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 O₂ (VE/VO₂) does not increase above the gas exchange threshold (Tge), hypercapnia and hypoxemia accompany intense exercise in the TB compared with humans, in whom Ve/VCO₂ increases during supra-Tge work, which both removes the CO₂ produced by the HCO₃⁻ buffering of lactic acid and prevents arterial partial pressure of CO₂ (PaCO₂) from rising. We used breath-by-breath techniques to analyze the relationship between CO₂ output (VCO₂) and VO₂ [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 increased to 29.2 ± 1.9 mM/L. However, as neither VE/VO₂ nor Ve/VCO₂ increased, PaCO₂ increased to 56.6 ± 2.3 Torr at peak VO₂ (VO₂max). Despite the presence of a relative hypoventilation (i.e., no increase in VE/VCO₂ or Ve/VCO₂), a distinct Tge was evident at 62.6 ± 2.7% VO₂max. Tge occurred at a significantly higher (P < 0.05) percentage of VO₂max than the lactate (45.1 ± 5.0%) or pH (47.4 ± 6.6%) but not the bicarbonate (65.3 ± 6.6%) threshold. In addition, PaCO₂ was elevated significantly only at a workload > Tge. Thus, in marked contrast to healthy humans, pronounced V-slope (↑VCO₂/VCO₂) behavior occurs in TB concomitant with elevated PaCO₂ and without evidence of a ventilatory threshold. Equine; lactate threshold; ventilatory threshold; exercise-induced pulmonary hemorrhage; hypoxemia

IN CONTRAST TO HUMANS, THE VENTILATORY RESPONSE to incremental exercise is a close to linear function of speed and O₂ consumption (VO₂) in the Thoroughbred horse (TB) such that the ventilatory equivalent for O₂ (VE/VO₂) is necessary to prevent a rise in PaCO₂ during supralactate threshold and subrespiratory compensation threshold work (37), whereas an increase in the ventilatory equivalent for CO₂ (VE/VCO₂) drives PaCO₂ below resting levels during suprarespiratory compensation threshold work (34). Therefore, an increase in PaCO₂ to the level predicted for a linear ventilatory response to incremental exercise (i.e., 50–55 Torr; Ref. 36) is expected and found in TB during incremental exercise (3, 4, 33). In addition, experimental perturbations, such as He/O₂ (79:21%; Ref. 13) and hypoxic (16%; Ref. 25) gas breathing, which are designed to induce a greater ventilatory response, do not prevent hypercapnia during heavy exercise in TB.

Despite the absence of an elevated VE/VCO₂, TB exhibit an increase in VCO₂/N₂O and arterial lactate [i.e., LT] during incremental exercise (20, 22). In human subjects, the increase in VCO₂/VCO₂ [i.e., gas exchange threshold (Tge)] is thought to be a direct consequence of HCO₃⁻ buffering of lactic acid-generated H⁺ (36), leading to an elevated VE/VCO₂. This elevated ventilatory response clears additional CO₂ (produced by HCO₃⁻ buffering) and thus prevents PaCO₂ from rising. We speculate that, in the absence of an elevated VE/VCO₂ in TB, Tge is linked causally to an elevated blood lactate and PaCO₂. 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 VO₂, VCO₂, 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 VO₂ at which VE/VCO₂ begins to rise systematically), an elevated PaCO₂ must precede Tge. In addition, it was hypothe-
sized that LT and Tge would not occur at similar time points because \( \text{Paco}_2 \) 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 for 1 min, after which the speed was increased 1 m/s from 1 to 7 m/s for 1 min. 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). \( \dot{V}_e \) was converted to \( \dot{V}\text{EFD} \) by using standard equations for determination of \( \dot{V}\text{O}_2 \) and \( \dot{V}\text{CO}_2 \). Alveolar ventilation \( \dot{V}_A \) was calculated by using the \( \dot{V}\text{A} \) equation: \( \dot{V}_A = (\dot{V}\text{CO}_2/\text{Paco}_2)k \), where \( k = 0.863 \) when converting from \( \text{STPD} \) to \( \text{STPD} \) \( \dot{V}\text{CO}_2 \).

