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Research article

Ventilation behavior in trained and untrained men during incremental test: evidence of one metabolic transition point

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Abstract
This study aimed to describe and compare the ventilation behavior during an incremental test utilizing three mathematical models and to compare the feature of ventilation curve fitted by the best mathematical model between aerobically trained (TR) and untrained (UT) men. Thirty five subjects underwent a treadmill test with 1 km·h^{-1} increases every minute until exhaustion. Ventilation averages of 20 seconds were plotted against time and fitted by: bi-segmental regression model (2SRM); three-segmental regression model (3SRM); and growth exponential model (GEM). Residual sum of squares (RSS) and mean square error (MSE) were calculated for each model. The correlations between peak VO_2 (VO_{2PEAK}), peak speed ($Speed_{PEAK}$), ventilatory threshold identified by the best model (VT_{2SRM}) and the first derivative calculated for workloads below (moderate intensity) and above (heavy intensity) VT_{2SRM} were calculated. The RSS and MSE for GEM were significantly higher ($p < 0.01$) than for 2SRM and 3SRM in pooled data and in UT, but no significant difference was observed among the mathematical models in TR. In the pooled data, the first derivative of moderate intensities showed significant negative correlations with VT_{2SRM} ($r = -0.58$; $p < 0.01$) and $Speed_{PEAK}$ ($r = -0.46$; $p < 0.05$) while the first derivative of heavy intensities showed significant negative correlation with VT_{2SRM} ($r = -0.43$; $p < 0.05$). In UT group the first derivative of moderate intensities showed significant negative correlations with VT_{2SRM} ($r = -0.65$; $p < 0.05$) and $Speed_{PEAK}$ ($r = -0.61$; $p < 0.05$), while the first derivative of heavy intensities showed significant negative correlation with VT_{2SRM} ($r= -0.73$; $p< 0.01$), $Speed_{PEAK}$ ($r = -0.73$; $p < 0.01$) and VO_{2PEAK} ($r = -0.61$; $p < 0.05$) in TR group. The ventilation behavior during incremental treadmill test tends to show only one threshold. UT subjects showed a slower ventilation increase during moderate intensities while TR subjects showed a slower ventilation increase during heavy intensities.

Key words: Ventilatory threshold, mathematical modeling, ventilatory responses, aerobic training status.

Introduction

The theoretical model traditionally describes two metabolic transition points in the response of ventilation (VE) during incremental exercise, frequently called first (VT1) and second (VT2) ventilatory thresholds (Meyer et al., 2005; Skinner and Mclellan, 1980). When VE is plotted against VO_2 or workload, VT1 and VT2 are often determined in the first and second break point on the VE curve, respectively (Meyer et al., 2005; Skinner and Mclellan, 1980).

However, several researchers frequently make the assumption that there is only one break point (Crescêncio et al., 2003; Higa et al., 2007), not allowing to know if this point corresponds to VT1 or VT2. This supposition would impact on the training prescription because VT1 is used for aerobic training in elderly and diseased population (Meyer et al., 2005; Koufaki et al., 2000) while VT2 is employed for aerobic training in healthy subjects and elite athletes (Meyer et al., 2005; Lucía et al., 2000).

Divergences between the theoretical model and researcher's assumptions make the conceptual and methodological understanding difficult. Orr et al. (1982) and Dennis et al. (1992) tried to solve this divergence through mathematical modeling which could bring valuable information about the phenomenon characteristics. For instance, Orr et al. (1982) found a better fitting on the $\dot{V}E$ data with a bi-segmental linear regression model (2SRM) than with a three-segmental linear regression model (3SRM). On the other hand, Dennis et al. (1992) reported better fitting with an exponential model than with 2SRM or 3SRM. The 2SRM as well as the exponential fitting produce only one metabolic transition point through intersection point between two segments or derivatives, respectively (Higa et al., 2007; Hughson et al., 1987; Orr et al., 1982; Morton, 1989; Santos e Giannella-Neto, 2004). Thus, these results are in contrast with the theoretical model in which two transitions points are expected.

Nevertheless, methodological issues may restrict the inferences from these studies (Myers and Ashley, 1997; Morton, 1993). Orr et al. (1982) did not compare 2SRM and 3SRM with the exponential model while Dennis et al. (1992) did not determine the intersection point(s) mathematically of 2SRM and of 3SRM fitting and did not use enough points on each curve due to the employment of 60 seconds averages on VE values. Then, the divergences between theory and experimental data concerning VE behavior could not be completely answered by results of these studies and a better mathematical description of VE data during incremental exercise remains to be determinate.

These divergences between theory and empirical data can be more confusing when the training status is taken into account. While aerobic training changes the VT1 and VT2 to the right and promotes lower VE values (Esteve-Lanao et al., 2007; Zapico et al., 2007), anaerobic training produces a longer bicarbonate buffering (distance between VT1 and VT2) (Röcker et al., 1994). Thus, it could be hypothesized that aerobically trained subjects

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should have a more smooth VE increase, mainly in the initial stages of an incremental test usually employed in practical situations. Consequently, the VE behavior should be exponential with only one ventilatory threshold. In addition, untrained subjects and anaerobically trained subjects should have an abrupt and less smooth VE increase which could allow the presence of two thresholds.

