Assessment of ventilatory thresholds during graded and maximal exercise test using time varying analysis of respiratory sinus arrhythmia

G Blain, O Meste, T Bouchard, S Berman


Objective: To test whether ventilatory thresholds, measured during an exercise test, could be assessed using time varying analysis of respiratory sinus arrhythmia frequency (fRSA).

Methods: Fourteen sedentary subjects and 12 endurance athletes performed a graded and maximal exercise test on a cycle ergometer: initial load 75 W (sedentary subjects) and 150 W (athletes), increments 37.5 W/2 min. fRSA was extracted from heart period series using an evolutive model. First (T1) and second (T2) ventilatory thresholds were determined from the time course curves of ventilation and ventilatory equivalents for O2 and CO2.

Results: fRSA was accurately extracted from all recordings and positively correlated to respiratory frequency (r=0.96 (0.03), p<0.01). In 21 of the 26 subjects, two successive non-linear increases were determined in fRSA, defining the first (RSA1) and second (RSA2) RSA thresholds. When expressed as a function of power, RSA1 and RSA2 were not significantly different from and closely linked to T1 (r=0.99, p<0.001) and T2 (r=0.99, p<0.001), respectively. In the five remaining subjects, only one non-linear increase was observed close to T2. Significant differences (p<0.04) were found between athlete and sedentary groups when RSA1 and RSA2 were expressed in terms of absolute and relative power and percentage of maximal aerobic power. In the sedentary group, RSA1 and RSA2 were 150.3 (18.7) W and 198.3 (28.8) W, respectively, whereas in the athlete group RSA1 and RSA2 were 247.3 (32.8) W and 316.0 (28.8) W, respectively.

Conclusions: Dynamic analysis of fRSA provides a useful tool for identifying ventilatory thresholds during graded and maximal exercise test in sedentary subjects and athletes.

Methods

Subjects

Fourteen sedentary healthy men (mean (SD) age: 24.5 (2.3) years) and 12 endurance athletes (age: 25.7 (2.8) years; >12 h of training/week) (characteristics shown in table 1) participated in the study. All subjects were non-smokers and none was taking medication. Physical activity and consumption of alcohol and caffeinated beverages were prohibited 24 h before the exercise testing session. Written informed consent was obtained prior to participation and ethical approval was granted by the Local Ethics Committee.

Experimental design

Subjects performed a graded and maximal exercise test on a cycle ergometer (Ergomedic 824 E, Monark Exercise, Vansbro, Sweden) in a quiet room at a controlled temperature of 21°C, at least 3 h after the last meal. In the sedentary and the athlete groups, subjects performed a graded and maximal exercise test on a cycle ergometer (Ergomedic 824 E, Monark Exercise, Vansbro, Sweden) in a quiet room at a controlled temperature of 21°C, at least 3 h after the last meal. In the sedentary and the athlete groups.

Abbreviations: AT, anaerobic threshold; HP, heart period; HPV, heart period variability; RSA, respiratory sinus arrhythmia.
groups, the initial load was fixed at 75 and 150 W, respectively, and increased by 37.5 W every 2 min until exhaustion. The pedalling rate was kept constant at 75 rev/min.

Ventilatory indices and gas exchanges were measured using an automatic ergospirometer on a breath by breath basis (Metasys TR-M, Brainware, Toulon, France). Subjects breathed through a silicon facemask connected to a two-way non-rebreathing valve (Hans Rudolph, Kansas City, MO). Inspired and expired O₂ and CO₂ concentrations were measured using paramagnetic and infrared sensors, respectively. Averages every 10 s were then established for V˙I (l/min), O₂ uptake (V˙o₂, l/min), CO₂ production (V˙co₂, l/min), respiratory ratio (R), and ventilatory equivalents for O₂ (V˙O₂), and CO₂ (V˙CO₂). fRSA was calculated on a breath by breath basis. Before each test, the gas analysers were calibrated with gases of known composition and an accurate controlled volume syringe was used to adjust the pneumotachograph. During the exercise tests, a one lead ECG (Cardiocap II, Datex Engstrom, Helsinki, Finland) was recorded and digitised on line by a 12 bit analog-to-digital converter (DAS 1600, Keithley Instruments, Taunton, MA) at a sampling rate of 1000 Hz, on a personal computer. Oxygen uptake was considered maximal (V˙o₂max) if three of the following criteria were met: levelling off of V˙O₂ despite increasing load, R greater than 1.10, and inability to maintain the fixed pedalling rate. The power corresponding to V˙o₂max defined the maximal aerobic power (Wmax).

