ANALYSIS OF HEART RATE DEFLECTION POINTS TO PREDICT THE ANAEROBIC THRESHOLD BY A COMPUTERIZED METHOD

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ABSTRACT
Marques-Neto, SR, Maior, AS, Maranhão Neto, GA, and Santos, EL. Analysis of heart rate deflection points to predict the anaerobic threshold by a computerized method. J Strength Cond Res 26(7): 1967–1974, 2012—Many studies have used the heart rate deflection points (HRDPs) during incremental exercise tests, because of their strong correlation with the anaerobic threshold. The aim of this study was to evaluate the profile of the HRDPs identified by a computerized method and compare them with ventilatory and lactate thresholds. Twenty-four professional soccer players (age, 22 ± 5 years; body mass, 74 ± 7 kg; height 177 ± 7 cm) volunteered for the study. The subjects completed a Bruce-protocol incremental treadmill exercise test to volitional fatigue. Heart rate (HR) and alveolar gas exchange were recorded continuously at ≥1 Hz during exercise testing. Subsequently, the time course of the HR was fit by a computer algorithm, and a set of lines yielding the lowest pooled residual sum of squares was chosen as the best fit. This procedure defined 2 HRDPs (HRDP1 and HRDP2). The HR break points averaged 43.9 ± 5.9 and 89.7 ± 7.5% of the VO₂peak. The HRDP1 showed a poor correlation with ventilatory threshold (VT; r = 0.50), but HRDP2 was highly correlated to the respiratory compensation (RC) point (r = 0.98). Neither HRDP1 nor HRDP2 was correlated with LT1 (at VO₂ = 2.26 ± 0.72 L·min⁻¹; r = 0.26) or LT2 (2.79 ± 0.59 L·min⁻¹; r = 0.49), respectively. LT1 and LT2 also were not well correlated with VT (2.93 ± 0.68 L·min⁻¹; r = 0.20) or RC (3.82 ± 0.60 L·min⁻¹; r = 0.58), respectively. Although the HR deflection points were not correlated to LT, HRDP2 could be identified in all the subjects and was strongly correlated with RC, consistent with a relationship to cardiorespiratory fatigue and endurance performance.

KEY WORDS anaerobic threshold, ventilatory threshold, lactate threshold, heart rate deflection points, respiratory compensation point

INTRODUCTION
In sports sciences, the determination of ventilatory threshold (VT) is important for the adjustment of exercise intensities in training programs (36). This respiratory variable is highly correlated with endurance performance, fatigue, and anaerobic thresholds (ATs) (13,30). To assess these cardiorespiratory parameters, advanced ergospirometry equipment is required. Classical studies have shown that the heart rate (HR) and oxygen uptake (VO₂) both increase during progressive and maximal exercise but not exactly in parallel (2,33,36,37). In 1982, Conconi et al. (8) proposed a noninvasive method to detect AT, based on the observation that above the AT intensity, the HR exhibits a disproportional response pattern to further increases in VO₂. The authors proposed the existence of an HR deflection point (HRDP), which may be associated conveniently with AT intensity.

The HRDP has potential as a parameter for exercise prescription and can be measured with relatively simple equipment; however, its reliability was questioned in studies showing that HRDP could not be observed in all subjects (5,19,21,35). Various attempts have been made to assess the validity of using HRDP as a predictor of AT intensity. Although some studies reported consistent agreement between HRDP and AT intensities (8,12,18,25), others have shown the opposite results (5,21,23,27), and different authors have suggested that HRDP may be associated with other physiological events, such as a decline in the left ventricular ejection fraction (LVEF), increased vagal tone (27), thicker heart walls (22), hyperkalemia (23), and reduced stroke volume (21).
Hoffman et al. (18) observed 2 HR deflection points (HRDP1 and HRDP2), which could be correlated with blood lactate concentrations of 2 and 4 mM, respectively. Techniques based on the visual inspection of HR and on respiratory gas exchange plots have been described as a means of identifying HRDP, VT, and respiratory compensation (RC; [2,12,20]). Even though for HRDP the visual inspection method may not be the most accurate, it is the most practical. However, some studies have reported that HRDP assessment is not readily achieved with all subjects and that there are difficulties in discerning the deflection point visually, even for experienced observers (29). Other studies have applied regression analysis to HRDP data to minimize errors in the detection of HRDP1 and HRDP2, but no one has established a reproducible criterion to define the point where an HRDP occurs (18,22).

