Ventilatory threshold concordance between ergoespirometry and heart rate variability in female professional cyclists

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ABSTRACT

The ventilatory threshold (VT) is the point at which ventilation intensifies disproportionately concerning to the oxygen uptake, this parameter is essential within the training methodology to optimize performance; the purpose of this work is to measure the degree of agreement between the determination of the ventilatory threshold determined by for oxygen uptake and four methods by Heart Rate Variability (HRV). Methodology: Twelve professional female cyclists between 18 and 28 years of age were evaluated through a maximal incremental test on a cycle ergometer; gas exchange was measure with a portable telemetric device (Cosmed K4b2®) and simultaneously the HRV was registered by R-R recording trough a Polar RS800CX® (Polar, Finland) heart rate monitor. Statistics: The degree of precision and accuracy established between the variables mentioned and normality test Shapiro-Wilk. Results: rho = 1.00 with p-value < .05, and Lin correlation and concordance coefficients of .99 with r = 0.98 for ventilatory threshold 2 (VT2), constituting a level of precision and accuracy almost perfect; by contrast, ventilatory threshold 1 (VT1) did not show significant precision and accuracy. This study suggests that VT2 can be identified from The time series. RR using the analysis of frequency and temporal domains likewise allows. Us to have a reference measure for future research in other sports.

Keywords: Ventilatory threshold; Ergospirometry; Heart rate variability; Cyclists; Women.

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INTRODUCTION

The intensity of the races in professional cycling requires resistance training and load control that allows them to meet sporting achievements (Peinado, et al., 2016), for this purpose ventilatory thresholds are a tool for feedback, which includes study variables in the laboratory and in the field, allowing coaches to build an effective training plan for each cyclist.

Ventilation thresholds have been defined as the point at which a disproportionate increase in ventilation (VE) is generated concerning oxygen uptake (VO₂), secondary to an increase in the production of carbon dioxide (VCO₂) during exercise (Wasserman, Hansen, Sue, Whipp, & Froelicher, 1987) (Wasserman, Beaver, & Whipp, 1990); the changes of these gases (O₂ and CO₂) in arterial blood are registered by the Autonomic Nervous System (ANS) through the central and peripheral chemoreceptors (Boron & Boulpaep, 2015), which are in charge of transmitting the information to the brain stem, to regulate the intensity of the stimulus and determine changes in the depth and frequency of the ventilatory pattern. Likewise, send references to the node sinus to modulate heart rate (HR) rhythmically and automatically without any conscious effort (Tiller, McCraty, & Atkinson, 1996) (Potts, 2006) (Blain, Meste, & Bermon, 2005).

During the development of an incremental test, before reaching ventilatory threshold one (VT1), a decrease in arterial PO₂ occurs, which is the main stimulus for peripheral chemoreceptors, generating an early response in ventilation (Naimark, Wasserman, & McIlroy, 1964); however, when arterial gas parameters are controlled, central chemoreceptors are the main source of feedback to evaluate the efficacy of this change. It should be mentioned, also, that this receptor is more sensitive to an increase in PCO₂ and a decrease in pH, if this occurs during an incremental test, the athlete would be close to ventilatory threshold two (VT2) (Wasserman, Hansen, Sue, Whipp, & Froelicher, 1987), which would result in a proportional increase in VE (Cottin, et al., 2006) (Cottin, et al., 2007) (Mourot, Fabre, Savoldelli, & Schena, 2014). These changes generate expansion of the rib cage, allowing the lung stretch receptors to inhibit the vagal nuclei in the brainstem, and thus, the increase in alveolar ventilation decreases the PCO₂ caused by hypoxia, raising the pH and inhibiting the nuclei vagal, this generating an increase in HR (Blain, Meste, & Bermon, 2005). Thus, is why the cardiorespiratory coupling during exercise (Serrato & Galeano, 2015) (Cottin, et al., 2006) can be interpreted through the variation of the beat-beat interval (RR).