\( \dot{V}_\text{O}_2 \) and \( \dot{V}_\text{CO}_2 \) were calculated as the product of \( \dot{V}_e \) (\( \text{STPD} \)) and \( \Delta F\text{O}_2 = (f\text{O}_2 - f\text{\textsubscript{E}}\text{O}_2) \) and \( \Delta F\text{CO}_2 = (f\text{CO}_2 - f\text{\textsubscript{E}}\text{CO}_2) \), respectively. \( \Delta F\text{O}_2 \) and \( \Delta F\text{CO}_2 \) 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 with 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., \( \dot{V}_\text{O}_2 \)) where the slope of the \( \dot{V}\text{CO}_2 \) identity line departs from linearity with the slope of the \( \dot{V}_\text{O}_2 \) identity line.

LT was determined by visually selecting the point where a slope change in the BL\textsubscript{a}–work rate relationship occurred. This was then verified by plotting the linear segments of BL\textsubscript{a} against \( \dot{V}_\text{O}_2 \) and using least-squares regression analysis to choose the point of intersection, which was then recorded as LT. The pH (pH\textsubscript{T}) and HCO\textsubscript{3} thresholds (HCO\textsubscript{3}-T) were determined visually as the \( \dot{V}_\text{O}_2 \) 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, pH\textsubscript{T}, HCO\textsubscript{3}-T). Statistical significance was preselected to correspond to a \( P \) value of \( \leq 0.05 \).
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 $\dot{V}O_2\text{max}$ as identified and defined by a distinct plateau in the $\dot{V}O_2$-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 $\dot{V}O_2$. Furthermore, $\dot{V}E/\dot{V}O_2$ fell (Fig. 3C; comparing equine with human; Ref. 33) progressively throughout exercise concomitant with a widening of the alveolar-arterial $P_O_2$ difference ($A-aD_O_2$; 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 $\dot{V}O_2\text{max}$, which was significantly greater than both LT ($45.1 \pm 5.0\%$) and pH$_T$ ($47.4 \pm 6.6\%$; 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 $P_{aCO_2}$ significantly greater and arterial partial pressure of $O_2$ ($P_{aO_2}$) 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 $\dot{V}O_2$ substantially above LT but not significantly different from HCO$_3^-$ or the $\dot{V}O_2$ at which $P_{aCO_2}$ 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 $\dot{V}O_2$ to ~45 to 55% of $\dot{V}O_2\text{max}$ (i.e., LT), after which the $\dot{V}E/\dot{V}O_2$ ratio increases systematically (8, 36), which serves to prevent a rise in $P_{aCO_2}$ (37). At higher work rates (~75% $\dot{V}O_2\text{max}$), the $\dot{V}E/\dot{V}CO_2$ ratio rises, driving $P_{aCO_2}$ 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 (~40%; 3–4 vs. 2–3 l·kg$^{-1}$·min$^{-1}$ for TB and human, respectively) compared with human athlete, relative $\dot{V}O_2\text{max}$ is much greater (~135%; 150–180 vs. 60–80 ml·kg$^{-1}$·min$^{-1}$ for TB and human, respectively). Therefore, $\dot{V}E/\dot{V}O_2$...
equivalents for \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) (\( \dot{V}E/\dot{V}O_2, \dot{V}E/\dot{V}CO_2, V_A/\dot{V}O_2, \) and \( V_A/\dot{V}CO_2 \)) do not increase during progressive work in TB (33), \( \dot{V}CO_2/\dot{V}O_2 \) must increase via a different mechanism than that described above for humans. According to the relation \( \dot{V}CO_2 = V_A \times F_{ACO_2} \), where \( F_{ACO_2} \) is the alveolar \( CO_2 \) fraction, \( \dot{V}CO_2 \) can increase out of proportion to \( \dot{V}O_2 \) in the face of a linear rise in \( \dot{V}E \) (and \( V_A \); Fig. 3) because of an alinear increase in \( F_{ACO_2} \). As an increase in \( F_{ACO_2} \), surely accompanies an increase in \( P_{ACO_2} \), it is logical that the substantial arterial hypercapnia occurring above LT in TB (Figs. 7 and 8) causes Tge.

