

Daily variation in plasma electrolyte and acid–base status in fasted horses over a 25 h period of rest

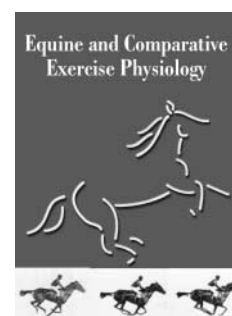
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Abstract

Measurement and interpretation of acid–base status are important in clinical practice and among racing jurisdictions to determine if horses have been administered alkalinizing substances for the purpose of enhancing performance. The present study used the physicochemical approach to characterize the daily variation in plasma electrolytes and acid–base state that occurs in horses in the absence of feeding and exercise. Jugular venous blood was sampled every 1–2 h from two groups ($n = 4$ and $n = 5$) of Standardbred horses over a 25 h period where food and exercise were withheld. One group of horses was studied in October and one in December. The time course and magnitude of circadian responses differed between the two groups, suggesting that subtle differences in environment may manifest in acid–base status. Significant daily variation occurred in plasma weak acid concentration ($[A_{\text{tot}}]$) and strong ion difference ($[SID]$), $[Cl^-]$, $[K^+]$, $[Na^+]$ and $[lactate^-]$, which contributed to significant changes in $[H^+]$ and TCO_2 . The night-time period was associated with a mild acidosis, marked by increases in plasma $[H^+]$ and decreases in TCO_2 , compared with the morning hours. The night-time acidosis resulted from an increased plasma $[A_{\text{tot}}]$ due to an increased plasma protein concentration ($[PP]$), and a decreased $[SID]$ due to increases in $[Cl^-]$ and decreases in $[Na^+]$. An increased plasma $[K^+]$ during the night-time had a mild alkalotic effect. There were no differences in pCO_2 . It was concluded that many equine plasma electrolyte and acid–base parameters exhibit fluctuations in the absence of feeding and exercise, and it is likely that some of these changes are due to daily variation.

Keywords: equine; H^+ ; TCO_2 ; diurnal; circadian

Introduction

Many animals and plants are influenced by diurnal and circadian variations and, in animals, many blood parameters fluctuate with ~ 24 h periodicity. Circadian rhythm refers to a cyclical pattern of biological variation in response to light–dark cycles over a 24 h period, while daily variation refers to biological responses to daylight (diurnal) and night (nocturnal)^{1,2}. Horses have been shown to exhibit daily variations in heart and respiratory rates, blood pressure and hormones, plasma protein concentration ($[PP]$), haematocrit, electrolytes and serum enzymes^{1–5}. For example, $[PP]$ has been shown to increase for 4 h during the night-time period⁶, while plasma $[K^+]$ also

exhibits distinct increases at night^{1,5,6}. In contrast, reports of daily variation in plasma $[Na^+]$ and $[Cl^-]$ are inconsistent, with some studies showing no change⁶ while others have reported changes in $[Na^+]$ but not in $[Cl^-]$ ¹ or vice versa⁵.

Because there are daily variations in plasma electrolyte and protein concentrations, it may be expected that blood acid–base parameters will also exhibit daily variation and should thus be taken into consideration when assessing equine acid–base status. Interpretation of the effects of circadian changes in plasma protein, electrolyte and CO_2 on acid–base balance is facilitated by the use of a comprehensive physicochemical approach that recognizes independent and dependent

determinants of acid-base state⁷. According to the physicochemical approach, the independent variables that determine acid-base status are the partial pressure of carbon dioxide ($p\text{CO}_2$), the concentrations of strong ions in solution represented by the strong ion difference ($[\text{SID}]$) and the concentrations of weak ions (primarily plasma albumin) in solution, defined as the total weak acid concentration ($[\text{A}_{\text{tot}}]$). The dependent variables $[\text{H}^+]$, bicarbonate ($[\text{HCO}_3^-]$) and total carbon dioxide (TCO_2) change only when one or more of the independent variables are altered. It is hypothesized that daily variation in plasma electrolyte, protein and CO_2 concentrations could have effects on acid-base status.

