Mechanistic basis for the gas exchange threshold in Thoroughbred horses

PAUL MCDONOUGH, CASEY A. KINDIG, HOWARD H. ERICKSON, AND DAVID C. POOLE
Departments of Anatomy, Physiology, and Kinesiology, Kansas State University, Manhattan, Kansas 66506-5802

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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) drives PACO2 below resting levels during suprerespiratory compensation threshold work (37), whereas an increase in the ventilatory equivalent for CO2 (VE/VCO2) drives PACO2 below resting levels during suprerespiratory 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/VO2, TB exhibit an increase in VCO2/VO2 and arterial lactate [i.e., LT] during incremental exercise (20, 22). In human subjects, the increase in VCO2/VO2 [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/VO2. This elevated ventilatory response clears additional CO2 (produced by HCO3\(^{-}\) buffering) and thus prevents PACO2 from rising. We speculate that, in the absence of elevated VE/VO2 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/VO2 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 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 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 to 7 m/s for 1 min, after which the speed was increased 1 m/s \( \cdot \) 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). \( \dot{V}_{e} \) was converted to \( \dot{V}_{T} \) by using standard equations for determination of \( \dot{V}_{O2} \) and \( \dot{V}_{CO2} \). Alveolar ventilation (\( \dot{V}_{A} \)) was calculated by using the \( \dot{V}_{A} \) equation: \( \dot{V}_{A} = (\dot{V}_{CO2}/\text{PaCO}_2)k \), where \( k \) is 0.863 when converting from STPD (\( \dot{V}_{A} \)) to STPD (\( \dot{V}_{CO2} \)).

\( \dot{V}_{O2} \) and \( \dot{V}_{CO2} \) were calculated as the product of \( \dot{V}_{e} \) (STPD) and \( \Delta F_{O2} = (f_{O2} - f_{eO2}) \) and \( \Delta F_{CO2} = (f_{CO2} - f_{eCO2}) \), respectively. \( \Delta F_{O2} \) and \( \Delta F_{CO2} \) 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., \( V_{O2} \)) where the slope of the \( V_{CO2} \) identity line departs from linearity with the slope of the \( V_{O2} \) 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 \( V_{O2} \) and using least-squares regression analysis to choose the point of intersection, which was then recorded as LT. The pH (pH_T) and HCO\(_3\)- thresholds (HCO\(_3\)-T) were determined visually as the point 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_T, HCO\(_3\)-T). 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, $V_E/V_O2$ fell (Fig. 3C; comparing equine with human; Ref. 33) progressively throughout exercise concomitant with a widening of the alveolar-arterial $P_O2$ difference (A-aDO2; Fig. 3C). All TB exhibited distinct metabolic LT and Tge behavior during incremental exercise (Figs. 4A and 5B), with Tge occurring at 62.6 ± 2.7% of $V_O2_{max}$, which was significantly greater than both LT (45.1 ± 5.0%) and pHf (47.4 ± 6.6%; Figs. 4A and 6A) but not HCO3-T (65.3 ± 6.6%; Figs. 4A and 6B). Each TB demonstrated a pronounced arterial hypoxemia and hypercapnia (Figs. 4B and 7). Tge occurred at a $P_{ACO2}$ significantly greater and arterial partial pressure of $O_2$ ($P_{AO2}$) 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 HCO3-T or the $V_O2$ at which $P_{ACO2}$ 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 ~45 to 55% of $V_O2_{max}$ (i.e., LT), after which the $V_E/V_O2$ ratio increases systematically (8, 36), which serves to prevent a rise in $P_{ACO2}$ (37). At higher work rates (~75% $V_O2_{max}$), the $V_E/VCO2$ ratio rises, driving $P_{ACO2}$ 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 $V_E$ (i.e., $V_E$/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 $V_O2_{max}$ is much greater (~135%; 150–180 vs. 60–80 ml·kg$^{-1}$·min$^{-1}$ for TB and human, respectively). Therefore, $V_E/V_O2$...
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 (40% less than in human athletes per kilogram), which, when coupled with voluminously large airways, mandates that the dead space fraction [dead space volume (VD)/VT] is much greater in TB compared with humans (7, 33). Because VD/VT is so high, VA is relatively low at maximal effort (Fig. 3B). Therefore, because of this relative hypoventilation in TB (33), VE/VO2 falls and VE/VCO2 fails to increase above LT, which causes Tge.

