Mechanistic basis for the gas exchange threshold in Thoroughbred horses

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McDonough, Paul, Casey A. Kindig, Howard H. Erickson, and David C. Poole. Mechanistic basis for the gas exchange threshold in Thoroughbred horses. J Appl Physiol 92: 1499–1505, 2002.—The exercising Thoroughbred horse (TB) is capable of exceptional cardiopulmonary performance. However, because the ventilatory equivalent for O_2 (Ve/Vo_2) does not increase above the gas exchange threshold (Tge), hypercapnia and hypoxemia accompany intense exercise in the TB compared with humans, in whom Ve/Vo_2 increases during supra-Tge work, which both removes the CO_2 produced by the bicarbonate buffering of lactic acid and prevents arterial partial pressure of CO_2 (PaCO_2) from rising. We used breath-by-breath techniques to analyze the relationship between CO_2 output (V˙CO_2) and V˙O_2 [V-slope lactate threshold (LT)] estimation] during an incremental test to fatigue (7 to ~15 m/s; 1 m s^{-1} min^{-1}) in six TB. Peak blood lactate was evidenced at 62.6 ± 2.7% Vo_2max; Tge occurred at a significantly higher (P < 0.05) percentage of Vo_2max than the lactate (45.1 ± 5.0%) or pH (47.4 ± 6.6%) but not the bicarbonate (65.3 ± 6.6%) threshold. In addition, PaCO_2 was elevated significantly only at a workload > Tge. Thus, in marked contrast to healthy humans, pronounced V-slope (↑ VCO_2/Vo_2) behavior occurs in TB concomitant with elevated Paco_2 and without evidence of a ventilatory threshold.

IN CONTRAST TO HUMANS, THE VENTILATORY RESPONSE to incremental exercise is a close to linear function of speed and O_2 consumption (Vo_2) in the Thoroughbred horse (TB) such that the ventilatory equivalent for O_2 (Ve/Vo_2) increases during supra-Tge work, which both removes the CO_2 produced by the bicarbonate buffering of lactic acid and prevents arterial partial pressure of CO_2 (PaCO_2) from rising.

Despite the absence of an elevated Ve/Vo_2, TB exhibit an increase in VCO_2/Vo_2 and arterial lactate [i.e., LT] during incremental exercise (20, 22). In human subjects, the increase in VCO_2/Vo_2 [i.e., gas exchange threshold (Tge)] is thought to be a direct consequence of HCO_3^{-} buffering of lactic acid-generated H^{+} (36), leading to an elevated Ve/Vo_2. This elevated ventilatory response clears additional CO_2 (produced by HCO_3^{-} buffering) and thus prevents PaCO_2 from rising. We speculate that, in the absence of elevated Ve/Vo_2 in TB, Tge is linked causally to an elevated blood lactate and PaCO_2. However, the proximity of these events has not been determined to date. In this regard, it is pertinent that breath-by-breath technology, essential to resolving the temporal profiles of these events with high fidelity, has not, to date, been used for this purpose in exercising TB.

Currently, humans are the sole species in which the breath-by-breath gas exchange response to exercise has been related mechanistically to metabolic and humoral events. It is likely that analysis of such relationships in another species will provide a novel and insightful perspective on respiratory control during exercise. The purpose of this investigation was to analyze the relationship between Vo_2, VCO_2, and Ve on a breath-by-breath basis during incremental treadmill exercise in TB. We tested the hypothesis that for Tge to occur during treadmill exercise in the absence of a ventilatory threshold (VT; defined as the Vo_2 at which Ve/Vo_2 begins to rise systematically), an elevated Paco_2 must precede Tge. In addition, it was hypothe-
sized that LT and Tge would not occur at similar time points because PaCO₂ will only start to rise progressively after LT.