To date, there appear to be no studies that have specifically examined the daily variation of all the plasma variables that contribute to acid-base state in horses. Measurement and interpretation of acid-base status is particularly important among racing jurisdictions to determine if horses have been administered alkalizing substances for the purpose of enhancing performance. Previous studies showing daily variation in plasma protein and electrolytes were of limited time course and confounded by feeding or exercise. Studies designed to examine circadian variation must control for confounding variables such as food, activity and environment, otherwise a true underlying circadian rhythm may not be identified or a false one reported. Therefore, the purpose of the present study was to detail the time course and magnitude of daily variation in the primary plasma constituents that determine acid-base state in horses over a 25 h period where food and exercise are withheld. It was hypothesized that daily variation in strong and weak ions occur, and that these contribute to daily variation in plasma acid-base state independent of feeding and exercise.

Materials and methods

Animals

Two groups of five healthy Standardbred horses (eight mares, two geldings; body weight: 441–536 kg, age: 5–12 years) from the University of Guelph research herd were used. Horses underwent a 21-day acclimation period prior to sampling, during which they were housed in individual box stalls overnight, with 7–8 h of paddock (3 acres, minimal forage) turnout during the day. The first group of five horses was studied in October (Group 1; temperature range: 3–12°C), and the other 7 weeks later in December (Group 2; temperature range: –7 to 1°C). One mare in the first group of horses was unwell on the sampling day, and thus this horse was removed from the study so that data for Group 1 are for four horses. Horses were exercised on a mechanical walker and by free lunging 5–6 days per week during the 21-day acclimation period. The duration and intensity of

exercise were increased over this period such that, during the final week, the horses walked for 30 min, trotted for 20 min and cantered for 5 min. The purpose of the moderate exercise conditioning was so that body mass of the horses be maintained constant while consuming a typical Standardbred racehorse diet. Body mass of the horses was measured bi-weekly and there were no significant changes in the horses' body mass during the trial. The horses were not exercised during the 2 days immediately prior to the sampling days in order to avoid post-exercise recovery effects, but did receive their regular turnout. The animal care and procedures used were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

Diet

The diet provided in the present study was intended to mimic the diet of a typical North American racehorse, and consisted of grass hay and a pelleted grain concentrate fortified with minerals and vitamins (Purina Request: 14% protein, 6% fat and 10% fibre). The same batches of hay and grain were fed during both trials. A detailed nutritional analysis of the diet has been published in a companion paper⁸. The horses were fed daily at 8 am and 7 pm. The proportion of concentrate to feed was increased gradually over the initial 14 days of the acclimation period, such that the grain:hay ratio was 50:50 for the third week. During the third week of acclimation and the sampling period, the horses were fed 2.5 kg of hay and 2.5 kg of grain simultaneously twice a day. Although horses were turned out to a 3-acre paddock daily, there was very minimal forage available. Water was provided *ad libitum*. Feed dietary cation-anion difference ($\text{DCAD} = \text{Na}^+ + \text{K}^+ - \text{Cl}^-$) was 274 meq kg^{-1} dry matter.

Sampling

The horses were fed as per normal at 7 pm on the evening prior to the experiment. On both sampling days beginning at 7 am, EMLA cream (2.5% lidocaine and 2.5% prilocaine; Astra Pharma, Mississauga, ON, Canada) was applied 25–30 min before insertion of catheters to desensitize the skin. The initial blood sample was taken by venipuncture, following which the hair coat over the jugular vein, 10–20 cm below the mandible, was clipped short to the skin on both sides of the neck. Each jugular vein catheterization site was aseptically prepared for insertion of catheters. Local anaesthetic (2% xylocaine; Astra Pharma) was injected subcutaneously to complete the anaesthesia. Catheters (14-gauge, 5.25 inch; Angiocath, Becton-Dickinson, Mississauga, ON, Canada) were inserted antegrade into the left and right jugular veins, secured with tape and stitched to the skin. Four-way

stopcocks with 50 cm extensions were attached to the catheters for ease of indicator infusion and blood sampling. The patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2000 IU l⁻¹ NaCl).