However, how the aerobic training status would change the feature of VE behavior during an incremental exercise and whether the occurrence of one or two metabolic transitions would depend upon the training status still needs to be experimentally answered. A more specific mathematical analysis of the VE curve could provide valuable practical information to elucidate this issue (Mader and Heck, 1986; Newell et al., 2006). Therefore, the objectives of this study were: 1) to describe and to compare VE behavior during incremental testing by mathematical modeling; 2) to compare the feature of VE curve fitted by the best mathematical model between untrained and aerobically trained subjects. To reach these goals, 2SRM, 3SRM and grow exponential model (GEM) as well as the derivatives of the best mathematical model were compared within and between groups.

Methods

Subjects

Fifteen trained soccer players (TR) (26.8 ± 2.8 years; 75.6 ± 10.0 kg; 1.78 ± 0.11 m) from a Brazilian professional team and twenty healthy untrained men (UT) (28.9 ± 3.8 years; 80.6 ± 13.8 kg; 1.76 ± 0.07 m) volunteered to take part in the study. After giving information about the risks, an informed consent was obtained from each participant (study has approved by the Ethics Committee of School of Physical Education and Sport, São Paulo University).

Protocol and measurements

After a 4-minute warm-up, the subjects underwent an incremental test on a treadmill with initial speed between 5.0-6.0 (UT) and 10.0 $km \cdot h^{-1}$ (TR) and increases of 1 $km \cdot h^{-1}$ every minute, until exhaustion (the slope was kept at 0%). Subjects wore a nose clip and mouthpiece connected to a heated wire flow meter with precision of ± 0.05 L and dead space lower than 100 ml (SensorMedics, CA, USA). The flow signal was digitally integrated to give tidal volume and inspired gases were continuously analyzed by rapidly responding O_2 (paramagnetic) and CO_2 (infrared) analyzers with response time lower than 130 ms (SensorMedics Vmax 229, SensorMedics, CA, USA). The analyzers were checked prior to each test using a calibration syringe containing precision O_2 and CO_2 gas mixes and a rapidly switching solenoid valve (SensorMedics, CA, USA). Expiratory flow, VE (BTPS) and fractional concentrations of expired O_2 and CO_2 were measured and digitally integrated on a breath by breath basis to provide estimates of O_2 uptake (VO_2) and expired CO_2 volume (VCO_2) at real time.

Data analysis

The VE, expired O_2 fraction (FeO_2) and expired CO_2 fraction ($FeCO_2$) data were smoothed to averages of 20 seconds and were plotted against the time (approximately 30 points over each curve). Three evaluators identified VT1 and VT2 by visual inspection following the hierarchic criteria's: VT1: first linearity break point of VE curve and the break point of FeO_2; VT2: second linearity break point of VE curve and the break point of $FeCO_2$ (Meyer et al., 2005). The FeO_2 and $FeCO_2$ were utilized only when it was not possible to identify VT1 and VT2 utilizing VE curve. VT1 and VT2 identified by evaluators were useful as reference points for the beginning calculation of bi-segmental regression and three-segmental regression since these mathematical fittings can produce artificial break points in the curve, without physiological significance. Peak VO_2 (VO_{2PEAK}) was determined by averaging the three highest values found in last two speeds (Weston et al., 2002), and peak speed ($Speed_{PEAK}$) was identified as the highest speed reached.

Fitting of curves

The behavior of VE curve was fitted by 2SRM, 3SRM and GEM. The 2SRM was attained by linear regression with an initially unknown intercept calculated from every possible intersection between the three points immediately before to VT1 and three points immediately after to VT2. This criterion was employed to provide physiological limits in which the intercept(s) should be identified, avoiding intercept with artificial values, without physiological significance. The intercept that best shared the curve in two linear segments was assumed when the highest R^2 and the lowest residual sum of squares (RSS) were attained. The curve segments were predicted by the following equations:

$$y = a' + b' (x) \quad (1)$$

where x is the time, y is the predicted value of VE to each segment, a' is the constant for the 1^{st} or 2^{nd} segment, and b' is the slope of the 1^{st} or 2^{nd} segment, respectively (Figure 1).

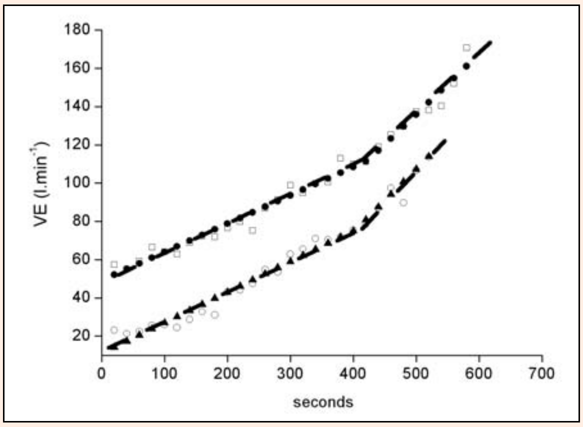

Figure 1. Graphic representation of bi-segmental regression model (2SRM) on the VE curve. Data series from a trained and an untrained subject (□: obtained values from athletes; ●: estimated values from athletes; ○: obtained values from untrained; ▲: estimated values from untrained).