The ergospirometry, is the gold standard, that through measurement of exhaled gases (VO₂ and VCO₂) provides parameters that allow visual identification of ventilatory thresholds through different methods (Mourot, Fabre, Savoldelli, & Schena, 2014). However, this measurement strategy is expensive, must be done in the laboratory, and requires additional logistics. On the other hand, HRV is a non-invasive method, which allows evaluating autonomic modulation by instantaneously measuring variations in RR intervals using a HR monitor (Font, Pedret, Ramos, & Ortís, 2008) (Camm, et al., 1996) (Cottin, et al., 2004). Identification is carried out through two methods: the first is the temporal domain, which considers the RR intervals, using statistical tools (Malek, Berger, Housh, Coburn, & Beck, 2004) (Ruiz, 1999); and the second one is a spectrum that is obtained from a mathematical transformation (Jarvis, et al., 1999) of the RR intervals, it is called frequency and its waves are in the range of 0 to 0.4 Hz but it extends at 2 Hz during their exercise assessment, mainly since respiratory rate and high frequency (HF) in HRV are linked (Anosov, Patzak, Kononovich, & Persson, 2000) (Grossman & Taylor, 2007) (Kenney, 1985). Plus, the previously mentioned mechanical associations (Cottin, et al., 2007) (Mourot, Fabre, Savoldelli, & Schena, 2014) (Karapetian, Engels, & Gretebeck, 2008). Associated with this, this measurement strategy is inexpensive and allows a higher percentage of access by athletes.
All of the above has led to research in the sports field establishing the degree of association between the measurement of thresholds by ergospirometry and HRV \( r = 0.98 \ p\text{-value} = .05 \) (Malek, Berger, Housh, Coburn, & Beck, 2004) (Ruiz, 1999) (Karapetian, Engels, & Gretebeck, 2008) (Cottin, et al., 2006) (Mourot, Fabre, Savoldelli, & Schena, 2014) (Dourado & Guerra, 2013) (Zoladz, Duda, & Majerczak, 1998) (Ramos-Campo, et al., 2017), however the estimation of the accuracy quantified by the concordance has not been considered, which allows us to know the capacity a measurement of bringing it to the value of the real magnitude (Cabrera, et al., 2018) (Cabrera, et al., 2018) (Arvidsson, Kawakami, Ohlsson, & Sundquist, 2012), on the other hand, is not our knowledge that research has been carried out in women's cycling for these variables (Cottin, et al., 2006) (Cottin, et al., 2007) (Mourot, Fabre, Savoldelli, & Schena, 2014) (Ramos-Campo, et al., 2017); for this reason, the purpose of this study is to establish the degree of concordance between HRV and ergospirometry for the determination of ventilatory thresholds in professional female cyclists.

METHODOLOGY

Subjects
Twelve \((n=12)\) elite cyclists between 18 and 28 years old, were evaluated voluntarily and with previous experience in testing. They were informed verbally and in writing about the possible risks and benefits, and all signed informed consent. This study was carried out under the codes of ethics of the Declaration of Helsinki and was approved by the ethics committee of the University Institution National School of Sport, Cali. Colombia.

Maximum Test
The selected protocol was: warm-up: five (5) minutes at 70 Watts, cadence less than 70 revolutions per minute (rpm). The test started at 70 W, 30 W increments every two minutes (70W/30W/2min) until the rider reached volitional exhaustion and met at least two criteria of maximum oxygen uptake, cadence greater than 70 rpm (85-100 rpm). Subsequently, 5 minutes of recovery at 50 W with a comfortable cadence (50-60 rpm) (Serrato & Galeano, 2015).

**Determination of ventilatory thresholds**

**Maximum oxygen uptake**
The maximum oxygen uptake \( (\overline{V}O_2) \) was defined as the average of the last 30 seconds of the test, in which a plateau is presented in \( \overline{V}O_2 \) despite the increase in working power, in the same way, the volitional exhaustion of the cyclist assured by a respiratory exchange ratio \( (VCO_2/VO_2) \) greater than 1.05 or a pedalling rate less than 70 rpm (Serrato & Galeano, 2015). \( VO_2max \) was considered as the achievement of at least two of the above criteria.

**Ventilatory Threshold determined by Ergospirometry**
The ventilatory threshold \( (VT1, VT2) \) was determined according to the method of Wasserman (1987) (Wasserman, Hansen, Sue, Whipp, & Froelicher, 1987) through four independent evaluators in different cities.

