

**Acid-Base Balance in Three Theoretical Models of Anaerobic Threshold**F.A.M.S. Pompeu¹, P.S.C. Gomes²¹Physical Education Graduate Program, Rio de Janeiro Federal University (PPGEF/UFRJ), Rio de Janeiro, RJ, 21941-590, Brazil²Graduate Program in Exercise and Sport Sciences, Rio de Janeiro State University (PPG/UERJ), Rio de Janeiro, RJ, 20740-280, Brazil**ABSTRACT**

Pompeu FAMS, Gomes PSC. Acid-Base Balance in Three Theoretical Models of Anaerobic Threshold. **JEPonline** 2017;20(2): 64-72. Few theoretical models for anaerobic threshold assessment are truly axiomatic and causative. The main goal of the present study was to compare three kinds of such theoretical models that are widely used to assess the anaerobic threshold. Ten subjects (mean $\pm$ SD: age, 23 ± 1 yrs ; weight, 61.2 ± 4.9 kg; VO_2 max = 37.6 ± 2.8 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹) were exposed to the original graded exercise test (GXT) to determine Wasserman's Anaerobic Threshold (AT), Stegmanan's Individual Anaerobic Threshold (IAT), and Mader's 4 mmol $\cdot$ L⁻¹ Anaerobic Threshold (4mM). Then, they were submitted to three square wave tests (SWT), one for each anaerobic threshold workload, in order to compare acid-base balance and metabolic parameters at each workload. Repetitive two-way ANOVA with Green-House correction for alpha inflation and HSD-Tukey *post hoc* tests were used for statistical comparisons among AT, IAT, and 4mM, GXT and SWT and their interactions ($P \leq 0.05$). The 4mM anaerobic threshold occurs at higher values (Workload = 142 ± 16 W) than the threshold based on the AT (Workload = 86 ± 11 W) or the IAT (Workload = 95 ± 8 W) approach. The AT model shows the lowest mean squared error (watt/lactate curve), the strongest correlation with VO_2 max and the least disturbance in acid-base balance during the SWT. The findings indicate that the Wasserman's AT theory for anaerobic threshold offered better predictions than both the IAT and the 4mM models.

Key Words: Blood Lactate, Lactate Threshold, Metabolic Acidosis, Ventilatory Threshold

INTRODUCTION

Over the last few years, several models have been proposed to determine the beginning of anaerobic metabolism based on the graded exercise tests (GXT). The main criticism for the majority of the anaerobic threshold (AT) assessments is related to the lack of knowledge about the causative mechanisms associated with metabolic integration. On the other hand, Wasserman et al. (19) pointed out the cause-effect theoretical model based on the oxygen deficit and acid-base disturbances. However, the muscular hypoxia hypothesis for this anaerobic threshold is not well accepted for its intensity level, e.g., 40 to 60 %VO₂ max (15). Another causative model was proposed by Stegmann and colleagues (17). The authors demonstrated mathematically, based on effort and recovery time/lactate curve that the point of lactate appearance and disappearance rates was equal and this was the same as the Individual Anaerobic Threshold (IAT). However, this model does not consider any of the metabolic characteristics, like the distribution of monocarboxylate transporters or other factors that are known to influence lactate flow, such as increased rate in load, conditioning, and metabolic rate on active or passive recovery. Moreover, in the fixed lactate point (4mM) model by Mader and Heck (12), the authors employed several estimates to calculate the distribution of lactate between two compartments. Although this model takes a larger number of factors into consideration, it nevertheless requires considerable extrapolation of “average” values as well as assumptions that collectively can generate a substantial degree of error.

Since the AT, IAT, and 4mM models are based on protocols of progressive effort, the specific recruitment order of motor units and the mechanical efficiency must be considered (11). This means the original GXT is necessary to establish the anaerobic threshold workloads. Hence, the purpose of this study was to determine whether Wasserman’s Anaerobic Threshold (AT), Mader’s 4 mmol·L⁻¹ Anaerobic Threshold (4mM), and Stegmann’s Individual Anaerobic Threshold (IAT) models are valid and precise markers to maintain acid-base balance and metabolic steady-state during a non-stop 1-hr exercise. In order to reduce biological variation among the subjects, a counterbalanced experimental design was employed.

METHODS

Sample Characteristics

Ten volunteers (7 males), age 23 ± 1 yrs, weight 61.2 ± 4.9 kg, and height 176 ± 3 cm, were instructed to maintain a mixed diet. They did not eat for 3 hrs beforehand and did not do strenuous effort (above 5 METs) during the 48 hrs preceding the tests. Subjects were not involved in any physical training programs. A written informed consent was obtained from the subjects. All procedures were approved by the institutional Ethics Committee.