The 3SRM fitting was calculated by linear regression with two initially unknown intercepts calculated from every possible intersection between the three points immediately before to VT1 and three points immediately after to VT2. The intercepts that best shared the curve in three linear segments were assumed when the highest R^2 value and the lowest RSS were attained. The curve segments were predicted by the same equation of 2SRM (equation 1), where *x* is the time, *y* is the predicted value of VE to each segment, *a'* is the constant for the 1st or 2nd or 3rd segment, and *b'* is the slope of the 1st or 2nd or 3rd segment, respectively. More than thirty possible combinations were tested from each linear regression model to each curve (Figure 2).

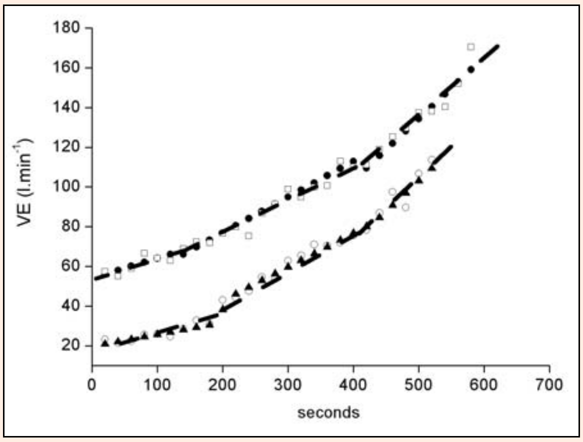

Figure 2. Graphic representation of three-segmental regression model (3SRM) on the VE curve. Data series from a trained and an untrained subject (□: obtained values from athletes; ●: estimated values from athletes; ○: obtained values from untrained; ▲: estimated values from untrained).

The exponential characteristic of VE was tested by the GEM, through the following equation:

$$y = y0 + A\,(e^{((x - x0)/t1)}) \quad (2)$$

where x is the time, y is the predicted value of VE, y0 is the offset of VE values, A is the amplitude of curve, x0 is the delay of VE response and t1 is the grow time constant of VE (Figure 3).

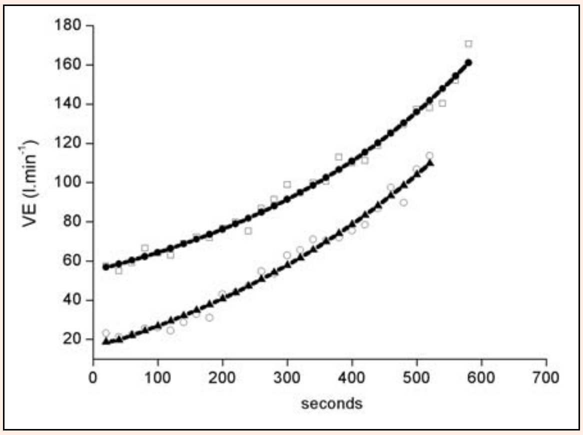

Figure 3. Graphic representation of growth exponential model (GEM) on the VE curve. Data series from a trained and untrained subject: (□: obtained values from athletes; ●: estimated values from athletes; ○: obtained values from untrained; ▲: estimated values from untrained).

The GEM was chosen considering important parameters such as the offset, delay and time constant of the data series, after comparison of the R^2, RSS and mean square error (MSE) with the continuous exponential function described by Hughson et al. (1987).

The parameters of each model were determined by least squares methods, which evaluate the sum of squares residuals for each combination. The sweep calculations were employed to obtain the minimized sum of squares residuals and to determine the parameters *a'* and *b'* in 2SRM and 3SRM. The Levenberg-Marquardt algorithm was used to determine the parameters in GEM.

Residual analysis

The residuals, RSS and MSE were calculated for each mathematical model. To verify the robustness of mathematical models, the original residuals were plotted against the VE measured values and analyzed by graphic inspection, by Shapiro Wilk's test and by Run test. Subsequently, the residuals were fitted by a linear regression fitting and the constant (α) and slope (β) obtained were used to give an approximation concerning the location and behavior of estimated VE data by mathematical models.

Relationship between fitting curve and aerobic fitness

After the best mathematical model had been found, the first derivative was calculated to the points corresponding to workloads below and above ventilatory threshold with the assumption that lower workload derivatives (LWD) should indicate moderate intensities and upper workload derivatives (UWD) should indicate heavy intensities. The first derivative obtained in moderate and heavy intensities of data fitted by the best mathematical fitting were employed to investigate the relationship between VE behavior during the incremental test and aerobic fitness. The $Speed_{PEAK}$ ($km \cdot h^{-1}$), VO_{2PEAK} ($ml \cdot kg^{-1} \cdot min^{-1}$), VT1 and VT2 ($ml \cdot kg^{-1} \cdot min^{-1}$, $km \cdot h^{-1}$ and $\%Speed_{PEAK}$) were accepted as aerobic fitness markers.