METHODS

Animals. Six healthy geldings (all TB; age = 4–10 yr; weight = 470–600 kg) were used in this study. Animals were housed in enclosed dry lots, with a shed and free access to water and salt, and were fed alfalfa, grass hay, and concentrate twice daily. They were dewormed and vaccinated at regular intervals and exercised on a high-speed treadmill (SATO, Uppsala, Sweden) at least twice weekly. All procedures herein were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animal preparation. Before the experimental protocol, each horse had one 7-Fr introducer catheter placed in the right jugular vein and one 18-gauge, 2.0-in. catheter placed either in a previously elevated left carotid artery or the transverse facial artery (20 gauge, 1.5 in., two TB) by using aseptic techniques. A thermistor catheter (Columbus Instruments, Columbus, OH) was advanced through the 7-Fr introducer catheter into the right pulmonary artery, 8 cm past the pulmonary valve, for measurement of core body temperature. The thermistor was calibrated by using a Physitemp thermocouple thermometer (BAT-10, Physitemp, Clifton, NJ). A cannula (1.6 mm ID, 3.2 mm OD) was connected to the arterial catheter to facilitate withdrawal of arterial blood.

Experimental protocol. Each TB completed one maximal run on the level treadmill. After collection of resting cardiorespiratory measurements and blood samples, TB were warmed up at a trot (3 m/s for 800 m). After this warm-up, the speed was increased 1 m/s⁻¹·min⁻¹ to maximal effort. TB were then cooled down (3 m/s) for at least 4 min. Cardiorespiratory measurements were collected continuously throughout exercise and cool-down, whereas blood samples were collected during the last 10 s of each stage and during minutes 2 and 4 of recovery.

Ventilation. Ventilation was measured by using an ultrasonic phase-shift flowmeter (model FR-41eq, Flowmetrics-BRDL, Birmingham, UK) that has been discussed in detail elsewhere (38). Briefly, a light fiberglass mask (<1 kg) is placed on the muzzle of TB. This mask is fitted internally with silicone rubber and foam gaskets to provide an airtight seal. Flow tubes are then placed in the front of the mask, approximately opposite each nostril, so that airflow for each nostril is measured. Flow tubes are fitted with two ultrasonic transducers, which quantify the velocity of airflow at a resonant frequency of 40 kHz. In addition, the effects of temperature and gas composition on zero stability are negated by the dual transducer design (38). Each transducer pair was calibrated before each experiment as per manufacturer’s standards (38). Ve was converted to STPD by using standard equations for determination of VO₂ and VCO₂. Alveolar ventilation (VA) was calculated by using the VA equation: VA = (VCO₂/PaCO₂)k, where k = 0.863 when converting from STPD to STPD (VCO₂).

VO₂ and VCO₂. VO₂ and VCO₂ were calculated as the product of Ve (STPD) and ΔFO₂ = (fO₂ ≈ feO₂) and ΔFCO₂ = (fCO₂ ≈ feCO₂), respectively. ΔFO₂ and ΔFCO₂ were determined from the difference between inspired and expired gas measured via mass spectrometry (Perkin-Elmer, model 1100, Pomona, CA) by sampling from a port located on the Fiberglas mask midway between the nares.

Blood analysis. After anaerobic withdrawal (~5 ml into plastic, heparinized syringes), blood samples were placed immediately on ice. After completion of the experimental protocol (within 1–2 h), arterial blood gases, pH, and plasma lactate were quantified by a blood-gas analyzer (Nova Stat Profile, Waltham, MA). Blood gases and pH were corrected to the TB pulmonary arterial blood temperature (14). The above measurements were performed on each occasion by a single technician to maintain internal consistency. Equipment was calibrated before and after each exercise test according to manufacturer’s standards.

Threshold analyses. Tge was determined by using a simplified version (30) of the V-slope method of Beaver et al. (5). The method identifies the point (i.e., VO₂) where the slope of the VCO₂ identity line departs from linearity with the slope of the VO₂ identity line.

LT was determined by visually selecting the point where a slope change in the BLa⁻ work rate relationship occurred. This was then verified by plotting the linear segments of BLa⁻ against VO₂ and using least-squares regression analysis to choose the point of intersection, which was then recorded as LT. The pH (pH₅) and HCO₃⁻ thresholds (HCO₃⁻) were determined visually as the VO₂ where each variable departed from linearity, and this point was then confirmed by least-squares regression analysis.