We have observed that very effective computerized methods have been developed to identify VT; these involve pulmonary ventilation (VE) (26,32); the ventilatory equivalent for O₂ (VE/V̇O₂), and the ventilatory equivalent for CO₂ (VE/V̇CO₂) (14,24); the V̇CO₂/V̇O₂ ratio (v-slope) (2); the respiratory exchange ratio (Ṙ) (11); the end-tidal PO₂ (PET, O₂) and the end-tidal PCO₂ (PET, CO₂) (10).

Because no consistent quantitative method has been described to determine precisely the point at which an HRDP occurs, the main goal of this study was to apply a computerized method similar to those mentioned above for gas exchanges (26) to identify HRDPs and test for a possible relationship with ventilatory and lactate thresholds during incremental exercise.

**METHODS**

**Experimental Approach to the Problem**

Visual inspection is currently the method most used for HRDP detection (5,20,38). According to Ballarin et al. (1), there is a significant correlation (r > 0.94) between computer-determined HRDP and visual inspection by experienced observers, and they concluded that visual HRDP analysis is reliable if it is carried out by an experienced observer.

On the other hand, several automatic techniques have been proposed for assessing VT and RC (26,30,36), and these have been useful for clinical practice and exercise prescription. Orr et al. (26) compared 2 methods for detecting VT, one based on a single linear regression and the other designed to fit 2 lines to the data. The regression lines are calculated for all possible assignments to 2 contiguous segments, and the pair of lines yielding the lowest pooled residual sum of squares is chosen as the best fit. As far as we know, this computerized method has not been applied for the assessment of HRDP, and for this reason, the HR, V̇O₂, and lactate samples were all processed using Matlab-based algorithms similar to those of Orr et al. (26).

A second feature of our approach was the use of an adequate sampling rate (≥1 Hz), to avoid errors associated with HR data sampled at a lower rate (31).

**Subjects**

Twenty-four male soccer players (age, 22 ± 5 years; body mass, 74 ± 7 kg; height, 177 ± 7 cm) with at least 5 years of aerobic and resistance training experience were asked to participate in this study. The experiments were conducted in the midseason. All the subjects (N = 24) were considered healthy on the basis of their health history, physical examination, and normal resting electrocardiogram (ECG). All were nonsmokers, nonalcohol users, and nonillicit drug users (cocaine, marijuana, and heroine). All the subjects answered the Physical Activity Readiness Questionnaire and signed an informed consent. The experimental procedures were approved by the Ethics Committee of the Federal University of Rio de Janeiro.

The following additional exclusion criteria were adopted: (a) use of drugs that could affect the cardiorespiratory responses; (b) bone-, joint-, or muscle-diagnosed problems that could limit the execution of the maximal exercise testing; (c) systemic hypertension (≥140/90 mm Hg or use of antihypertensive medication); and (d) metabolic disease.

**Exercise Testing**

All testing was performed between 1:00 and 3:00 PM. The subjects were instructed to eat a normal light lunch 2 hours before the test so that they would not feel any gastrointestinal discomfort. Coffee, tea, and alcohol intake was prohibited for 12 hours beforehand, and the subjects avoided formal and strenuous exercise for 48 hours before testing. The tests were performed on a motor-driven treadmill (Inbramed 10200, Brazil) using Bruce’s protocol (6). The subjects were allowed sufficient practice during preliminary testing to become familiar with the treadmill. Ambient air temperature was 22 to 24°C, and mineral water was freely available.

The tests were preceded by a 3-minute warm-up followed by incremental increases in speed and incline every 3 minutes. The test was terminated when the participant stopped because of exhaustion. However, the subjects were encouraged to exercise as long as possible. Achieving target HR alone (i.e., >85% of maximal age-predicted HR) was not used as justification for terminating the exercise.