**Determination of Ventilatory Threshold \((VT)\) using Heart Rate Variability (HRV)**
Interval RR values of warm-up, effort and recovery were recorded using a Polar RS800CX® (Polar, Finland). The vagal activity was determined by time-domain variables: 1. The standard deviation of the RR (SD) and 2. The difference of the mean of successive RRs (MSD); the frequency-domain variables used: 1. The High-
frequency \( f_{HF} \) peak (ms\(^2\)) and the High-frequency HF power (Hz). The thresholds were identified through four methods; one method for VT1 and three methods for VT2, defined as follows:

Method 1 (HRVT1M1): In this method, the values of SD, MSD were plotted as a function of the work rate (W), for each stage of the test. The threshold was determined similarly way in that performed for the visual identification of the ventilatory threshold employing the measurement of gases with the ergospirometer, looking for the point where the lowest value of the SD, MSD, was presented, which reflects a complete removal of vagal activity during exercise.

Method 2 (HRVT2M2): This method varies over time and is most effective during lower limb activity (Cottin, et al., 2006). HF is taken from the series of RR intervals, using a short-term Fourier transform, variable in time with moving windows of 64 seconds, and a displacement of 3 seconds (Kubios HRV Premium version 3.3.1, Kuopio, Finland\(^{\circledR}\)). The HF range was extended to 2-Hz (Mourot, Fabre, Savoldelli, & Schena, 2014). Software corrected in \( f_{HF} \) artifacts and enabled automatically obtains an \( f_{HF} \) modelling (\( f_{HFm} \)). The \( f_{HFm} \times \) HF product was calculated and logarithmically transformed to increase the availability of its instantaneous changes over time (\( \text{Ln}(f_{HFm} \times \text{HF}) \)). The threshold from this method was identified in the final abrupt increase in \( \text{Ln}(f_{HFm} \times \text{HF}) \) as a function of time (Mourot, Fabre, Savoldelli, & Schena, 2014).

Method 3 (HRVT2M3): For this method RR intervals are established every 60 seconds with an HF extended to 2 Hz, using an autoregressive model of order 12 (Kubios HRV Premium version 3.3.1, Kuopio, Finland\(^{\circledR}\)). The threshold is determined taking into account the entire HF band (0.15 – 2 Hz) as a function of time when there are one or two abrupt increases in the trends of each curve (Mourot, Fabre, Savoldelli, & Schena, 2014).

Method 4 (HRVT2M4): For this method and perform the steps of method 2, the only threshold is visually determined from the curve as a function of time.

Procedures
The tests were carried out during the preseason. All the cyclists were instructed to maintain their usual nutritional intake the day before the test, to abstain from consuming food and beverages (other than water) two hours before the test, sleep at least six hours without consuming stimulants or alcoholic beverages and do not perform intense physical activity 24 hours before the test. Before the riders performed the tests, they were instructed to exert as much effort as possible and were verbally encouraged to pedal as long as possible.

The tests were carried out in a closed room, with a temperature that ranged from 22°C to 24°C, and relative air humidity between 50% and 59%. A sport physician, the coach, and the lead investigator were present. Body Composition records were obtained using a Tanita BC-585F FitScan Body Composition Monitor\(^{\circledR}\), and height on a CE 0123 UK \(^{\circledR}\) DRY stadiometer. The chainring-pinion ratio was 52/11 and did not change during the test. The ventilatory threshold was measured with a portable telemetric device (Cosmed K4b2\(^{\circledR}\)). Before each test, the device was calibrated according to the manufacturer's recommendations. The HR and RR record was recorded using a Polar RS800CX\(^{\circledR}\) (Polar, Finland) from the start of the warm-up to the end of the tests including recovery time.

Statistical analysis
Applied a normality test Shapiro-Wilk, the nonparametric statistics are presented through the median, standard error, correlation coefficient of Spearman (rho), and Kendal concordance coefficient (Coef.).
Parametric statistics are presented using the mean, standard error, Pearson’s correlation coefficient (r), and Lin’s concordance correlation coefficient (CCC), with a 95% confidence interval. The statistical program was SPSS 22.0®.