**Blood-gas responses during incremental exercise: human and TB.** During incremental exercise in healthy humans, \( P_{ACO_2} \) does not rise (due to the increased \( \dot{V}E/\dot{V}O_2 \)) and \( P_{AO_2} \) stays constant or falls slightly (11). In trained human athletes (Fig. 7), \( P_{AO_2} \) often falls significantly and the hypoxemia that develops appears to be directly related to the degree of alveolar hyperventilation, \( \dot{V}O_2 \) max, and the A-aDO2 (11). The reduced hyperventilatory response is thought to be because of a combination of the increased work of breathing as \( \dot{V}O_2 \) max increases (10), mechanical constraints to flow (18), and a reduced sensitivity of the peripheral chemoreceptors to \( CO_2 \) (15). Increased A-aDO2 has been attributed to an increased \( V_A \)-cardiac output (Q) mis-
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 (D O₂/VO₂; Ref. 28), where D O₂ is the lung diffusing capacity for O₂ and VO₂ is the mean slope of the linear portion of the O₂-dissociation curve. Variables on both sides of this equation increase with exercise. However, VO₂ increases to a greater degree than does D O₂ because of the massive Q of TB (11), and thus hypoxemia results from the lowered D O₂/VO₂ ratio. In addition, at least part of the hypoxemia must also be because of the hypercapnia according to the alveolar gas equation approximated as

\[
\text{PaO}_2 = \text{PtO}_2 - \left(\frac{\text{PaCO}_2}{R}\right)
\]

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/VECO₂) 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. 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.

\[
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Fig. 8. Arterial blood-gas response in the horse. *Significant change (P < 0.05) in both PaCO₂ and PaO₂ from LT.
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 \(R \dot{V} / \dot{V} \text{CO}_2\)) and its effect on \(PaO_2\) in these subjects.

**Metabolic responses during incremental exercise: human and TB.** The inflection points of plasma lactate, pH, and \(HCO_3^-\) 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 \(\dot{V}O_2_{max}\) 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 \(HCO_3^-\). Whereas LT and pHr occurred at a similar percentage of \(\dot{V}O_2_{max}\) and \(PaCO_2\), began to rise after LT, \(HCO_3^-\) occurred at a significantly greater percentage of \(\dot{V}O_2_{max}\) in the TB. Because \(PaCO_2\) 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 \(HCO_3^-\) 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 suggest that the TB has evolved a very powerful buffering system as part of a strategy that acts to minimize acid-base disturbances without the requirement of additional ventilation and, therefore, respiratory muscle work during intense exercise. Because TB cannot adequately control acid-base status through the respiratory system during incremental exercise, this adaptation is extremely beneficial and serves to constrain acid-base perturbations in the face of the substantial acid load this animal generates (17).

**Mechanistic explanation for exercise responses in TB.** One potential explanation for the arterial hypoxemia and hypercapnia in TB is that the coupling of stride and breathing frequency results in relatively small \(V_t\) and thereby limits \(Va\). However, the elite Standardbred (a breed genetically related to TB) can uncouple stride and breathing frequency when trotting at very high speeds. This uncoupling results in a substantially greater \(V_t\) (and reduced \(Vd/Vt\)) compared with TB (2) but does not constrain the magnitude of the blood-gas perturbations common to both of these equid breeds. These data suggest that locomotory-ventilatory coupling cannot singularly be responsible for the hypoxemia and hypercapnia that attend heavy exercise in TB.

Pertinent to this issue, Wagner and colleagues (31) splenectomized TB and, in concert with a reduced hematocrit and \(\dot{V}O_2_{max}\) (both ~24% lower), found that this procedure completely abolished both arterial hypoxemia and hypercapnia at maximal exercise. In addition, peak pulmonary arterial pressure (~45%; Ref. 31) and right ventricular (~20%) and atrial (~85%) pressures (25) were substantially reduced, whereas \(V_e/Vo_2\) was ~65% higher in splenectomized horses (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., \(V_e/Vo_2\) falls at increased \(Vo_2\), 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 discernable Tge in the absence of a VT. However, the insensitivity of TB to elevated \(PaCO_2\) (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 \(\dot{V}O_2_{max}\), it may be construed as beneficial, as an increase in \(V_e\) 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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