On the experimental day, blood sampling by venipuncture commenced at 7 am, and the horses were sampled every 1–2 h after by jugular venous catheters for a 25 h period. Food was withheld and horses were kept in their stalls throughout the sampling period. Water was provided *ad libitum*; while water intake was not measured, horses were observed to drink little to no water in the absence of feed. These research horses were accustomed to the sampling procedure and no visible excitement occurred. Barn lighting was maintained on the normal 12 h light and 12 h dark cycle.

Sample analyses

The volume of blood sampled was ~8 ml. Blood samples were immediately transferred from the syringe into 5 ml heparinized vacutainers (Lithium-heparin, Becton-Dickinson) and immediately analysed for plasma pH, the partial pressures of carbon dioxide (*p*CO₂) and oxygen (*p*O₂) and the plasma concentrations of Na⁺, Cl⁻, K⁺, Ca²⁺, lactate⁻ using a Nova Stat Profile 9⁺ (NOVA Biomedical, Waltham, MA). [HCO₃⁻] and total carbon dioxide (TCO₂) concentration were calculated using the Henderson-Hasselbach equation by the Nova Stat Profile 9⁺ (coefficient of variation [CV] 0.21–0.61%).

Heparinized blood was transferred into two 1.5 ml Eppendorf centrifuge tubes and centrifuged for 5 min at 15 000 × *g* to separate the plasma. Plasma protein concentration ([PP]) was determined (CV 0.83%) by refractometry (Atago clinical refractometer model SPR-T2; Atago, Tokyo, Japan).

Calculations

Plasma [H⁺] was calculated from measured pH, such that:

$$H^+ = 10^{-pH}$$

Plasma strong ion difference ([SID]) was calculated as the sum of the plasma concentrations of the strong cations minus the strong anions (Stewart, 1983⁴), such that:

$$[SID] = [Na^+] + [K^+] - [Cl^-] - [lactate^-]$$

In practice, the concentrations of the divalent cations and anions (Ca²⁺, Mg²⁺, PO₄²⁻ and SO₄²⁻) are small and the sum of their charges close to zero and thus can be ignored⁹.

Plasma [A_{tot}] was calculated by multiplying the [PP] (g dl⁻¹) by 2.24¹⁰.

Calculations of dependent acid-base parameters ([H⁺], TCO₂) were made using AcidBasics II software (©2003, PD Watson), using the equation:

$$\begin{aligned} [H^+] + (K_A + [SID]) [H^+]^3 + \{K_A([SID] - [A_{tot}]) \\ - (K_C^* pCO_2 + K_W)\} [H^+]^2 - \{K_A(K_C^* pCO_2 + K_W) \\ + (K_3)^* K_C^* pCO_2\} [H^+] - K_A K_3 K_C^* pCO_2 = 0 \end{aligned}$$

where *K_w*, *K_A*, *K₃* and *K_C* are the equilibrium constants for dissociations of water, weak acids, carbonic acid and bicarbonate, respectively:

$$K_W = 4.4 \times 10^{-14} (\text{eq l}^{-1})$$

$$K_A = 2.22 \times 10^{-7} (\text{eq l}^{-1})$$

$$K_3 = 5.76 \times 10^{-11} (\text{eq l}^{-1})$$

$$K_C = 2.45 \times 10^{-11} (\text{eq l}^{-1})^2 / \text{mm Hg}$$

Statistics

Data are presented as mean ± standard error. Changes over time within a group were assessed by one-way repeated measures ANOVA. When a significant F-ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when *P* ≤ 0.05 at a power ≥ 0.8.