Statistical analysis

The data distribution was verified by Shapiro Wilk's test and the data were reported as mean and standard deviation (SD) to make future comparisons easier, even when they did not show normal distribution. Factorial ANOVA (3 x 2) was carried out to detect interaction effects between the factors (mathematical fitting *versus* groups). *F* test was utilized to determine the differences between RSS and MSE for comparison intra-group and in the pooled data. The tests of Mann-Whitney, Wilcoxon and Student were carried out when necessary, for comparisons among derivatives, α and β parameters, $Speed_{PEAK}$, VO_{2PEAK}, VT1 and VT2 intra-group or between UT and TR. Pearson correlation coefficient determined the degree of association between α and β parameters of residuals, and between the derivatives of the best mathematical model and the aerobic markers. The analysis was carried out in SPSS (15.0) and in Origin (6.0) softwares and the significance was accepted at $p < 0.05$.

Results

VO_{2PEAK}, VT1 and VT2 (expressed as $ml \cdot kg^{-1} \cdot min^{-1}$) were, respectively, 39.7 ± 6.3, 19.1 ± 5.1 and 31.5 ± 4.4

Table 1. Speed$_{PEAK}$, VT1, VT2 and VT$_{2\text{-}SRM}$ (absolute and relative) in UT and TR groups. Data are means (±SD).

	Speed$_{PEAK}$ km·h^{-1}	VT1 km·h^{-1}	VT2 km·h^{-1}	VT$_{2\text{-}SEM}$ km·h^{-1}	VT1 %Speed$_{PEAK}$	VT2 %Speed$_{PEAK}$	VT$_{2\text{-}SEM}$ %Speed$_{PEAK}$
UT	13.7 (1.6) *	7.7 (1.1) *	10.2 (1.0) *	8.9 (3.0) *	55.1 (5.8)	76.4 (6.8) *	65.8 (18.0) *
TR	18.9 (.4)	12.2 (3.5)	16.0 (.7)	14.9 (1.7)	64.5 (18.5)	84.8 (3.8)	78.8 (8.1)

* $p < 0.05$ (significantly different from TR).

ml·kg^{-1}·min^{-1} in UT and 54.7 ± 3.2, 38.6 ± 3.8 and 46.1 ± .41 ml·kg^{-1}·min^{-1} in TR. These variables showed significant differences between UT and TR groups. Other aerobic parameters are listed in the Table 1.

Fitting of curves

The distribution of residuals of 2SRM, 3SRM and GEM showed a Gaussian behavior in almost every subject and the fittings attained significant R^2 (R^2 = 0.97 to 0.99 to more than 90% of values). Some subjects did not show Gaussian behavior with 2SRM (n = 5), 3SRM (n = 2) and GEM (n = 5) when analyzed by Shapiro Wilk's test. These values were reduced for 2SRM (n = 1), but not for 3SRM (n = 3) and GEM (n = 6), when analyzed by Run test. No interaction effect was observed between the mathematical fittings (2SRM, 3SRM and GEM) and groups (UT and TR).

The RSS and MSE for GEM in pooled data were higher than 2SRM and 3SRM ($p < 0.01$). In intra-group comparisons, RSS and MSE for GEM were higher than 2SRM and 3SRM in the UT, but no difference was observed among mathematical fittings in the TR (Table 2).

The parameter β obtained on the residuals was not different from zero for 2SRM, 3SRM and GEM fittings in UT, while the constant α was negative and different from zero (a= -2.01 ± 3.20 l·min^{-1}) for GEM. Together, the parameters α and β of GEM showed a modest tendency of estimated VE values to be located below measured $\dot{V}$E values, throughout the whole curve (Table 2). In TR group, α and β did differ from zero, with negative values for α and positive values for β. Together, the parameters α and β of three mathematical models showed a modest tendency for underestimation of VE values during moderate intensities, and this tendency was attenuated according to increasing of VE (Figure 4 and Table 2).

Relationship between fitting curve and aerobic fitness

The 2SRM was chosen as the best model once it is more parsimonious due to the employment of fewer estimative parameters than 3SRM and GEM models. So, the first derivative was calculated for workloads below and above intersection point of two regression lines. In this case, we accepted the intersection point as ventilatory threshold (VT$_{2SRM}$) (Higa et al., 2007; Orr et al., 1982), with LWD and UWD indicating moderate and heavy intensities, respectively.