RESULTS

In Table 1 shows the parameters determined by the cyclists’ condition, it was found that they had 8.3 ± 8.2 years of training with 6.2 ± 0.6 sessions per week each of 3.4 ± 1 hours, 23.7 ± 5.8 years of age, aerobically trained and familiar with exercise by cycle, with a height of 161.1 ± 3.6 cm and a weight of 54.2 ± 4.8 kg that allows determining a BMI of 20.8 ± 4.2 Kg/m². After applying the maximum cycle ergometer test in Table 2, with the maximum possible effort verbally encouraged, we found that the cyclists reached an absolute maximum power of 262.5 ± 29.8 watts; with maximum values of oxygen consumption relative to weight 55.5 ± 7.3 ml. kg⁻¹ min⁻¹ and heart rate of 187 ± 9.5 BPM.

Table 1. Demographic characteristics (media ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>23.7 (5.8)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>54.2 (4.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.1 (3.6)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>20.8 (4.2)</td>
</tr>
<tr>
<td>Years of training</td>
<td>8.3 (8.2)</td>
</tr>
<tr>
<td>Session per week (session/Wk.)</td>
<td>6.2 (0.6)</td>
</tr>
<tr>
<td>Hours of Training per Week (HRS/Wk.)</td>
<td>3.4 (1)</td>
</tr>
</tbody>
</table>

Table 2. Cyclo ergometer maximal Test (media ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_max (watts)</td>
<td>262.5 (29.8)</td>
</tr>
<tr>
<td>VO₂max (ml. Kg⁻¹ min⁻¹)</td>
<td>55.5 (7.3)</td>
</tr>
<tr>
<td>HR_max (BPM)</td>
<td>187 (9.5)</td>
</tr>
</tbody>
</table>

DISCUSSION

The main results of this work were:

1. The threshold power, maximum power, and maximum HR values coincide with those recorded in the literature for professional female cyclists.
2. Almost perfect agreement between the ventilatory threshold by ergospirometry and determination methods 2 and 4.
3. There was no agreement between the first ventilatory threshold (VT1) due to ergospirometry and HRV.

Characterization of women’s professional cycling

Malek et al., 2004 evaluated 12 cyclists and 19 triathletes with an average training level of 6.6 sessions per week with a total of 11 HRS/Wk., similar in our subjects that trained 6.2 sessions per week, but with a total of 21.08 HRS/Wk. These weekly hours allow establishing a better adaptation to the training load, suggesting that when an athlete endures more training hours, they can have a better self-concept, regarding optimization of power production by improving tolerance to time, accompanied by your emotional and motor intelligence (Ruiz, 1999).
The maximum power in cycling consists of better development of force on the pedals, contributing to the rider's performance in a positive way. In a 2005 study carried out in the Women's Road Cycling World Cups, from 1999 to 2004 with 15 cyclists of the Australian national team, and Power average of $W_{\text{max}} = 310 \pm 25$ W was observed. The final powers reached by our sample were lower than this level, with a power average of $W_{\text{max}} = 262 \pm 29.8$ W was obtained, a difference of 16% (Ebert, et al., 2005). Similarly, in 2017 a study described the demand for the races for the Women's Cycling World Cup between 2012 and 2015 to seven cyclists during 49 races, of which 25 finished in the first ten positions, for this purpose during the first 20 minutes of the test was obtained an average of $4.4 \pm 0.4$ W/Kg; comparatively our sample had an average of $4.8 \pm 0.5$ W/Kg; which represents a better ratio of work per kilogram of weight (Menaspà, Sias, Bates, & La Torre, 2017).