Results

An initial inspection of the data showed that the time course of responses differed between groups for many of the parameters measured. Differences between groups were confirmed by two-way repeated measures ANOVA (group and time). Therefore results for each group were analysed and plotted separately.

Diurnal variation of plasma strong ions is shown in Fig. 1. Plasma [Na⁺] (Fig. 1a) reached a minimum at 12 h (137.7 ± 0.54 meq l⁻¹) and 19 h (137.9 ± 0.40 meq l⁻¹), and reached a maximum at 1 h (140.3 ± 0.43 meq l⁻¹), and 4–5 h (139.6 ± 0.64 meq l⁻¹) for Groups 1 and 2, respectively. Plasma [K⁺] (Fig. 1b) reached a minimum at 5 h (3.3 ± 0.17 meq l⁻¹) and 2–3 h (3.4 ± 0.11 meq l⁻¹), and reached a maximum at 16 h (4.1 ± 0.00 meq l⁻¹ NS) and 12–14 h (4.0 ± 0.07 meq l⁻¹) for Groups 1 and 2, respectively. Plasma [Cl⁻] (Fig. 1c) reached a minimum at 4 h (98.9 ± 1.09 meq l⁻¹) and 1 h (98.3 ± 0.86 meq l⁻¹), and reached a maximum at 16 h (104.0 ± 0.00 meq l⁻¹)- and 17 h (102.0 ± 0.71 meq l⁻¹) for Groups 1 and 2, respectively. Plasma [lactate⁻] (Fig. 1d) reached a minimum at 5 h (0.3 ± 0.04 meq l⁻¹) and 19 h