VO₂ Max Tests

The subjects performed 7 tests on a mechanically braked cycle ergometer (Monark®, Brazil): (a) 1 test established the subjects’ VO₂ max; (b) 3 tests were carried out with graded exercise loads (GXT); and (c) 3 tests were done with fixed loads (SWT). There was a recovery interval of 2 to 7 d. The sequence of the AT, IAT, and 4mM models was randomized.

The VO₂ max test was conducted in accordance with the procedures by Astrand et al. (1) in which at least three of the following criteria were required to establish the subjects’ maximum

effort: (a) a plateau of VO_2 ($\leq 150 \text{ mL} \cdot \text{min}^{-1}$); (b) blood lactate $\geq 8 \text{ mmol} \cdot \text{L}^{-1}$; (c) heart rate (HR) $\geq 10\%$ HRmax predicted for the subjects' age; (d) respiratory exchange ratio (RER) ≥ 1.10 ; and (e) at least 18 on the Borg's scale.

Graded Test for AT Model

After 4 min riding unloaded, the workload was increased by $15 \text{ W} \cdot \text{min}^{-1}$ to the limit of tolerance. Lactate was measured in hyperemic earlobes blood samples at the end of every stage. The AT was determined by using the $\log \text{VO}_2 / \log [\text{Lac}]$ and, additionally, the V-slope and the V_E / VO_2 break points techniques (3,4).

Graded Test for 4mM Model

After 10 min of riding at 50 to 100 W, the workload was increased by 50 W every 5 min to the limit of tolerance. Lactate was measured in hyperemic earlobes blood samples at the end of every stage and at 1, 2, 3, 5, and 7 min during the recovery (10). The 4mM point was interpolated from the work-rate / blood lactate curve, fitted into a mono-exponential function (10).

Graded Test for IAT Model

After 3 min of 25 to 100 W, the workload was increased by 30 to 50 W every 3 min to the limit of the subjects' tolerance. Lactate was measured in hyperemic earlobes blood samples at the end of every stage, and at 1, 3, 5, 10, and 15 min during the recovery (17,18). The IAT was determined from a graph of time/blood lactate (17). Since the individuals in the original study (17) exhibited higher VO_2 max values, the test protocol was adapted to obtain the same relative increments in workload. Thus, the first load was $1.33 \text{ W} \cdot \text{kg}^{-1}$ (27.3% VO_2 max) for men, and the increments were $0.67 \text{ W} \cdot \text{kg}^{-1}$ (13.7% VO_2 max). For women, the first load was $0.81 \text{ W} \cdot \text{kg}^{-1}$ (19.1% VO_2 max) and the increments were $0.80 \text{ W} \cdot \text{kg}^{-1}$ (29.1% VO_2 max).

Square Wave Tests

Each subject performed 3 SWT up to 60 min or exhaustion at an intensity that was at AT, IAT, and 4mM of his or her GXT. After unloaded warm-up for 4 min, the load was increased for up to 5 min to adjust the constant workload. The same cardiorespiratory measurements were taken as in the GXT.

The lactate steady-state (LSS) was defined when the blood lactate concentration did not change more than $0.5 \text{ mmol} \cdot \text{L}^{-1}$ from the 15th-min until the 60th-min. The plateau of blood lactate was obtained when variation was lower than 99% lactate analyzer's typical error ($0.1942 \times 2.58 = 0.501 \text{ mmol} \cdot \text{L}^{-1}$, $r^2 = 0.9999$, $N = 24$). The precision of the lactate analyzer was confirmed following the manufacture's guide. Also, measuring the lactate solutions prepared from standards provided by the manufacturer (2.5 to $30 \text{ mmol} \cdot \text{L}^{-1}$) 4 times in order to test error. The reliability of measurements from 0.8 to $9.8 \text{ mmol} \cdot \text{L}^{-1}$ in whole-blood samples was $0.14 \text{ mmol} \cdot \text{L}^{-1}$.