**Determination of ventilatory threshold by HRV**

Regarding the study object, a low correlation was evidenced between VT1 and HRVT1M1 in watts with rho = 0.45 p-value = .139 and in time r = 0.53 p-value = .072; however for HR there was a good correlation (r = 0.88 p-value = .000), similar results found by Cottin and collaborators in 2007 (Cottin, et al., 2007), which presented a high correlation in VT1 and VT2 through ergospirometry and HRV, through the multiplication of f/HF and HF, with an r = 0.94 for VT1 and r = 0.96 for VT2 with a p < .001 in 12 professional soccer players of 25 ± 3 years, with domains frequencies between 0.04 - 0.15 Hz for low frequency (LF) and 0.15-1.5Hz for HF. On the contrary, this study used four methods, the first was through the values of SD, and HF, with an r = 0.94 for VT1 and r = 0.96 for VT2 with a p < .001 in 12 professional soccer players of 25 ± 3 years, with domains frequencies between 0.04 - 0.15 Hz for low frequency (LF) and 0.15-1.5Hz for HF. On the contrary, this study used four methods, the first was through the values of SD, MSD as a function of the work rate only for VT1, and for the following methods the HF band was extended to 2 Hz in the evaluation. Of VT2 using different mathematical models previously described. Similarly, Cottin et al., (Cottin, et al., 2006) on another occasion evaluated 11 well-trained subjects during an incremental test carried out on a cycle ergometer, for which purpose they extended the HF to 2 Hz and identified VT1 in watts an r = 0.97 p < .001, in contrast in our study in watts and rho = 0.45 p = 0.139 was found, with a Coef. = 0.41, for HR an r = 0.88 p = .000 with CCC = 0.87 and finally in time an r = 0.53 p = .072 with CCC = 0.94; for VT2 per watts in HRVT2M2 and HRVT2M4 and rho = 0.90 p = .000 and a rho = 1.0 p = .000 respectively were found, with a Coef. = 1 for both. For HR and time, the correlations are above r = 0.95 with a CCC = 0.95 (Table 3 and 4), except for method 3.

<table>
<thead>
<tr>
<th>Ventilatory Threshold (VT) (media ± SD)</th>
<th>VT by HRV (media ± SD)</th>
<th>Spearman (p-value)</th>
<th>Kendall (Coeff.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT1 (W) 195.0 (7.2)</td>
<td>HRVT1M1 (W) 190.0 (5.2)</td>
<td>0.45 (.139)</td>
<td>0.41§</td>
</tr>
<tr>
<td>VT2 (W) 247.5 (10.0)</td>
<td>HRVT2M2 (W) 247.5 (9.3)</td>
<td>0.90 (.000)</td>
<td>1.00*</td>
</tr>
<tr>
<td>VT2 (W) 247.5 (10.0)</td>
<td>HRVT2M3 (W) 250.0 (9.0)</td>
<td>0.72 (.008)</td>
<td>0.65§</td>
</tr>
<tr>
<td>VT2 (W) 247.5 (10.0)</td>
<td>HRVT2M4 (W) 247.5 (10.0)</td>
<td>1.00 (.000)</td>
<td>1.00*</td>
</tr>
</tbody>
</table>

*Note. p < 0.05 *Almost perfect concordance. †Substantial concordance. §Poor concordance. W = watts.

<table>
<thead>
<tr>
<th>Heart Rate at Ventilatory Threshold by EE (media ± SD)</th>
<th>Heart Rate at Ventilatory Threshold by HRV (media ± SD)</th>
<th>Pearson (p-value)</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT1 (BPM) 159.9 (2.6)</td>
<td>HRVT1M1 (BPM) 156.8 (5.2)</td>
<td>0.88 (.000)</td>
<td>0.87§</td>
</tr>
<tr>
<td>VT2 (BPM) 176.7 (3.0)</td>
<td>HRVT2M2 (BPM) 175.7 (2.8)</td>
<td>0.95 (.000)</td>
<td>0.99*</td>
</tr>
<tr>
<td>VT2 (BPM) 176.7 (3.0)</td>
<td>HRVT2M3 (BPM) 175.6 (2.4)</td>
<td>0.87 (.000)</td>
<td>0.96†</td>
</tr>
<tr>
<td>VT2 (BPM) 176.7 (3.0)</td>
<td>HRVT2M4 (BPM) 174.8 (2.7)</td>
<td>0.98 (.000)</td>
<td>0.97†</td>
</tr>
</tbody>
</table>

*Note. p < 0.05 *Almost perfect concordance. †Substantial concordance. §Poor concordance. BPM = Beats per minute.
Karapetian (Karapetian, Engels, & Gretebeck, 2008) evaluated 24 adults to determine the VT2 in cycle ergometer, the HRV, and lactate; the HRV was based on a continuous record RR, in which they took the lowest values of MSD and SD, finding a correlation between .82 and .89 for identifying the threshold and the three variables with p < .05 (Karapetian, Engels, & Gretebeck, 2008), however our study showed a correlation above .95 for VT2 in HRVT2M2 and HRVT2M4 (CCC = 0.95 p < .05).