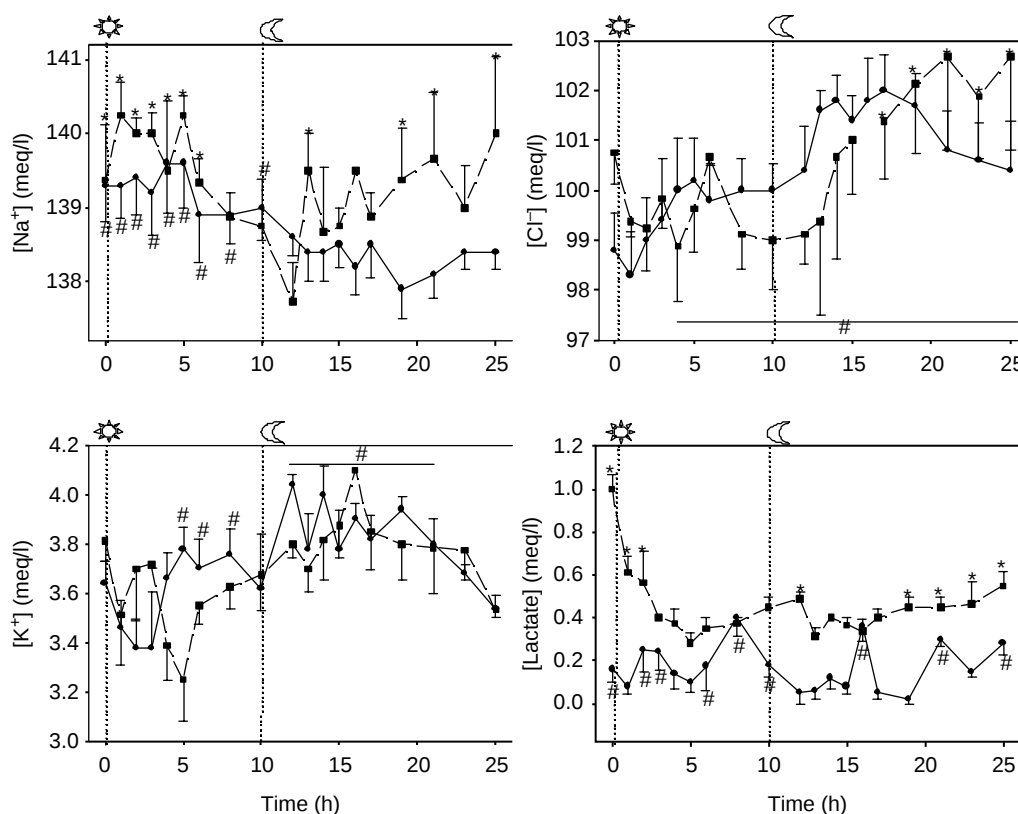


FIG. 1 The time course of the plasma strong ion concentrations (a) Na^+ , (b) Cl^- , (c) K^+ and (d) lactate $^-$. Feed was withheld for the 25 h sampling period. ■ Group 1 horses. ● Group 2 horses; ☆ denotes sunrise (~6:50 am Group 1, ~7:48 am Group 2). ☾ denotes sunset (~5:13 pm Group 1 horses, ~4:45 pm Group 2 horses). Values are mean $\pm$ SE for four horses (Group 1) or five horses (Group 2); *, #: significantly greater than the minimum value (a: 12, 19 h), (b: 4, 1 h), (c: 5, 2–3 h), (d: 5, 19 h) for Groups 1 and 2, respectively

(0.0 meq l^{-1}), and reached a maximum at 0 h ($1.0 \pm 0.07 \text{ meq l}^{-1}$) and 8 h ($0.4 \pm 0.03 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively.

The changes in the independent acid-base variables are shown in Fig. 2. Plasma [SID] (Fig. 2a) reached a minimum at 16 h (39.2 ± 0.00 and $39.9 \pm 0.55 \text{ meq l}^{-1}$) for both Groups 1 and 2, and reached a maximum at 2 h ($43.9 \pm 0.70 \text{ meq l}^{-1}$) and 1 h ($44.4 \pm 0.66 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively. pCO_2 (Fig. 2b) reached a minimum at 25 h ($31.2 \pm 0.35 \text{ mm Hg}$) and 15 h ($33.0 \pm 2.14 \text{ mm Hg}$) and reached a maximum at 6 h ($38.1 \pm 2.34 \text{ mm Hg}$) and 16 h ($39.5 \pm 1.88 \text{ mm Hg}$, NS) for Groups 1 and 2, respectively. Plasma $[A_{\text{tot}}]$ (Fig. 2c) reached a minimum at 1 h (12.7 ± 0.42 and $13.4 \pm 0.36 \text{ meq l}^{-1}$) for both Groups 1 and 2, and reached a maximum at 21 h ($15.2 \pm 0.47 \text{ meq l}^{-1}$) and 19 h ($15.1 \pm 0.32 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively.

The changes in the dependent acid-base variables are shown in Fig. 3. Plasma $[H^+]$ (Fig. 3a) reached a minimum at 6 h ($34.35 \pm 0.82 \text{ neq l}^{-1}$) and 0 h ($37.09 \pm 0.60 \text{ neq l}^{-1}$), and reached a maximum at 21 h ($37.77 \pm 0.36 \text{ neq l}^{-1}$) and 17 h ($46.30 \pm 0.55 \text{ neq l}^{-1}$) for Groups 1 and 2, respectively. Plasma TCO_2 (Fig. 3b) reached a minimum at 15 h ($30.8 \pm 0.99 \text{ meq l}^{-1}$) and 16 h ($25.0 \pm 0.60 \text{ meq l}^{-1}$), and reached a maximum at

4 h ($36.6 \pm 0.48 \text{ meq l}^{-1}$) and 0 h ($35.3 \pm 0.64 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively.

Plasma $[Ca^{2+}]$ (Fig. 4a) reached a minimum at 16–17 h ($1.37 \pm 0.01 \text{ meq l}^{-1}$) and 25 h ($1.38 \pm 0.01 \text{ meq l}^{-1}$), and reached a maximum at 0 h ($1.51 \pm 0.05 \text{ meq l}^{-1}$) and 14 h ($1.47 \pm 0.02 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively.