**ECG preprocessing**

R wave peak occurrence was estimated using a threshold technique applied to the filtered and demodulated ECG signal. Thresholds were expressed in terms of absolute (W) and relative (W/kg) power and percentage of Wmax.

**Statistical analysis**

Differences between the sedentary and athlete groups were tested using unpaired Student’s t test. Comparison and relationship between ventilatory and fRSA thresholds were tested using paired Student’s t test and a linear regression analysis, respectively. Individual relationships between fRSA and f were tested by calculating Pearson’s r correlation coefficients. The mean (SD) of all individual correlation coefficients was then calculated. Statistical significance was set at p<0.05. Results are means (SD). Statistical analysis was performed using Statistica software 5.5 (StatSoft, Tulsa, OK).

**RESULTS**

Athletes showed significantly higher values of V˙O₂ and Wmax when compared to sedentary subjects (see table 1).

**Table 1** Anthropometric and maximal ergometric characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Sedentary group (n = 14)</th>
<th>Athlete group (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.4</td>
<td>9.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.3</td>
<td>9.9</td>
</tr>
<tr>
<td>V˙O₂max (l/min/kg)</td>
<td>44.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Wmax (W)</td>
<td>266.6</td>
<td>27.1</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>197.6</td>
<td>7.8</td>
</tr>
</tbody>
</table>

HR, heart rate; NS, not significant; V˙o₂max, maximal oxygen uptake; Wmax, maximal aerobic power.

**Table 2** First and second thresholds obtained from fRSA and ventilatory indices, in sedentary and athlete groups

<table>
<thead>
<tr>
<th></th>
<th>Sedentary group (n = 12)</th>
<th>Athlete group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Absolute power (W)</td>
<td>150.3</td>
<td>18.7</td>
</tr>
<tr>
<td>TV1</td>
<td>151.0</td>
<td>19.5</td>
</tr>
<tr>
<td>TV2</td>
<td>200.3</td>
<td>29.4</td>
</tr>
<tr>
<td>Relative power (W/kg)</td>
<td>2.21</td>
<td>0.33</td>
</tr>
<tr>
<td>TV1</td>
<td>2.22</td>
<td>0.34</td>
</tr>
<tr>
<td>TV2</td>
<td>2.97</td>
<td>0.38</td>
</tr>
<tr>
<td>% Wmax</td>
<td>56.5</td>
<td>6.0</td>
</tr>
<tr>
<td>TV1</td>
<td>56.8</td>
<td>6.7</td>
</tr>
<tr>
<td>TV2</td>
<td>75.1</td>
<td>7.5</td>
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</tbody>
</table>

% Wmax, percentage of maximal aerobic power; TV1, first ventilatory threshold; TV2, second ventilatory threshold.

**Determination of ventilatory and RSA thresholds**

Ventilatory thresholds were determined from the time course curves of V˙I, V˙I/V˙O₂, and V˙I/V˙CO₂ by a first independent operator. TV1 corresponded to the last point before a first non-linear increase in both V˙I and V˙I/V˙O₂. TV2 corresponded to the last point before a second non-linear increase in both V˙I and V˙I/V˙O₂, accompanied by a non-linear increase in V˙I/V˙CO₂. The fRSA thresholds were determined from the time course curve of fRSA by a second independent operator. The first fRSA threshold (TRSA1) corresponded to the last point before a first non-linear increase in fRSA. The second fRSA threshold (TRSA2) corresponded to a second non-linear increase in fRSA.

Thresholds were expressed in terms of absolute (W) and relative (W/kg) power and percentage of Wmax.
**fRSA extraction**

A conspicuous high frequency oscillation synchronous with fR was found in all ECG recordings, clearly indicating the persistence of RSA over the entire graded and maximal exercise protocol. The dynamic evolution of fRSA was accurately extracted from the HP series and fRSA positively correlated (r = 0.96 (0.03), p < 0.01) with fR (fig 1).