Testing was symptom limited and was terminated by the examiner if the subjects reported having dyspnea, fatigue, or chest pain or for medical reasons, such as horizontal or down-sloping ST-segment depression of ≥1 mm, ST-segment elevation >1 mm in non-Q wave lead, atrial fibrillation, or supraventricular tachycardia, suggestive of a left bundle-branch block, abnormal blood pressure response to exercise (blood pressure ≥220 × 120 mm Hg), fall in systolic blood pressure (≥20 mm Hg), variation in diastolic pressure >15 mm Hg, presyncope, severe arrhythmias, extrasystoles, ataxia, or ventricular ectopy (presence of ≥6 premature ventricular beats per minute), and development of bundle-branch block or intraventricular conduction delay that could not be distinguished from ventricular tachycardia (15).
Measurements of Pulmonary Gas Exchange and Lactate
The \( \dot{V}O_2 \) mask and equipment were donned after the subject was positioned on the motor-driven treadmill. A face mask (Hans Rudolph V Mask\textsuperscript{TM}, Cole Parkway Shawnee, KS, USA) that covered the mouth and nose was attached to a bidirectional digital flowmeter and fastened to the subject with a mesh hairnet and Velcro straps. The gas exchange analysis was first conducted with the subject at rest for 5 minutes with a VO-2000\textsuperscript{TM} ergospirometer (Medical Graphics, Saint Louis, MO, USA). Resting state was characterized as follows: \( \dot{V}O_2 \) at 3.5 \( \text{ml kg}^{-1} \text{min}^{-1} \), minute ventilation \( (\dot{V}E) \) between 8 and 15 \( \text{L min}^{-1} \), and respiratory quotient between 0.75 and 0.85 (15). The HR was monitored continuously using a V5-lead ECG monitor system (CONTEC, model 8000D, Quinhuangdao, China), and measurements of \( \dot{V}O_2 \), carbon dioxide production \( (\dot{V}CO_2) \), and \( \dot{V}E \) were assessed every 3 complete respiratory cycles at rest, during, and after treadmill incremental exercise testing. The ergospirometer was calibrated before each individual test according to the manufacturer’s guidelines.

A lactate analyzer (Lactate Pro LT-1710, Roche Bioelectronics, Basel, Switzerland) was used on fingertip capillarized blood microsamples, then blood lactate readings were collected during warm-up and during the 30 seconds after each stage of incremental exercise testing.

**Ventilatory Threshold and Respiratory Compensation Point**
The VT and RC point were detected automatically by searching for the breakpoints of \( \dot{V}E/\dot{V}O_2 \) (Figure 1A) and \( \dot{V}E/\dot{V}CO_2 \), respectively (Figure 1B). It is known that \( \dot{V}O_2, \dot{V}CO_2, \) and \( \dot{V}E \) increase similarly up to VT. However, above VT, buffering of the lactic acid leads to a disproportional increase in \( \dot{V}CO_2 \) relative to \( \dot{V}O_2 \) with a subsequent increase in \( \dot{V}E \). Furthermore, the ventilatory equivalent for \( \dot{V}CO_2 \) \( (\dot{V}E/\dot{V}CO_2) \) remains constant or decreases slightly, whereas the ventilatory equivalent for \( \dot{V}O_2 \) \( (\dot{V}E/\dot{V}O_2) \) increases. Above the RC point, \( \dot{V}E \) increases at a higher rate than \( \dot{V}CO_2 \), with a concomitant increase in \( \dot{V}E/\dot{V}CO_2 \). Briefly, the breath-by-breath values for \( \dot{V}E/\dot{V}O_2 \) and \( \dot{V}E/\dot{V}CO_2 \) were fitted by the least-squares method to a fifth-degree polynomial using a smooth spline curve (Figure 1). The minima obtained from the first-order derivatives of the fitted polynomials for \( \dot{V}E/\dot{V}O_2 \) and \( \dot{V}E/\dot{V}CO_2 \) were used to calculate the VT and RC values, respectively. Finally, the \( \dot{V}O_2 \) was computed as the maximal value of \( \dot{V}O_2 \) reached at the end of the test (30,37).