Statistical analysis. A one-way analysis of variance was used to determine whether differences existed between selected experimental variables. When significance was revealed, the point of significance was identified by using a Student-Newman-Keuls post hoc test. Multiple linear regression was used for threshold analysis (LT, pH₅, HCO₃⁻). Statistical significance was preselected to correspond to a P value of ≤0.05.
RESULTS

Breath-by-breath values for $\dot{V}O_2$, $\dot{V}E$, frequency of breathing, and tidal volume ($VT$) are shown graphically for a representative TB in Figs. 1 and 2. All TB attained $V_{O2\max}$ as identified and defined by a distinct plateau in the $V_{O2}$-speed relationship (Fig. 1). In addition, no TB exhibited a VT, as $\dot{V}E$ and $V_A$ (Fig. 3B) responses did not demonstrate an upward inflection (compared with humans; Ref. 33) even at the highest treadmill speeds and $V_{O2}$. Furthermore, $\dot{V}E/V_{O2}$ fell (Fig. 3C; comparing equine with human; Ref. 33) progressively throughout exercise concomitant with a widening of the alveolar-arterial $P_{O2}$ difference ($A-aD_{O2}$; Fig. 3C). All TB exhibited distinct metabolic LT and Tge behavior during incremental exercise (Figs. 4A and 5B), with Tge occurring at $62.6 \pm 2.7\%$ of $V_{O2\max}$, which was significantly greater than both LT (45.1 $\pm$ 5.0%) and pH (47.4 $\pm$ 6.6%; Figs. 4A and 6A) but not HCO$_3^-$T (65.3 $\pm$ 6.6%; Figs. 4A and 6B). Each TB demonstrated a pronounced arterial hypoxemia and hypercapnia (Figs. 4B and 7). Tge occurred at a $P_{A_{CO2}}$ significantly greater and arterial partial pressure of $O_2$ ($P_{A_{O2}}$) significantly less than that found at or below LT (Fig. 8).

DISCUSSION

Analysis of breath-by-breath gas exchange, ventilation, and the associated metabolic and blood-gas profiles during maximal incremental exercise in TB produced several novel findings. Specifically, in TB, Tge can occur without a discernible VT. In addition, Tge occurred at a $V_{O2}$ substantially above LT but not significantly different from HCO$_3^-$T or the $V_{O2}$ at which $P_{A_{CO2}}$ began a progressive and sustained increase above baseline values. Thus it appears that Tge in TB occurs via mechanisms that are qualitatively different from those described for healthy humans.

Ventilatory responses during incremental exercise: human and TB. In the human athlete, ventilation increases linearly with respect to $V_{O2}$ to $\sim45$ to $55\%$ of $V_{O2\max}$ (i.e., LT), after which the $\dot{V}E/V_{O2}$ ratio increases systematically (8, 36), which serves to prevent a rise in $P_{A_{CO2}}$ (37). At higher work rates ($\sim75\%$ $V_{O2\max}$), the $\dot{V}E/V_{CO2}$ ratio rises, driving $P_{A_{CO2}}$ downward in an attempt to constrain the incipient acidemia (35). Therefore, in humans, the ventilatory system plays an important role in arterial pH homeostasis during incremental exercise.

In the equine athlete, the ventilatory response to incremental work is markedly different from that in humans. Whereas relative $\dot{V}E$ (i.e., $\dot{V}E$/body weight) at maximal exercise is elevated in the equine (340%; 3-4 vs. 2-3 l·kg⁻¹·min⁻¹ for TB and human, respectively) compared with human athlete, relative $V_{O2\max}$ is much greater (135%; 150-180 vs. 60-80 ml·kg⁻¹·min⁻¹ for TB and human, respectively). Therefore, $\dot{V}E/V_{O2}$...
(33) does not increase (and in fact falls systematically) during incremental exercise in TB. As TB must couple stride and breathing frequency (6), relative VT is small \((\sim 40\% \text{ less than in human athletes per kilogram})\), which, when coupled with voluminously large airways, mandates that the dead space fraction \([\text{dead space volume} / \text{VT}]\) is much greater in TB compared with humans (Fig. 3A) (7, 33). Because \(V_{\text{E}}/V_{\text{O}2}\) is so high, \(V_{\text{A}}\) is relatively low at maximal effort (Fig. 3B). Therefore, because of this relative hypoventilation in TB (33), \(V_{\text{E}}/V_{\text{CO}_2}\) fails to increase above LT, such that \(P_{\text{ACO}_2}\) rises in the TB to 55–60 Torr (1, 4, 7, 13, 23, 33). This \(P_{\text{ACO}_2}\) level is consistent with that predicted for humans should an elevated \(V_{\text{E}}/V_{\text{O}2}\) not occur above LT (37).