**fRSA dynamic behaviour**

Two non-linear increases were observed in fRSA in 21 of the 26 subjects. These non-linear increases coincided with TV1 and TV2, respectively (see fig 2) and no statistical difference was observed between TRSA1 and TV1 (absolute power: p = 0.98; relative power: p = 0.96; percentage of Wmax: p = 0.91) and TRSA2 and TV2 (absolute power: p = 0.57; relative power: p = 0.79; percentage of Wmax: p = 0.78). Power values and percentages of Wmax at TRSA1, TRSA2, TV1, and TV2 are presented in table 2. When expressed as absolute or relative power and percentage of Wmax, TRSA1, TRSA2, TV1, and TV2 were significantly higher in athletes than in their sedentary peers. Linear regression analysis showed high correlation between TRSA1 and TV1 (absolute power: r = 0.99, p < 0.001) and TV2 (absolute power: r = 0.99, p < 0.001; percentage of Wmax: r = 0.95, p < 0.001) with TV1. This is confirmed by the study of James et al who concluded that the first ventilatory threshold (referred as the AT in their study) could be detected by fR analysis.

In the five remaining subjects (three athletes and two sedentary subjects) only one non-linear increase was clearly identifiable and occurred close to TV2 (fig 4).

**DISCUSSION**

To assess HPV and RSA during non-stationary exercise conditions, we developed and validated an original method.6 In the present study, this method was used to process the cardiac electrical signal during a maximal and graded exercise test.

Using our original approach, the dynamic pattern of fRSA was accurately extracted from R-R interval series; RSA and breathing have been shown to develop dynamically at the same frequency. This result confirms previous findings5,6 which showed that during exercise, heart rate is modulated by breathing at the fR. When fRSA was considered, we were able to point out two successive non-linear increases in 81% of our population. First, we observed that TRSA1 was closely related to TV1. This finding is consistent with those of Anosov et al who reported that significant changes in the behaviour of fRSA occurred in the region of the AT. As the fRSA pattern is closely linked to fR, we could state that the first disproportionate increase in VI observed at TV1 is mainly induced by an increase in fR. This is confirmed by the study of James et al who concluded that the first ventilatory threshold (referred as the AT in their study) could be detected by fR analysis.

Second, we observed that TRSA2 was closely related to TV2, suggesting that the second disproportionate increase in VI is again related to fR increase. It has been reported that TV2 determines the workload before a marked fall in capillary pH.7 This exercise induced metabolic acidosis then causes ventilation increase through an increase in fR.8

The concept of ventilatory thresholds is closely linked in the literature to the concept of AT. AT is defined as the intensity of exercise, involving a large muscle mass, above which the oxidative metabolism cannot account for all the required energy and the anaerobic contribution to energy demand increases.21 Numerous studies have been conducted to detect one or two thresholds in metabolic (lactate for instance) or ventilatory indices time course curves. This diversity in methods of detection as well as lack of consensus on the theoretical basis have led to confusion and misinterpretation (see Bosquet et al20 and Svedahl and MacIntosh21 for reviews). Using blood lactate concentration is probably the most direct and reliable method to detect the AT.19 However, this method is invasive and requires

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**Figure 1** Representation of the dynamic behaviour of RSA frequency (fRSA, solid line) and respiratory frequency (fR, dashed line) recorded in one subject during graded and maximal exercise test.
frequent blood sampling which is uncomfortable during continuous exercise. The indirect technique using ventilatory indices could be thus preferable. Indeed, although disagreement exists,\textsuperscript{10,11} ventilatory thresholds are known to be closely related to lactate thresholds.\textsuperscript{12–16} Ventilatory threshold detection is usually based on assessment of successive disproportionate increases in $V_I$ and $f_R$ is known to play a major role in these increases.\textsuperscript{8,9} It is also known that heart activity is modulated by breathing at the $f_R$ and this modulation represents the RSA which is vagally mediated at rest.\textsuperscript{22–24} Although cardiac vagal tone is totally abolished over $\approx 60\%$ of $V_{o2\max}$\textsuperscript{25} to adapt heart activity to cell metabolic demand,\textsuperscript{26,27} RSA was retrieved over our entire exercise test. This finding confirms that RSA persistence at intense exercise could be related to enhancement of a non-neural mechanism in response to $V_I$ increase. Indeed, changes in thoracic pressure induced by breathing influence filling of the right ventricle.\textsuperscript{29} Increased right ventricle filling during inspiration consequently increases transmural pressure and stretches the sinus node, thus activating positive chronotropic response via mechanosensitive Cl$^-$ channels.\textsuperscript{30,31}