Plasma [glucose] (Fig. 4b) reached a minimum at 25 h ($4.70 \pm 0.20 \text{ meq l}^{-1}$) and 21 h ($4.74 \pm 0.13 \text{ meq l}^{-1}$), and reached a maximum at 5 h ($5.45 \pm 0.17 \text{ meq l}^{-1}$) and 2 h ($5.70 \pm 0.42 \text{ meq l}^{-1}$) for Groups 1 and 2, respectively.

Plasma pO_2 (Fig. 4c) reached a minimum at 15 h ($43.0 \pm 1.26 \text{ mm Hg}$) and 8 h ($39.5 \pm 1.60 \text{ mm Hg}$), and reached a maximum at 0 h ($50.0 \pm 0.78 \text{ mm Hg}$) and 19 h ($57.5 \pm 1.09 \text{ mm Hg}$) for Groups 1 and 2, respectively.

Discussion

The present study measured and detailed the time course of daily variation in key independent and dependent acid-base variables in horses over a 25 h period. The circadian responses differed in some respects between the two groups of horses studied,

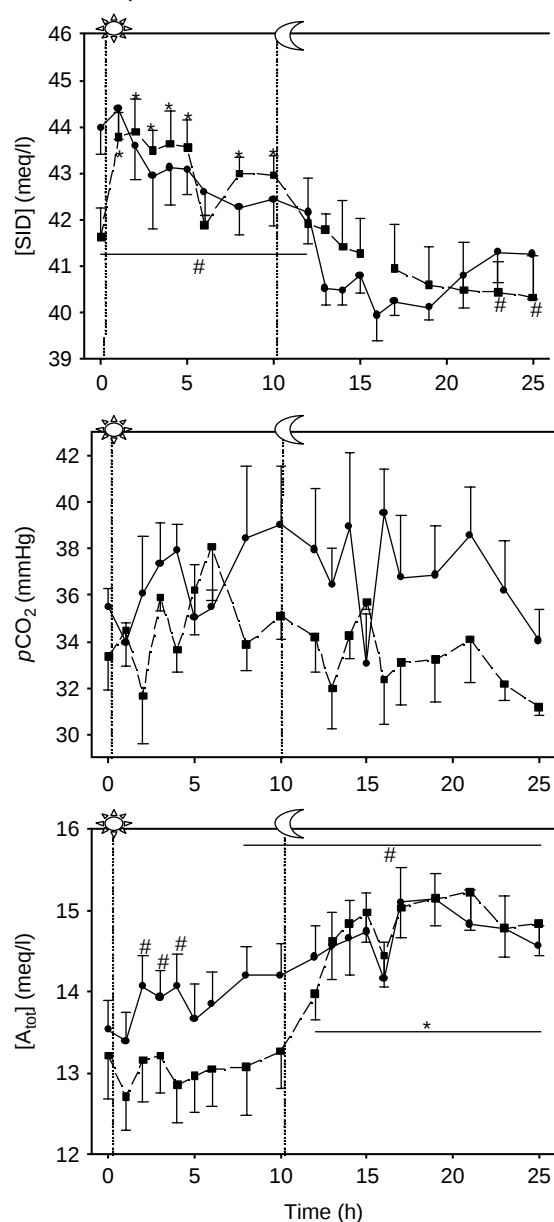


FIG. 2 The time course of the independent plasma acid-base variables (a) [SID], (b) $p\text{CO}_2$ and (c) $[A_{\text{tot}}]$. Feed was withheld for the 25 h sampling period. ■ Group 1 horses. ● Group 2 horses; ☆ denotes sunrise (~6:50 am Group 1, ~7:48 am Group 2). ☾ denotes sunset (~5:13 pm Group 1 horses, ~4:45 pm Group 2 horses). Values are mean $\pm$ SE for four horses (Group 1) or five horses (Group 2); *, #: significantly greater than the minimum value (a: 16, 16 h), (b: 25, 15 h), (c: 1, 1 h) for Groups 1 and 2, respectively

suggesting that subtle differences in environment and season may manifest in acid-base status. The night-time period was associated with a mild acidosis, marked by increases in plasma $[\text{H}^+]$ and decreases in TCO_2 , compared with the morning hours. The night-time acidosis resulted from an increased $[A_{\text{tot}}]$ (due to increased [PP]) and a decreased [SID] (due to increases in $[\text{Cl}^-]$ and decreases in $[\text{Na}^+]$), as there were no differences in $p\text{CO}_2$ during the sampling period.