Dourado (Dourado & Guerra, 2013), evaluated the ventilatory threshold using the HRV in 31 healthy adults, 14 men and 17 women in a maximum, incremental test and was compared with the ventilatory threshold determined by gas measurement, finding a correlation of .92 between both methods (Dourado & Guerra, 2013), this study confirms the correlations but in this case for highly trained women taking into account that for VT2 it correlated (r > 0.95).

**HRV to determine the first ventilatory threshold**

Mendia-Iztueta et al., (Mendia-Iztueta, Monahan, Kyröläinen, & Hynynen, 2016), assessed the thresholds by HRV in ten National Level Elite skiers 5 men and 5 women), aged 19 to 30 yr., incremental test, in five techniques of cross-country skiing were performed; during the test, averages of VT1 were not significantly different, the Nordic walking test was the only trial that showed a significant statistical difference-in VT1 (p = .007). These variables were significantly correlated only in the DS (diagonal stride) (r = 0.77; p = .009) and NW (Nordic walking) tests (r = 0.68; p = .031), Mendia-Iztueta had a better statistical correlation than that evidenced by our study; on the other hand, for the VT2, the variables were significantly correlated only in the DS tests (r = 0.80; p = .006) and V2 (skating) (r = 0.81; p = .008), for this case our study evidenced a better degree of correlation rho = 0.90 and Coef. 1.00 agreement with p < .05.

Finally, Ramos and collaborators (Ramos-Campo, et al., 2017) determined if the HRV during an incremental test could be used to estimate the ventilatory thresholds in 24 professional basketball players (age: 23.4 ± 4.9 years; height: 195.4 ± 9.8 cm; body mass: 92.2 ± 11.9 Kg), between the results for VT1 there were no significant differences between VO2 and HR, corresponding to VT1 calculated by gas analysis and HRV. However, the speed was significantly higher with the inflection compared to the HRV. The following correlations between HRV and ergospirometry were observed (r = 0.54 in VO2; r = 0.57 in HR and r = 0.47 in speed), in the same way our study confirmed the low correlation with significant differences and poor agreement (rho = 0.45 p = .139 Coef. = 0.41 in watts), low correlation and without significant difference (r = 0.88 p = .000 with CCC = 0.87 in FC, r = 0.53 p = .072 with CCC = 0.94 in time). On the other hand, for VT2, the correlation in HR values was higher between R (respiratory exchange) and HRV (r = 0.96 p < .005), VE and VFC (r = 0.96 p < .005), in the same way our study confirmed not only a high precision (rho = 0.9 p < .05), but it accompanied an almost perfect accuracy (Coef. = 1.0 p < .05).

**CONCLUSIONS**

When comparing the results of this study, we have found few published investigations in cycling with professional women that establish the accuracy between ergospirometry (EE) and HRV, they have only been found about correlations. This leads us to determine that the evaluation of the thresholds by HRV is a field of investigation that should be continued in-depth. This study confirmed that the second ventilation thresholds can be detected from the cardiac RR series using HRV Time-frequency analysis during an incremental exercise test in cyclists due to changes in arterial gases detected by chemoreceptors. Furthermore, it has been shown that the high frequency (HF) as a response to changes from the baroreflex - induced effect mechanical respiration, depending on the absolute power represented in watts provides a reliable index, in
which decreases as you increase the respiratory rate and increasing according to the tidal volume in the athlete.

These findings provide a practical and economical approach to assess specific training loads when determining VT2 in Cyclists. Therefore, HRV is an alternative method to determine VT2 without the application of expensive technology that limits its use to laboratories.

AUTHOR CONTRIBUTIONS

Mina, Tafur, Garcia-Vallejo and Tejada wrote and verified the abstract and the introduction. Mina, Tafur, Povea and Cabrera wrote and verified the methods, the results. Mina, Tafur, Cabrera and Garcia-Corzo the discussion and conclusions. All authors reviewed the final document and assisted in its construction.

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No potential conflict of interest was reported by the authors.

REFERENCES