Thus, using $f_{RSA}$ to detect ventilatory thresholds has the advantages of being non-invasive and cheap and may have field application in ambulatory heart rate monitors. Moreover, this technique appears to be reliable in most athletes and sedentary subjects. $f_{RSA}$ thresholds of athletes were detected at higher values than those of their sedentary peers, whatever the mode of expression, confirming that the AT is significantly improved with endurance training.\textsuperscript{32,33} Thus, this $f_{RSA}$ method could be used for the determination of human ventilatory thresholds over a broad range of physical abilities. However, in 19% of our population only one increase close to $TV_2$ was clearly identifiable in $f_{RSA}$, whereas two ventilatory thresholds were detected. As $V_I$ is the product of $f_R$ and $V_T$, it could be expected that the first non-linear increases in $V_I$ and $V_I/V_{o2\max}$ were mainly related to $V_T$ increase. Indeed, as shown in fig 4, no clear change in $f_R$ was observed around absolute power corresponding to $TV_1$.

Visual detection of both ventilatory and $f_{RSA}$ thresholds can lead to subjective results and may represent a methodological limitation of our study design. Indeed, it has been shown that different evaluators can choose different ventilatory thresholds from the same data.\textsuperscript{36} However, reliability of the ventilatory method is known to be enhanced when test conditions are kept constant and evaluators are experienced,\textsuperscript{21} which was the case in our study. Detection of ventilatory threshold is known to be dependant both on stage duration and load increase in graded exercise.\textsuperscript{37} As no exercise protocol test seems consensual, the standard protocol test used in our laboratory was thus preferred.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Relationships between absolute power measured at $T_{RSA1}$ and $TV_1$ (A) and $T_{RSA2}$ and $TV_2$ (B). Solid lines represent the regression lines. ● Athlete group; ▲ sedentary group. $T_{RSA1}$, first respiratory sinus arrhythmia frequency ($f_{RSA}$) threshold; $T_{RSA2}$, second $f_{RSA}$ threshold; $TV_1$, first ventilatory threshold; $TV_2$, second ventilatory threshold.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Example of lack of clear change in $f_{RSA}$ (A) and $f_R$ (B) in the region of $TV_1$. $f_R$, respiratory frequency; $f_{RSA}$, respiratory sinus arrhythmia frequency; $T_{RSA2}$, second $f_{RSA}$ threshold; $TV_1$, first ventilatory threshold.}
\end{figure}
Respiratory sinus arrhythmia results from modulation of sinus node activity by breathing and during exercise is the main mechanism regulating short term heart period fluctuations. Strong correlations have been found between the centred frequency of respiratory sinus arrhythmia and respiratory frequency.

We have shown that, in most of our subjects, two successive non-linear increases are observed in RSA. These thresholds are closely related to the first and second ventilatory thresholds, respectively. Thus, the method we developed provides a useful tool for identifying the ventilatory thresholds during graded and maximal exercise test in athletes and sedentary subjects as well as for assessing endurance levels. The next step could be to process HP series recorded during an adapted field test using modern heart rate monitors and time varying modelling.

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7 Reinhard U, Muller PH, Schmutz RM. Determination of anaerobic threshold by the ventilatory equivalent in normal individuals. Respirilation 1997;38:36–42.

What is already known on this topic

Two successive non-linear increases observed in respiratory sinus arrhythmia frequency are closely related to the first and second ventilatory thresholds, respectively. We have developed a useful method for identifying the ventilatory thresholds during graded and maximal exercise test in athletes and sedentary subjects as well as for assessing endurance levels.

What this study adds

During the past 20 years, very many studies have indicated that parameters measured during submaximal exercise may be better markers of endurance performance than VO2max, the anaerobic (or ventilatory) and lactate thresholds being useful parameters to evaluate functional capability in various types of endurance performance. Both gas analysis and ventilatory flow measurements, as well as blood lactate determinations, can be used to estimate the anaerobic threshold as a predictor of endurance capacity. A procedure that would be simple, relatively inexpensive, and non-invasive would be welcome. Procedures based on maximal heart rate (or a percentage of it) are simple but not reliable. Thus, the determination of ventilatory thresholds by time varying analysis of respiratory sinus arrhythmia, as proposed in this paper, appears to be quite promising, providing that it can be used with data obtained by ambulatory heart rate monitors.

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