**Blood-gas responses during incremental exercise: human and TB.** During incremental exercise in healthy humans, \(P_{\text{ACO}_2}\) does not rise (due to the increased \(V_{\text{E}}/V_{\text{O}2}\) and \(P_{\text{AO}_2}\) stays constant or falls slightly (11). In trained human athletes (Fig. 7), \(P_{\text{AO}_2}\) often falls significantly and the hypoxemia that develops appears to be directly related to the degree of alveolar hyperventilation, \(V_{\text{O}2_{\text{max}}}\) and the \(A-aD_{\text{O}2}\) (11). The reduced hyperventilatory response is thought to be because of a combination of the increased work of breathing as \(V_{\text{O}2_{\text{max}}}\) increases (10), mechanical constraints to flow (18), and a reduced sensitivity of the peripheral chemoreceptors to \(\text{CO}_2\) (15). Increased A-aD_{\text{O}2} has been attributed to an increased \(V_{\text{A}}\)-cardiac output (Q) mis-

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**GAS EXCHANGE IN THE THOROUGHBRED HORSE**

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**Fig. 4.** Arterial lactate \((L_a)\), bicarbonate \((\text{HCO}_3^-)\), and pH \((A)\), and arterial partial pressure of \(\text{O}_2\) \((P_{\text{AO}_2})\), arterial partial pressure of \(\text{CO}_2\) \((P_{\text{ACO}_2})\), and hematocrit \((\text{Hct})\) \((B)\) as a function of treadmill speed in all horses.

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**Fig. 5.** Gas exchange threshold (Tge) for human \((A; \text{Ref. 24})\) and equine \((B)\) subjects. Note broad similarity of response. \(V_{\text{CO}_2}, \text{CO}_2\) output.
match, an alveolar end capillary diffusion limitation, or a combination of both (11).

Compared with humans, TB blood-gas response is markedly different. PaCO₂ rises to 55 to 65 Torr (Figs. 4B and 7) and PaO₂ falls to a greater degree than seen even in elite human athletes (Fig. 7). The rise in PaCO₂ is caused primarily by a relative hypoventilation (4), which is at least partly due to the coupling of stride and respiratory frequency during the gallop. In addition, the peripheral chemoreceptors of TB are comparatively insensitive to CO₂ (21). The hypoxemia (and the rise in A-aD O₂) is thought to be primarily due to a diffusion limitation, according to the relationship of Piiper and Scheid (DO₂/Qt; Ref. 28), where D O₂ is the lung diffusing capacity for O₂ and Qt is the mean slope of the linear portion of the O₂-dissociation curve. Variables on both sides of this equation increase with exercise. However, Qt increases to a greater degree than does Do₂ because of the massive Qt of TB (11), and thus hypoxemia results from the lowered Do₂/Qt ratio. In addition, at least part of the hypoxemia must also be because of the hypercapnia according to the alveolar gas equation approximated as

\[
PaO_2 = PtO_2 - \left(\frac{PaCO_2}{R}\right)
\]

where PtO₂ is the inspired PO₂ and R is the respiratory exchange ratio (see Ref. 10).