In the pooled data, the LWD showed significant correlation with VT$_{2SRM}$ and Speed$_{PEAK}$, while the UWD only showed significant correlation with VT$_{2SRM}$. When analyses were carried out separately, there was a significant correlation between LWD and VT$_{2SRM}$ and Speed$_{PEAK}$ in UT. However, in TR group a significant correlation was observed between UWD and VT$_{2SRM}$, Speed$_{PEAK}$ and VO$_{2PEAK}$ (Table 3). The intra-group comparisons showed that LWD and UWD differed significantly in UT (0.120 ± 0.062 l·min^{-1}·sec^{-1} versus 0.228 ± 0.058 l·min^{-1}·sec^{-1}) as well as in TR (0.117 ± 0.025 l·min^{-1}·sec^{-1} versus 0.251 ± 0.055 l·min^{-1}·sec^{-1}). The LWD and UWD did not differ between UT and TR groups.

Discussion

The main findings of this study were: 1) no interaction between the type of curve fitting and aerobic training status; 2) highest RSS and MSE of GEM in pooled data and in UT group; 3) change in the feature of $\dot{V}$E curve between untrained and aerobically trained men. The differences showed in RSS and MSE between GEM and segmental regression models in pooled data and the UT group and the absence of difference between 2SRM and 3SRM in pooled data as well as in UT and TR groups make us accept the 2SRM as the best fitting. This choice is based on the parsimony principle since this model is simpler than other models and it utilizes fewer estimative parameters.

Fitting of curves

Orr et al. (1982) and Dennis et al. (1992) were the only studies to employ mathematical fittings on VE data which could identify one or two metabolic transition points. Our results partially corroborate these findings since they suggested a VE behavior with only one transition point during incremental exercise. However, contradicting the Dennis et al. (1992) findings, a better exponential fitting was not observed here. One possible explanation for this discrepancy is the exponential equation type adopted here.

Table 2. RSS, MSE and residuals (α and β parameters) of mathematical models in pooled data and in UT and TR groups.

		2SRM				3SRM				GEM			
		RSS	MSE	α	β	RSS	MSE	α	β	RSS	MSE	α	β
Pooled	average	350	15.1	-1.34†	.02†	301	14.1	-1.37†	.01	490*	21.9*	-1.96†	.02†
	sd	198	8.3	.97	.01	176	7.9	1.30	.04	420	18.8	2.47	.03
UT	average	333	15.0	-1.11	.02	276	13.6	-0.91	.00	527*	23.8*	-2.01†	.03
	sd	205	9.0	1.07	.01	173	8.1	1.09	.05	496	21.5	3.20	.04
TR	average	374	15.2	-1.63†	.02†	335	14.9	-1.97†	.02†	441	18.0	-1.89†	.02†
	sd	192	7.7	.75	.01	180	7.9	1.34	.02	300	12.1	.98	.01

* $p< 0.05$ significant different from 2SRM and 3SRM; † significant different from zero; α is expressed as l·min^{-1}; β is expressed as l·min^{-1}·sec^{-1}.