During the tests, the subjects breathed room air through a low-resistance breathing valve (Hans Rudolph®, USA). The valve was connected to an ergospirometer (Aerosport®, USA). Measurements of exhaled air were integrated every 20 sec to determine expired ventilation (V_E), VO_2 , VCO_2 and RER. Before every test, the equipment was calibrated using a three-liter syringe (Hans Rudolph®, USA), and a certified gas mixture ($\text{O}_2 = 17.01\%$; $\text{CO}_2 = 5.00\%$ and balanced with N_2). Intraclass correlation coefficient for the test-retest gas exchange

measurements was 0.91 for V_E , 0.95 for VO_2 and 0.93 for VCO_2 (from 15 to 340 W). The HR was determined using a telemetric cardiometer (Polar®, Finland).

For the SWT, a 20 or 22 gauge catheter was used to sample 1 mL of blood from the back of the hand vein. The hand was placed for 15 min under water at 41 to 43 °C to arterialize blood samples (7). The lactate concentration in earlobe capillary blood did not differ from that in arterialized blood samples ($r^2 = 0.95$). Blood samples were collected at rest and at 5, 15, 25, 35, 45, 55, and 60 min during the SWT to assess PO_2 , PCO_2 and pH using electrodes (AVL® Compact III, USA) and [Lac] using electroenzymatic apparatus (YSI®, 1500 Sport, USA). These blood samples were stored at 5 °C for 2 hrs at most. The acid-base balance was determined using Davenport's diagram (9).

Statistical Analysis

The results are expressed as average $\pm$ SE. The repeated measures two-way (3 x 2) analysis of variance was applied with subsequent HSD-Tukey *post hoc* test. The Green-House alpha inflation correction was used. The regressions were applied to study workload curve adjustments and the relationship between the anaerobic threshold values as determined by different models and other metabolic variables. The significance level was set as $P \leq 0.05$.

RESULTS

Table 1 shows the data from the VO_2 max tests. During the progressive tests, AT occurred at $48.3 \pm 2.7\%$ VO_2 max ([Lac] = 1.29 ± 0.15 mmol·L⁻¹), IAT at $56.1 \pm 3.0\%$ VO_2 max ([Lac] = 1.39 ± 0.14 mmol·L⁻¹) and 4mM at $77.3 \pm 3.9\%$ VO_2 max. The residual sum of squares (RSS) and mean squared error (MSE) were both significantly lower when the threshold was determined by log VO_2 /log [Lac] than when the mono-exponential function was used to adjust the data (RSS AT = 0.01046 ± 0.002974 vs. 4mM = 0.1189 ± 0.04832 and MSE AT = 0.0072 ± 0.00219 vs. 4mM = 0.05269 ± 0.01730). The value for 4mM was significantly greater than for the AT and IAT models. There was no significant difference between AT and IAT in either absolute or relative intensities of effort or in the [Lac]. There were no significant correlations among anaerobic thresholds when expressed in % VO_2 max. However, correlations were found in absolute values between VO_2 max and each one of the three threshold values (VO_2 AT: $r = 0.92$, VO_2 IAT: $r = 0.86$, and VO_2 4mM: $r = 0.93$). The delta mechanical efficiency (VO_2/W) was correlated with VO_2 max (AT: $r = 0.74$, IAT: $r = 0.69$ and 4mM: $r = 0.66$). This efficiency was 12.39 ± 0.99 mL·W⁻¹ in the 4mM model, and 11.83 ± 1.01 , and 12.50 ± 0.83 mL·W⁻¹ for the AT and IAT models, respectively.

Table 1. Variables Observed in the Maximal Effort Test (N = 10).

Variables	Average $\pm$ SE
VO_2 max (L·min ⁻¹)	2.82 ± 0.30
VO_2 max (mL·kg ⁻¹ ·min ⁻¹)	37.6 ± 2.8
HR max (beats·min ⁻¹)	185 ± 2
Power max (watt)	202.9 ± 15.3

Table 2 shows the power output, VO_2 and heart rate in treatments (AT, IAT, and 4mM) and in blocks (GXT and SWT). Significant differences were observed among the treatments. All three variables were greater at the 4mM. No significant interactions were found. All subjects successfully completed the 60-min SWT in the AT and IAT models. However, only 5 subjects completed the 4mM test, which lasted on average 38 ± 7 min. Based on the RER values, there were no significant differences in the percentage of lipid metabolism between the AT ($57.5 \pm 4.8\%$) and IAT ($53.2 \pm 3.3\%$) models, but both values were higher than the 4mM model ($41.7 \pm 4.55\%$).

Table 2. Power (W), Aerobic Power (VO_2), and Heart Rate (HR) Averages at Anaerobic Thresholds Observed in Progressive and Square Wave Tests.