Figure 7 demonstrates the above points well. As exercise progresses, hypoxemia and hypercapnia become clearly evident, while VE/VO₂ (and VE/VCO₂) is falling (Fig. 3C). However, during the last few stages of exercise, the degree of hypoxemia and hypercapnia did not worsen (Figs. 4B and 7). Although this may seem strange, VE is increasing (Fig. 1A; due to increases in both frequency of breathing and Vt; Fig. 2), whereas VO₂ is beginning to plateau (Fig. 1B) at these time points. Thus an attenuation of the hypercapnia would be expected. In addition, the plateau in PaCO₂ should cause a similar plateau in PaO₂ (see alveolar gas equa-

![Fig. 6. Arterial pH (A), HCO₃⁻ (B), and plasma lactate (C) responses for trained equine (●, ■, ▲) and human (○, □, △; Refs. 18, 24) subjects. Tge is represented by a dashed line for humans and a solid line for horses. Note how the bicarbonate threshold (HCO₃⁻T) and Tge occur at a greater percentage of maximal VO₂ (VO₂max) than the lactate (LT) and pH (pHₗ) thresholds in the horse. This is contrasted with the human response, where LT and HCO₃⁻T occur at a similar percentage of VO₂max as Tge, but pHₗ occurs after Tge.](https://jap.physiology.org/)

![Fig. 7. PaO₂ (○, ■) and PaCO₂ (●, ▲) in trained equine (●, ▲) and human (○, ■) subjects (18). Note that progressive hypoxemia is in both humans and horses but hypercapnia is only in the horse. Dashed and solid lines, Tge for humans and horses, respectively.](https://jap.physiology.org/)

![Fig. 8. Arterial blood-gas response in the horse. *Significant change (P < 0.05) in both PaCO₂ and PaO₂ from LT.](https://jap.physiology.org/)
tion). This same phenomenon has been noted in trained human subjects as well (18) but is more likely due to respiratory compensation for metabolic acidosis (i.e., rise in VE/VCO₂) and its effect on PAo₂, in these subjects.

Metabolic responses during incremental exercise: human and TB. The inflection points of plasma lactate, pH, and HCO₃⁻ during incremental exercise demonstrate a close temporal association with VT (8, 35) and Tge (5) in humans. Although the nature of these associations has been argued (29), some degree of “threshold-like” behavior occurs in each of the above-mentioned variables at ~45 to 55% of VO₂ max in human subjects (35).

In contrast, the present investigation presents compelling evidence that metabolic and ventilatory events are dissociated in the TB because VT was not discernable in the presence of LT, Tge, pHᵣ, and HCO₃⁻. Whereas LT and pHᵣ occurred at a similar percentage of VO₂ max and PAO₂ began to rise after LT, HCO₃⁻ T occurred at a significantly greater percentage of VO₂ max in the TB. Because PAO₂ rose systematically above LT (Fig. 8), it appears that the TB does not have an isocapnic buffering period but what might instead be called “isocarbic buffering” (i.e., no change in HCO₃⁻ between LT and Tge; Fig. 6B) during incremental exercise. This phenomenon could only occur with the existence of an extraordinary nonbicarbonate muscle and blood buffering capacity (12, 19, 31). Indeed, compared with humans, TB has a much higher hemoglobin concentration (~50%; because of a contractile spleen; compare Refs. 22 and 31) and increased muscle carnosine levels (5- to 10-fold; Ref. 12). These observations compare Refs. 22 and 31) and increased muscle carnitine (31). These findings suggest that sequelae to pulmonary hypertension (i.e., perivascular cuffing, pulmonary edema, and exercise-induced pulmonary hemorrhage), very short pulmonary capillary red blood cell transit time (Ref. 9; due in part to the near doubling of systemic hematocrit; Fig. 4B), and relative hypoventilation (i.e., VE/VCO₂ falls at increased VO₂, in marked contrast to the rise seen in humans) may each contribute importantly to the arterial blood-gas derangement found in intact TB at maximal exercise.

In conclusion, TB exhibit a clearly discernible Tge in the absence of a VT. However, the insensitivity of TB to elevated PAO₂ (26) combined with high cellular (very high carnosine levels; Ref. 12) and intravascular (elevated exercise arterial hemoglobin concentration; Ref. 27) buffering capacities allows a breathing strategy that minimizes respiratory muscle work, while providing acid-base balance matching that of humans. Although this may cause profound arterial hypoxemia and likely reduce VO₂ max, it may be construed as beneficial, as an increase in VE to maintain blood-gas homeostasis would likely divert a substantial portion of Q from the locomotory to the ventilatory musculature (1, 4, 16), thereby causing early fatigue and reduced performance.

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