**Computerized Method for Detecting Heart Rate Deflection Point**
The \( \dot{V}O_2 \) and HR were resampled (cubic spline curve) at 1 Hz, resulting in equally spaced intervals, and were then filtered by a zero-crossing moving average of length 180 points. Each curve was then normalized to its maximum value.

After that, the HR time course was modeled by fitting 3 contiguous linear segments. This was done by dividing the HR as a function of time into 2 vectors, \( [1:j] \) and \( [(i+1):n] \), where \( i \) represents the intersection point and fitting 2 straight lines that yielded the lowest pooled residual sum of squares (Figure 2A). Then, starting from the intersection point \( (i,j) \), a third segment was extracted, varying \( i \) from 1 to \( n \), adding and subtracting points \( (i+j) \) or \( (i-j) \) to obtain the lowest pooled residual sum of squares for 3 lines. Finally, the i-th and j-th points (providing...
the least-square errors) were assigned as the deflection points (HRDP1 and HRDP2), and 3 linear segments (L1, L2, and L3) were obtained, as shown in Figure 2B. Evidence has been presented to show that after HRDP1, the HR vs. time curve should increase in slope and that after HRDP2 it should decrease (18). The coefficients extracted from the first linear segment (L1) of the HR time course were used to extrapolate this segment to the end of L2 (Figure 2C). This was followed by calculating the analytical integral of the difference between observed and estimated HRs along L2. Likewise, the coefficients extracted from the second linear segment (L2) were used to extrapolate this segment to the end of L3, and the decrease in slope after HRDP2 was calculated as the analytical integral of the difference between observed HR along L3 and the HR estimated from the coefficients extracted after L2 (Figure 2C). Figure 2D shows the result when the same computerized method was applied to detect lactate thresholds (LT1 and LT2).

### Statistical Analyses

The angular coefficients from each linear segment of the HR and lactate vs. time curves were compared with those extracted from VO\(_2\) using Student’s paired \(t\)-test, and the correlation was expressed by Pearson’s correlation coefficient (\(r\)). Similarly, the mean values of VO\(_2\) corresponding to HRDP1 and HRDP2 were compared with those for VT, RC, LT1, and LT2. The agreement between the points detected

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**TABLE 1.** Physical and anthropometric characteristics of the subjects at rest and during maximal exercise testing.*†

<table>
<thead>
<tr>
<th>Subjects’ characteristics (N = 24)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>74.05 ± 7.40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.32 ± 6.58</td>
</tr>
<tr>
<td>HR rest (b-min(^{-1}))</td>
<td>62.24 ± 9.63</td>
</tr>
<tr>
<td>HR peak (b-min(^{-1}))</td>
<td>187.66 ± 8.09</td>
</tr>
<tr>
<td>VO(_2) peak (ml kg(^{-1}) min(^{-1}))</td>
<td>62.95 ± 6.09</td>
</tr>
<tr>
<td>VO(_2) peak (L min(^{-1}))</td>
<td>4.08 ± 0.63</td>
</tr>
<tr>
<td>HRDP1 (% of VO(_2) peak)</td>
<td>43.98 ± 5.96</td>
</tr>
<tr>
<td>HRDP2 (% of VO(_2) peak)</td>
<td>89.66 ± 7.45</td>
</tr>
<tr>
<td>VT (% of VO(_2) peak)</td>
<td>71.65 ± 10.26</td>
</tr>
<tr>
<td>RC (% of VO(_2) peak)</td>
<td>93.68 ± 4.71</td>
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<tr>
<td>LT1 (% of VO(_2) peak)</td>
<td>55.70 ± 17.50</td>
</tr>
<tr>
<td>LT2 (% of VO(_2) peak)</td>
<td>68.92 ± 13.40</td>
</tr>
</tbody>
</table>

*BM = body mass; HRrest = resting heart rate; HRpeak = peak of the heart rate; VO\(_2\) peak = peak of the O\(_2\) uptake; HRDP1 = first heart rate deflection point; HRDP2 = second heart rate deflection point; VT = ventilatory threshold; RC = respiratory compensation point.

†Values are mean ± SD.