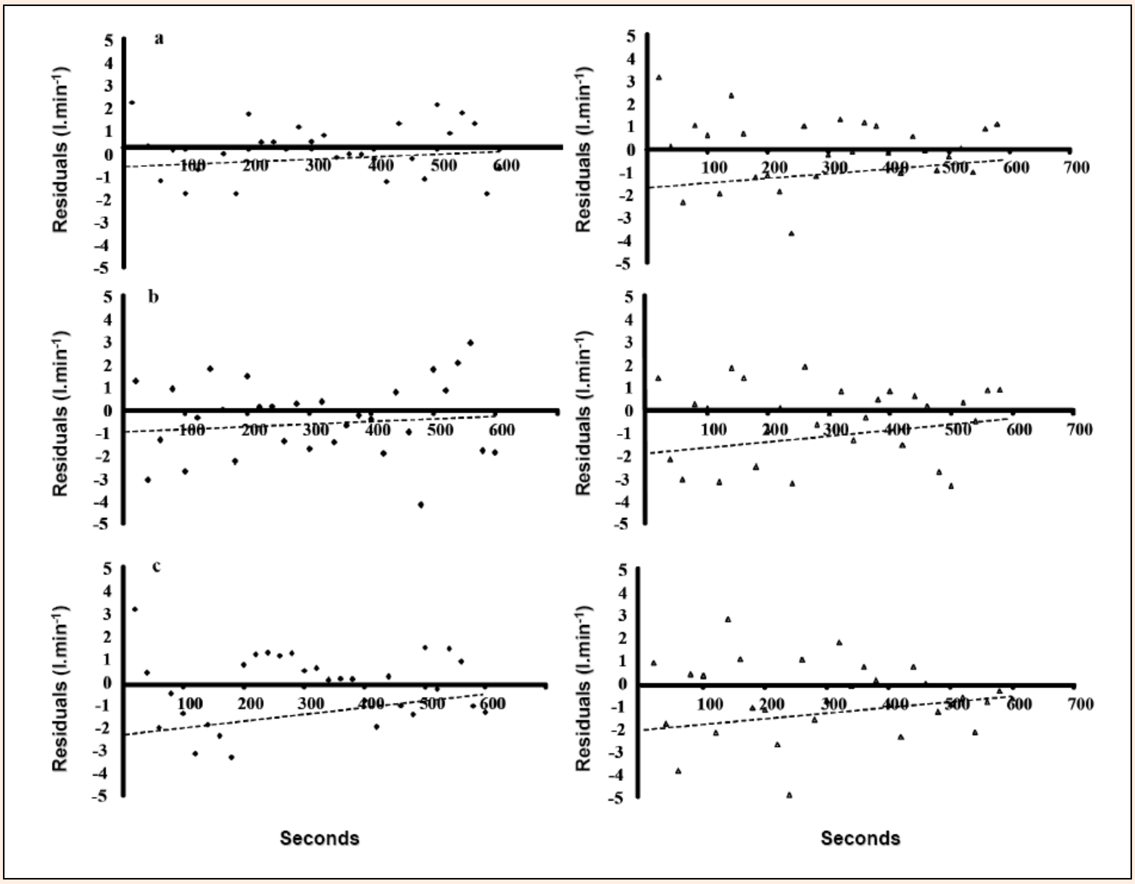

Figure 4. Residual distribution of three mathematical models in UT (●) and TR (Δ) groups. The panels *a*, *b* and *c* are 2SRM, 3SRM and GEM, respectively. The dotted line is the linear regression on the residuals.

The GEM equation was chosen after previous comparisons with the continuous exponential described by Hughson et al. (1987). As it was not observed difference between RSS and MSE, the GEM was chosen because it considers important parameters such as offset, delay and grow time constant of the data series, adding only one more parameter than the other exponential.

However, methodological differences between the studies must be a probable justification for the disagreement between them. Dennis et al. (1992) applied a protocol of 15 w·min^{-1} increments utilizing the average VE over each minute, which allowed around 15 points on each curve. Our experimental protocol with 1 km·h^{-1} increments allowed around 9 stages. As an average VE over each 20 seconds was employed, our experimental design allowed the analysis with around 27 points over each curve, which was almost twice that utilized by Dennis et al. (1992). As a number greater than 20 points is necessary for a successful segmental linear fitting (Orr et al., 1982) it is possible that the lower number of points in Dennis' study as well as others (Campbell et al., 1989; Orr et al., 1982), allowed a better continuous exponential

Table 3. Correlation coefficients among the derivatives of best mathematical fitting and the aerobic markers.

			VO_{2PEAK} km·h^{-1}	$Speed_{PEAK}$ km·h^{-1}	VT1 km·h^{-1}	VT2 km·h^{-1}	VT_{2SRM} km·h^{-1}
Pooled	LWD	r	-.32	-.46*	-.29	-.38	-.58*
		p (2-tailed)	.107	.015	.145	.054	.001
	UWD	r	-.32	-.23	-.18	-.27	-.43*
		p (2-tailed)	.101	.247	.363	.179	.025
UT	LWD	r	-.11	-.61*	-.20	-.42	-.65*
		p (2-tailed)	.712	.021	.503	.134	.012
	UWD	r	.04	.24	.49	.13	-.25
		p (2-tailed)	.885	.415	.078	.666	.390
TR	LWD	r	-.18	-.20	.82	.11	-.19
		p (2-tailed)	.554	.507	.001	.713	.524
	UWD	r	-.61*	-.73*	.08	-.36	-.73*
		p (2-tailed)	.028	.004	.788	.232	.004

* Significant correlation.

fitting since this model maximizes the correlation coefficient between x and y values by a linear coefficient ($y= a + b\ exp^{(cx)}$). Then, a better continuous exponential fitting should be observed whenever long time averages (≥ 1 minute) rather than short time averages (≤ 30 seconds) are employed for data analysis.

It is likely that the difference observed between the linear regression fitting and continuous exponential fitting is also linked to ergometer employed because a better linear regression model was found in treadmill (Myers et al., 1994) while a better continuous exponential model was found in cycle ergometer (Campbell et al., 1989; Dennis et al., 1992; Hughson et al., 1987). An incremental treadmill test with lowest initial velocity implies a change in energy expenditure of the transition phase between walking and running (Mercier et al., 1994). Due to lower mechanical efficiency during the transition between walking and running (around 7 $km \cdot h^{-1}$) there is a higher increase in VO_2 and VE than during velocities below (< 7 $km \cdot h^{-1}$) or above this phase (>7 $km \cdot h^{-1}$) (Mercier et al., 1994). This methodological detail can be responsible for a worse fitting with GEM in UT than in TR, similarly to Myers et al. (1994) results.