### Blood-gas responses during incremental exercise: human and TB

During incremental exercise in healthy humans, Paco2 does not rise (due to the increased VE/VO2) and PaO2 stays constant or falls slightly (11). In trained human athletes (Fig. 7), PaO2 often falls significantly and the hypoxemia that develops appears to be directly related to the degree of alveolar hyperventilation, VO2 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 VO2 max increases (10), mechanical constraints to flow (18), and a reduced sensitivity of the peripheral chemoreceptors to CO2 (15). Increased A-aDO2 has been attributed to an increased VA-cardiac output (Q) mis-

(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 (~40% less than in human athletes per kilogram), which, when coupled with voluminously large airways, mandates that the dead space fraction [dead space volume (VD)/VT] is much greater in TB compared with humans (Fig. 3A) (7, 33). Because VD/VT is so high, VA is relatively low at maximal effort (Fig. 3B). Therefore, because of this relative hypoventilation in TB (33), VE/VO2 falls and VE/VCO2 fails to increase above LT, such that Paco2 rises in the TB to 55–60 Torr (1, 4, 7, 13, 23, 33). This Paco2 level is consistent with that predicted for humans should an elevated VE/VO2 not occur above LT (37).

### Gas-exchange responses during incremental exercise: human and TB

In humans, during incremental exercise there is a distinct threshold of VCO2/VO2 (i.e., Tge) occurring at ~45 to 55% of VO2 max (36). Tge has been closely associated with LT (8, 36) and is thought to be the result of CO2 evolved through HCO3− buffering of H+ produced from the dissociation of lactic acid. It is this HCO3−-evolved CO2 that serves to drive VE/VO2 systematically upward.

In TB, a rise in VCO2/VO2 has been noted (22) during incremental work; however, Tge has never before been described with the same breath-by-breath techniques as those used in human subjects. Because ventilatory equivalents for O2 and CO2 (VE/VO2, VE/VCO2, VA/VO2, and VA/VCO2) do not increase during progressive work in TB (33), VCO2/VO2 must increase via a different mechanism than that described above for humans. According to the relation VCO2 = VA × FACO2, where FACO2 is the alveolar CO2 fraction, VCO2 can increase out of proportion to VO2 in the face of a linear rise in VE (and VA; Fig. 3) because of an alinear increase in FACO2.

As an increase in FACO2 surely accompanies an increase in PaCO2, it is logical that the substantial arterial hypercapnia occurring above LT in TB (Figs. 7 and 8) causes Tge.

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Fig. 4. Arterial lactate (La−), bicarbonate (HCO3−), and pH (A), and arterial partial pressure of O2 (PaO2), arterial partial pressure of CO2 (Paco2), and hematocrit (Hct) (B) as a function of treadmill speed in all horses.

Fig. 5. Gas exchange threshold (Tge) for human (A; Ref. 24) and equine (B) subjects. Note broad similarity of response. VCO2, CO2 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 DO₂ 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

\[ \text{PaO}_2 = \text{P}_{\text{I}O_2} - \left( \frac{\text{PaCO}_2}{R} \right) \]

where P_{\text{I}O_2} is the inspired O₂ 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 Vr; 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 equation).
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 \( \text{VE}/\text{VCO}_2 \)) and its effect on \( \text{PaO}_2 \) in these subjects.

**Metabolic responses during incremental exercise: human and TB.** The inflection points of plasma lactate, pH, and \( \text{HCO}_3^- \) 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 \( \sim 45 \) to \( 55\% \) of \( \text{V} \text{O}_2_{\text{max}} \) 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, pH_T, and HCO_T. Whereas LT and pH_T occurred at a similar percentage of \( \text{V} \text{O}_2_{\text{max}} \) and \( \text{PaCO}_2 \), began to rise after LT, HCO_T occurred at a significantly greater percentage of \( \text{V} \text{O}_2_{\text{max}} \) in the TB. Because \( \text{PaCO}_2 \) 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 (\( \sim 50\% \); 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 VT 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 VT (and reduced \( \text{V} \text{D}/\text{V} \text{T} \)) 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-respiratory 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 \( \text{V} \text{O}_2_{\text{max}} \) (both \( \sim 24\% \) lower), found that this procedure completely abolished both arterial hypoxemia and hypercapnia at maximal exercise. In addition, peak pulmonary arterial pressure (\( \sim 45\% \); Ref. 31) and right ventricular (\( \sim 20\% \)) and atrial (\( \sim 85\% \)) pressures (25) were substantially reduced, whereas \( \text{VE}/\text{V} \text{O}_2 \) was \( \sim 65\% \) 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., \( \text{VE}/\text{V} \text{O}_2 \) falls at increased \( \text{V} \text{O}_2 \)) 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 discernible Tge in the absence of a VT. However, the insensitivity of TB to elevated \( \text{PaCO}_2 \) (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 \( \text{V} \text{O}_2_{\text{max}} \), it may be construed as beneficial, as an increase in \( \text{VE} \) 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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