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for VT and HRDP1 or for HRDP2 and RC was assessed according to the intraclass correlation coefficient (ICC) and the limits of agreement (LOAs) as described by Bland and Altman (3). The reliabilities for the determination of the variables were similarly expressed as the differences between the values of \( \dot{V}O_2 \) obtained by the automatic method and the mean of the values detected on the abscissa. This allowed the 95% LOA to be defined as being within 1.96 SD of the mean difference (3). Statistical significance for the differences between mean values of HRDPs, L Ts, and VTs was set at \( p \leq 0.05 \). All data were processed using Matlab v. 7.4 R2007a (Mathworks, El Segundo, CA, USA).

**RESULTS**

Table 1 summarizes the physical characteristics of the subjects and the effects of performing an incremental exercise test on the respiratory gas exchange and HR.

Pearson’s correlation coefficient for HRDP1 vs. VT revealed a weak correlation when compared using absolute values of \( \dot{V}O_2 \) (Figure 3A). Similarly, LT1 was only weakly correlated with HRDP1 (\( r = 0.26 \)) and VT (\( r = 0.20 \)) and occurred at a significantly different \( \dot{V}O_2 \) value (\( p < 0.001 \), data not shown). Figure 3B shows that the mean absolute values of oxygen uptake (\( \dot{V}O_2 \)) shows none of the subjects outside the limits of agreement (LOAs), drawn at \( \pm 1.96 \text{SD} \).
Mean absolute values for $\dot{V}O_2$ at LT1 (2.26 $\pm$ 0.72 L.min$^{-1}$) were statistically greater than that for HRDP1 but lower than that for VT ($p < 0.001$). The Bland and Altman plot for the differences between the means of absolute values for VT and HRDP1 (Figure 3C) shows a good agreement between them, where only 1 subject appeared outside the 95% confidence limits ($SEE = 0.12$ L.min$^{-1}$).

Figure 4A shows a strong correlation ($r = 0.98$), and Figure 4B shows no significant difference between the absolute values of oxygen uptake for HRDP2 and RC ($p > 0.05$). In contrast, LT2 was only weakly correlated with HRDP2 ($r = 0.49$) and RC ($r = 0.58$, $p < 0.0001$) (data not shown). The VT correlated rather well with HRDP2 ($r = 0.75$), although the average value was significantly lower ($p < 0.001$, data not shown). Conversely, Figure 4B shows that the mean absolute $\dot{V}O_2$ was significantly lower for LT2 (2.79 $\pm$ 0.59 L.min$^{-1}$) than for HRDP2 (3.66 $\pm$ 0.65 L.min$^{-1}$; $p < 0.0001$) or RC (3.82 $\pm$ 0.60 L.min$^{-1}$; $p < 0.0001$). In Figure 4C, LOA analysis of $\dot{V}O_2$ for HRDP2 and RC revealed a good agreement as well ($SEE = 0.06$ L.min$^{-1}$).

Table 2 shows the agreement between the points detected by our computerized method, as assessed with ICCs. The mean values of $\dot{V}O_2$ detected at HRDP1 showed a poor agreement with VT (0.48) or with LT1 (0.04), whereas the $\dot{V}O_2$ values at HRDP2 agreed well with those at VT (0.75) and RC (0.98) but not with those at LT2 (0.35).

**DISCUSSION**

In the past, there has been no consensus about how to evaluate the anaerobic threshold (AT) during training exercises. The principle achievement in our study of a group of professional athletes was to show that there was a very good correlation between the second VT, RC, and the second HR deflection point, HRDP2, which can be readily measured with simple equipment. In addition, we found that these 2 variables occurred at identical $\dot{V}O_2$ values, whereas all other measures (VT, LT1, and LT2) were different from HRDP.

The 2 critical features that made this analysis possible were use of a computerized regression method to analyze HR and a relatively high HR sampling frequency ($\geq 1$ Hz).

