0 \pm 1.0 km.h\textsuperscript{-1} and 51.3 \pm 8.7\%, respectively) were lower than the values obtained at the VT by soccer players assessed in other studies, even when compared to those of players of lower athletic performance, who are expected to reach an earlier VT. For example, according to Ziogas et al.,\textsuperscript{25} soccer players of the first, second and third Greek division submitted to a CPX in the pre-season period reached the VT at a mean velocity of 13.2, 12.6 and 12.3 km.h\textsuperscript{-1}, respectively. Boone et al.,\textsuperscript{26} however, assessing 289 soccer players of the first Belgian division, have reported mean running velocities on the treadmill at the VT ranging from 12.7 \pm 1.4 in goalkeepers to 14.4 \pm 0.7 km.h\textsuperscript{-1} in center-defenders. Regarding the exercise intensity, Impellizzeri et al.\textsuperscript{27} and Helgerud et al.\textsuperscript{28} have reported that junior soccer players reached the VT at a mean percentage of VO\textsubscript{2}max greater than 80\%. Considering that the running velocity and the exercise intensity at which the VT is reached reflect the training status of the soccer players, future studies should assess whether COP is also useful to differentiate the physical performance of athletes.

When comparing the soccer players according to their field positions, goalkeepers, midfielders, left/right-backs, center-defenders and forwards did not differ regarding the COP. Manari et al.,\textsuperscript{29} comparing the VT and the VO\textsubscript{2}max of 450 European elite soccer players of different field positions, have found no differences regarding the VT, similarly to our study’s findings regarding VT and COP. However, similarly to our study’s findings, VO\textsubscript{2}max was lower in goalkeepers. Tonessem et al.,\textsuperscript{30} assessing 1,545 male soccer athletes, have found small to moderate differences in VO\textsubscript{2}max according to the athlete’s field position, with greater values in the midfielders, followed, in decreasing order, by the defense athletes, forwards and goalkeepers. Similarly, Balikian et al.,\textsuperscript{31} assessing 25 professional soccer players, have found lower mean VO\textsubscript{2}max values of goalkeepers (52.68 mL.kg\textsuperscript{-1}.min\textsuperscript{-1}) as compared to the mean values of soccer players of other field positions. However, in contrast to our study’s findings, the mean velocity at which the players reached the VT differed according to their field position, being lower for goalkeepers (12.66 km.h\textsuperscript{-1}) and higher for left/right-
backs (14.33 km.h⁻¹) and midfielders (14.11 km.h⁻¹). Nevertheless, it is worth noting that the heterogeneity of the methods used to measure VT hinders the comparison of the results between the studies.

Finally, the COP failed to show a linear association with the variables VT and VO₂max. Ramos et al.¹⁵ have not only described a moderate association with VO₂max (r=0.47) and VT (r=0.42), but have also observed that the combination of COP and VO₂max adds more prognostic information to all-cause mortality than each variable in isolation.¹⁷ Such findings suggest a possible independence and complementarity of COP regarding VO₂max and VT, which could contribute with additional information to the interpretation of the relationship between the cardiovascular and respiratory systems during a CPX. Thus, one can speculate that the submaximal variables – COP and VT – might better reflect the energetic demands of a soccer match in the current context, in which the differences in distance and in percentage of time spent in intense efforts are less evident in soccer players of different field positions.

The present study has some limitations in addition to those already mentioned. The CPX analyzed were limited to those performed in the pre-season period, not allowing us to assess the COP behavior in different training periods of the soccer players. In addition, this study only assessed male adult elite soccer players, which limits the extrapolation of the results to female soccer players, other age groups, different technical levels and other sport modalities.

Conclusion

The present study described the COP behavior and its absence of association with VO₂max and VT of male adult elite soccer players. Thus, future studies are required to assess whether COP can provide additional and relevant information to other sport contexts.

Author contributions

Conception and design of the research: de Souza e Silva CG, Castro CLB, Franca JF, Bottino A, Myers J, Araújo CGS; Acquisition of data: Castro CLB, Franca JF, Araújo CGS; Analysis and interpretation of the data, Statistical analysis and Writing of the manuscript: de Souza e Silva CG, Araújo CGS; Critical revision of the manuscript for intellectual content: Castro CLB, Franca JF, Bottino A, Myers J, Araújo CGS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Suprema - Faculdade de Ciências Médicas e da Saúde de Juiz de Fora under the protocol number 0218/11. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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