On the other hand, the exponential model seems to be more sensitive on VO_2 values above 28 $ml \cdot kg^{-1} \cdot min^{-1}$, approximately (Morton, 1993; Beaver et al., 1988). In previous works which found a better continuous exponential (Dennis et al., 1992; Hughson et al., 1987) the VO_2 value in the third minute (or third stage) was around 27 $ml \cdot kg^{-1} \cdot min^{-1}$ showing that the main part of the curve was fitted with values above this. Differently, other works that have found a better 2SRM employed an experimental protocol that allowed VO_2 values to be quite below 28 $ml \cdot kg^{-1} \cdot min^{-1}$ in the third minute, and a greater sampling frequency (Myers et al., 1994; Orr et al., 1982). In the present study, VO_2 values around 22 $ml \cdot kg^{-1} \cdot min^{-1}$ were found in UT group and 35 $ml \cdot kg^{-1} \cdot min^{-1}$ in TR group for the third minute ($p < 0.001$). So, a greater part of the curve built with values less than 28 $ml \cdot kg^{-1} \cdot min^{-1}$ in UT than TR can be the reason for the difference between the GEM and 3SRM in UT group. The modest tendency to underestimate VE values observed mainly in the beginning of the curve ($\alpha = -2.01 \pm 3.20\ l \cdot min^{-1}$) of UT corroborates this supposition. It is also likely that the utilization of VO_2 in x axis (rather than velocity or time) affects the quality of fitting because VO_2 has a curvilinear pattern in incremental tests (Kelly and Lindsey, 2004).

The 2SRM and 3SRM fittings also differed between our study and other studies (Dennis et al., 1992). Some studies employed the segmental regression fittings with intersection points previously determined by visual inspection, making the calculation of an R^2 possible for each segment (Campbell et al., 1989; Dennis et al., 1992; Hughson et al., 1987). In the present study the intersection points were determined mathematically after testing more than thirty possible combinations, allowing only one R^2 for the whole data series. These methodological differences may have influenced the quality of fittings because, while the studies which utilized intersection points previously determined by visual inspection found a better exponential fitting (Campbell et al., 1989; Dennis et al., 1992; Hughson et al., 1987), the studies which employed intersection points mathematically determined found a better segmental regression fitting (Myers et al., 1994).

It is not possible to justify statistically or mathematically why 3SRM was not better than 2RSM. Our results are close to other works (Dennis et al., 1992; Orr et al., 1982) and show that adding one more segment does not improve the fit of the data series. As this result was observed in UT group as well as TR group, it can be suggested that a better mathematical fitting is not related to aerobic training status. Therefore, at least in part, it is suggested that aerobic training does not change the identification possibility of one or two metabolic transition points when identified by VE data.

On the other hand, the absence of difference between 2SRM and 3SRM could also be due to type of incremental protocol employed here. The time constant of VE is around 59 s in moderate intensities (~40% VO_{2PEAK}) (Keslacy et al., 2005) and it increases according to exercise intensity (Casaburi et al., 1989). Thus, it is likely that the incremental protocol has not allowed a complete VE response during intermediate and heavy intensities (≥ 50% VO_{2PEAK}), leading a delayed response of VE in these stages. Consequently, the first linearity break point of VE could be carried towards the second linearity break point, providing only one break point and making the 3SRM unsuccessful. Nevertheless, as the time constant of VE depends on workload increase-duration relationship (Debigar et al., 2000), it is unlikely that the VE behavior observed in our data would be different from the majority of studies that identified the ventilatory threshold by VE curve in incremental treadmill tests once workload increase-duration relationship is very close between them (Bunc et al., 2001; Esteve-Lanao et al., 2007). Thus, if the workload increase is fixed, a behavior of VE curve different from that observed here could occur only when longer stages (≥ 2 minutes) are employed.

Fitting of curves and physiological significance

Mathematical models are frequently employed to aid the explanation about physiological mechanisms. Taking into account both previous results and the results obtained here, it would be suggested the presence of only one metabolic transition point in VE curve (Dennis et al., 1992; Orr et al., 1982). However, if it is a continuous process rather than a threshold model it can only be a methodological question.

Admitting a very close relationship between lactate production and ventilatory responses, the presence of only one metabolic transition point could be linked to a modification point on the lactate/pyruvate ratio around 65% VO_{2PEAK} (i.e., 4-6 $mmol \cdot l^{-1}$ of lactate) due to saturation on pyruvate dehydrogenase (PDH) activity and an elevation on lactate dehydrogenase (LDH) activity around twice or three times greater than the former (Spriet et al., 2000). Specifically, the saturation on PDH activity can be a result of modification of the redox state (NADH/NAD ratio), substrate availability and product accumulation relationship, and mitochondrial O_2 supply (Hollidge-Hovart et al., 1999; Parolin et al., 2000).

On the other hand, the control of VE during exercise is suggested as a multifactorial process (Ward, 2007). Potassium (K^+), catecholamine and ammonia (NH_3^-) exert humoral control on the ventilation by carotid body chemoreceptors, promoting an elevation in ventilatory stimulus as it increases (Hopkins et al., 2003; Paterson, 1997; Tong et al., 2003). Evidences show the presence of only one inflection point in K^+, catecholamine and NH_3^- in workloads around 40% and 70% of VO_{2PEAK} (or 60% and 80% maximal power output) during incremental exercise (Busse et al., 1992; Fraser et al., 2002; Mcmorris et al., 2000; Yuan et al., 2002). Whether the increasing pattern of K^+, catecholamine and NH_3^- in incremental exercise is linked to one metabolic transition point on VE is a matter that future studies should analyze.