What is the significance of HRDP1? In our experiments, the first breakpoint occurred at 44% of $V_o$peak, within the range of work rates associated with a decrease in the stroke volume (40–50% of $V_o$peak), associated with an increase in afterload. This compensatory HR response occurs to stabilize cardiac output (16,28). There is no evidence that the decrease in stroke volume has any relationship to LT1 or VT, and in fact, our data are consistent with idea that there is none (Figure 3).

In previous investigations, Conconi et al. (8,9) concluded that the increase in HR with incremental exercise was not a simple linear regression. Instead, there was a breakpoint between 80 and 90% of maximal exercise, which they called the deflection velocity. They suggested that when ATP is generated primarily anaerobically, the increase in $\dot{V}O_2$ occurs at a lower rate than that before AT; the work rate continues to increase, but the HR rises more slowly. Our data show, however, that the second breakpoint in the HR is not associated with either LT1 and LT2. According to previous studies, the point where HRDP2 occurs may be associated with LVEF (28), an increase in vagal tonus (27), thicker heart walls (22), hyperkalemia (23), or a reduced SV (21). All of these authors recorded the HR at low sampling rates (0.01 or 0.05 Hz).

According to Ballarin et al. (1), a computerized fitting method and visual inspection of the HR increase by experienced observers can both be used to identify HRDP, but others have reported considerable difficulty in the visual inspection of the HR (21,23). In this study, the HR data were fit to 3 contiguous lines chosen by minimizing the pooled residual sum of squares, and we were able to identify 2 HRDPs in all the subjects with a variability of approximately 10%. These points occurred, on average, at 44 and 90% of $V_o$peak. Only 1 previous study has identified 2 deflections in the HR time course, assessed by means of linear regression and located by fitting a second-degree polynomial to determine the differences of angles and selecting the HRDP visually. In that study, a strong correlation was observed between HRDP1 and LT1 and also between HRDP2 and LT2 (18).

Tokmakidis and Léger (34), on the other hand, determined the points corresponding to HRDP1 and lactate deflections using 2 different mathematical methods and, as in our study, found them only weakly correlated in both cases. They concluded that LT and HRDP thresholds were poor indicators of fatigue index and athletic performance.

A clue to the discrepancy between Ballarin’s results and ours, where HRDP2 occurred at a higher $\dot{V}O_2$ (equivalent to RC rather than to LT2), may lie in the different sampling rates ($\geq 1$ Hz in our case, 0.2 Hz for Conconi and coworker’s group (17)). During a progressive test leading to maximal effort, $>1,000$ beats are acquired in a beat-to-beat recording. When HR data are sampled only every 20 or 30 seconds (0.05 and 0.03 Hz, respectively), 50 points are acquired, and the exact
value of the HRDP will be underestimated. Recently, the values of power output acquired at 30 Hz during a Wingate anaerobic test were compared after resampling at 5 different frequencies (0.2, 0.5, 1, 2, and 5 Hz) (31). Peak effort was underestimated by 28% when the sampling rates were decreased from 1 to 0.2 Hz. Hence, the variables studied here were recorded at sampling rates equivalent to 1 Hz, that is, data were collected beat to beat and then resampled at 1-second intervals. It is noteworthy that \( \dot{V}_O_2 \) values at RC and HRDP2 were about 1 L·min\(^{-1} \) higher than \( \dot{V}_O_2 \) at LT2 (Figures 3 and 4), suggesting that exercise prescription at LT2 intensities might not be a successful strategy for a training program aimed at improving endurance performance, that is, one that requires accurate markers for cardiorespiratory fatigue but not lactic acidosis.

**Practical Applications**

Exercise prescription is one of the most important tools for the management of training programs. Since the 1970s (37), different AT markers have shown high correlations with cardiorespiratory fitness and the fatigue index. Either a sophisticated ergospirometry system or multiple blood lactate samples were considered crucial to determining the correct AT intensities for prescribing training sessions. Noninvasive, low-cost techniques based on HRDP have been proposed as an alternative (4,7,8,12,18), but there is a lack of agreement about how best to obtain HRDP and what it represents physiologically. We suggest that an automated, computerized regression analysis of the HR time course is preferable to visual inspection for obtaining HRDP. The HRDP2 will provide access to the RC point as long as a sampling rate of at least 1 Hz is used.

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**References**