	Variable	AT	IAT	4mM
Progressive	Power (W)	85.9 ± 11.0	95.4 ± 8.2	$141.9 \pm 15.5^*$
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	1.239 ± 0.152	1.414 ± 0.160	$2.269 \pm 0.416^*$
	HR ($\text{beats} \cdot \text{min}^{-1}$)	122 ± 4	127 ± 4	$160 \pm 4^*$
Square wave	Power (W)	85.6 ± 10.8	94.3 ± 7.3	$140.9 \pm 15.6^*$
	VO_2 ($\text{L} \cdot \text{min}^{-1}$)	1.330 ± 0.105	1.455 ± 0.099	$2.049 \pm 0.252^*$
	HR ($\text{beats} \cdot \text{min}^{-1}$)	112 ± 8	122 ± 7	$140 \pm 9^{*†}$

* $P \leq 0.05$ for differences among treatments. $^{\dagger}P \leq 0.05$ for differences between graded and square wave tests. Data show average ($\pm$ SE).

The SWT acid-base balance results are shown in Figures 1 and 2. At the AT model, the [Lac] at 60 min was significantly lower than at 15 min ($-0.62 \pm 0.26 \text{ mmol} \cdot \text{L}^{-1}$), and the criterion for LSS (see methods) was met in 6 subjects. At the AT, a mild compensated acidosis was showed in one subject during the first 5 min. Afterwards the normal acid-base balance was showed to continue during the effort. The rest of the subjects showed no evidence of acidosis. In the IAT model, the difference in lactate concentration at 60 vs. 15 min was $-0.74 \pm 0.19 \text{ mmol} \cdot \text{L}^{-1}$ and the LSS criterion was met in 5 subjects. Metabolic acidosis was absent in 7 subjects, and was compensated in the remaining IAT tests. For the 4mM load, the difference in [Lac] at 60 vs. 15 min ($-1.59 \pm 0.08 \text{ mmol} \cdot \text{L}^{-1}$) was greater than in the other two models, and LSS was not attained in any case. Four of the 5 subjects who were able to complete the 60 min SWT finished in the normal range of acid-base balance while 1 subject exhibited compensated metabolic acidosis.

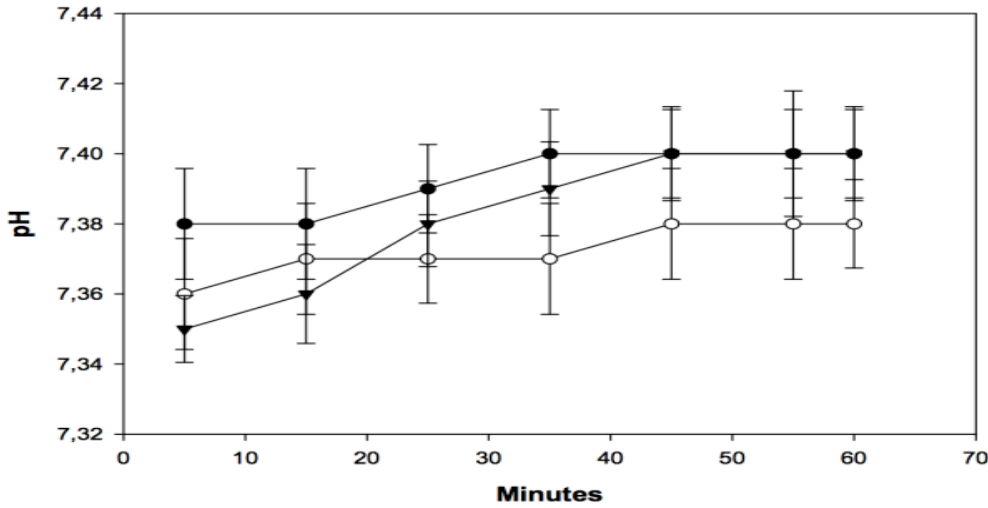


Figure 1. The pH Scale during the Square Wave Tests. Mean $\pm$ SE, AT (●) and IAT (○), 5 min, 15 min, 25 min, 35 min, 45 min, 55 min, and 60 min: n = 10; 4mM (▼), 5 min: n = 10; 15 min: n = 8; 25 min: n = 6; 35 min, 45 min, 55 min, and 60 min: n = 5.