The findings of present study have practical implications. Usually, VT1 is employed for aerobic training prescription in elderly and diseased population (Koufaki et al., 2000; Meyer et al., 2005) while VT2 is used for aerobic training prescription in healthy subjects and elite athletes (Lucía et al., 2000; Meyer et al., 2005), existing a broad difference of intensity between them. As it was experimentally observed only one ventilatory threshold, it is not possible to know if VT_{2SRM} would correspond at training intensity for elderly and diseased population or for healthy subjects and elite athletes since VT_{2SRM} was significantly higher than VT1 ($p < 0.01$) and significantly lower than VT2 ($p < 0.01$) in pooled data as well as in UT and TR. Consequently, VT_{2SRM} could be just employed as an upper intensity limit for aerobic training prescription in elderly and disease population and a lower intensity limit for aerobic training prescription in healthy subjects and elite athletes. While intensities around ~80% VT_{2SRM} could be employed for elderly and diseased population, intensities around ~120% VT_{2SRM} could be employed for healthy subjects and elite athletes. However, further studies should be realized to confirm this proposal.

Relationship between fitting curve and aerobic fitness

In the present study the relationship between parameters obtained by the best fitting and aerobic fitness level also was investigated. As proposed in pattern increasing on lactate curve (Mader and Heck, 1986), it was hypothesized that the aerobic training would allow a more smooth VE increase during incremental exercise, providing a different behavior in VE curve between untrained and aerobically trained subjects. As there was no difference in VE curve fitted between UT and TR groups, it could be suggested that the aerobic training status does not have effects on the mathematical description of VE behavior during incremental exercise. However, as the lower and upper derivatives of 2SRM were associated to markers of aerobic fitness, it could be suggested that these parameters of best mathematical fitting can be used to discriminate the aerobic training status.

In the pooled data, significant and negative correlations were observed among lower and upper derivatives of 2SRM and the markers of aerobic capacity (VT_{2SRM}) and aerobic power ($Speed_{PEAK}$). The results show that in a heterogenic group an improvement in the aerobic fitness also suggests an improvement in gas exchange leading the aerobically trained subjects to need a smaller elevation in the ventilatory responses over each increase of the mechanic power output during incremental exercise (Bickham et al., 2004; Ward, 2007). When analysis was carried out separately, differences were observed. In UT group the correlation coefficients showed that the subjects who had higher aerobic capacity and aerobic power also had a slower VE increase during moderate workloads (i.e. < VT_{2SRM}). In TR group, those who had higher aerobic capacity and aerobic power showed a slower VE increase in heavy workloads (i.e. > VT_{2SRM}).

It is not possible to establish any cause and effect relationship between slower VE increase and aerobic fitness markers in the present study. In spite of this, some suppositions should not be discarded: 1) for heterogenic groups the aerobic training seems to improve the duration of physical work in moderate workloads as well as heavy workloads, moving the whole VE-workload curve to the right with a smaller slope; 2) in untrained subjects the adaptations to aerobic training would mainly affect the work capacity in moderate workloads; 3) in endurance homogeny groups the time of permanence in heavy intensities can better distinguish the aerobic fitness level than VT_{2SRM}, once these subjects already show high values of ventilatory threshold. Actually, these results would corroborate other studies (Bickham et al., 2004; Ward, 2007), which can be a consequence of interval training carried out by soccer players (Bunc and Psotta, 2001).

The physiological mechanisms responsible for a slower VE increase in moderate (UT group) and heavy (TR group) intensities cannot be explained here. However, the following aspects linked to ventilation control should be investigated in future studies to aid in clarifying the possible mechanisms: 1) the association between the modifications on the bicarbonate buffering efficiency, PFK and PDH activity, NH_3^- and catecholamine levels, and the VE behavior in moderate intensities (i.e. < VT_{2SRM}) after aerobic training; 2) the association between the modifications on the tolerance to metabolic acidity, LDH isozyme expression (LDH-M isozyme and LDH-H isozyme), K^+ concentration, fiber recruiting pattern, and the VE behavior in heavy intensities (i.e > VT_{2SRM}) after aerobic training.

Conclusion

The behavior of VE during incremental treadmill test tends to show only one metabolic transition point. Although some possible physiological mechanisms can justify this increasing on VE data, the presence of a threshold process rather than a continuous process seems to be more of a methodological than physiological matter. When the feature of VE curve is analyzed according to the training status it is possible to verify that untrained subjects show a slower VE increase at moderate rather than heavy intensities and that aerobically trained subjects show a slower VE increase at heavy rather than moderate intensities.

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Key points

- The increase of ventilation during incremental exercise tends to show only one metabolic transition point.
- The presence of a threshold process or a continuous process in ventilation during incremental exercise seems to be only a methodological matter.
- The ventilatory efficiency can be employed to distinguish trained than untrained subjects once this index is associated with aerobic parameters. When analyzed the whole curve, trained subjects show a better ventilatory efficiency at heavy intensities and untrained subjects show a better ventilatory efficiency at moderate intensities.

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