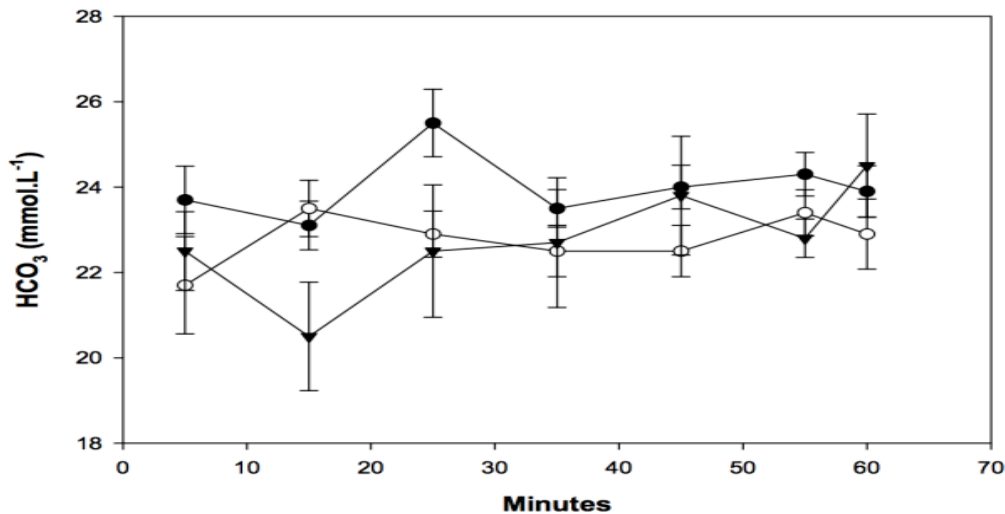


Figure 2. The Standard Bicarbonate (mmol/L) during the Square Wave Tests. Mean $\pm$ SE, AT (●) and IAT (○), 5 min, 15 min, 25 min, 35 min, 45 min, 55 min, and 60 min: n = 10; 4mM (▼), 5 min: n = 10; 15 min: n = 8; 25 min: n = 6; 35 min, 45 min, 55 min, and 60 min: n = 5.

DISCUSSION

Expressed as a percentage of VO_2 max, the anaerobic thresholds were similar to those reported by Beaver, Wasserman, and Whipp (3) for AT (55% VO_2 max), by Stegmann and Kindermann (18) for IAT (55 to 77% VO_2 max) and by Mader and Heck (12) for 4mM (77% VO_2 max). Like Gonzales-Hero (8) and Heck et al. (10), we found a side shift to the right in the workload/[Lac] curve with longer GXT.

Our data support the conclusions of Meyer et al. (13) who observed the $\log \text{VO}_2 / \log [\text{Lac}]$ to be the best mathematical adjustment. However, Bertuzzi et al. (5) estimated a continuous

VO₂ deficit without an anaerobic break point during the GXT. In this study, the glycolytic metabolic rate increased from 5 to 14% because of the VO₂ deficit.

Smith and colleagues (16) reported acidosis after 2 min of an exercise intensity of 4.9% above the AT; whereas, no change in pH was found below the AT. On the other hand, every subject experienced metabolic acidosis for 4mM and their [Lac] moved beyond the range for the steady-state. Even though the criterion of 1 mmol·L⁻¹ [Lac] variation from 10 to 30 min was applied, only 30% of the subjects could experience LSS. Furthermore, Myburgh et al. (14) demonstrated that the disturbance in the acid-base balance during effort at a fixed load varies greatly among individuals, and that it occurs at lactate concentrations above the AT.

Wasserman et al. (19) proposed that V_E could be driven from PCO₂ and pH to compensate the metabolic acidosis. Actually, we found that 87.6% of the variation in V_E at a fixed load can be explained by the variation in aerobic (VO₂) and anaerobic metabolism ([Lac]), and by a change in heart rate. According to Barstow et al. (2), the delta efficiency had a good correlation with type I muscle fiber recruitment and also with VO₂ max. Our findings indicate a moderate correlation between delta efficiency and anaerobic threshold workloads (VO₂AT: $r = 0.74$ and VO₂IAT: $r = 0.69$). Thereby, during SWT, no difference was found between zero and the slopes of linear regression between time and VO₂ after 5 min of effort for the AT load ($\beta = 0.0024 \pm 0.002$). Considering this, the slow phase of VO₂ kinetics presents a correlation with the recruitment of type II muscle fibers (6). Our results did not show the slow VO₂ kinetics and indicated the recruitment of type I motor units only.

CONCLUSION

In spite of our limited knowledge about the mechanisms involved in the anaerobic threshold phenomenon, the results presented here indicate that the AT theoretical model was the most efficient of the three models tested for an accurate determination of load during aerobic steady-state effort. An anaerobic threshold based on a fixed concentration of lactate (4mM) should be rejected.

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