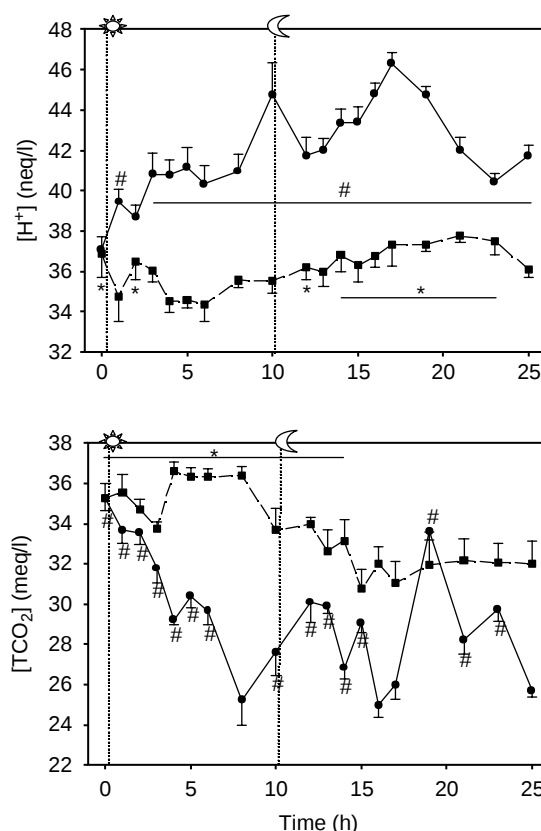


FIG. 3 The time course of the dependent plasma acid-base variables (a) $[\text{H}^+]$ and (b) TCO_2 . Feed was withheld for the 25 h sampling period. ■ Group 1 horses. ● Group 2 horses; ☆ denotes sunrise (~6:50 am Group 1, ~7:48 am Group 2). ☾ denotes sunset (~5:13 pm Group 1 horses, ~4:45 pm Group 2 horses). Values are mean $\pm$ SE for four horses (Group 1) or five horses (Group 2); *, #: significantly greater than the minimum value (a: 6, 0 h), (b: 15, 16 h) for Groups 1 and 2, respectively

Independent variables

Plasma $[\text{K}^+]$ showed a tendency to be increased during the night-time; however, this was only significant for Group 2. This nocturnal increase in $[\text{K}^+]$ has been demonstrated previously^{1,6,11} and is thought to be associated with a decreased activity of cellular Na K pumps during night-time resting periods¹². A major co-founder of the previous studies of plasma $[\text{K}^+]$ variation in horses is that all animals were fed at some point during the sampling period as plasma $[\text{K}^+]$ is affected by feeding^{3,8,13}. However, in a companion study to the present one, blood samples were taken from the same two groups of horses on days when the horses were fed normally (see Waller *et al.*⁸) and a decrease in plasma $[\text{K}^+]$ was still demonstrated during the daytime, demonstrating that plasma $[\text{K}^+]$ does exhibit diurnal variation.

Plasma $[\text{Na}^+]$ for Group 1 was highly variable amongst horses, and $[\text{Na}^+]$ for Group 2 was significantly decreased during the night-time period. This decrease in $[\text{Na}^+]$ could also be due to decreased cellular Na^+/K^+ -ATPase activity during the night-time. Previous studies of $[\text{Na}^+]$ variation in horses have shown either

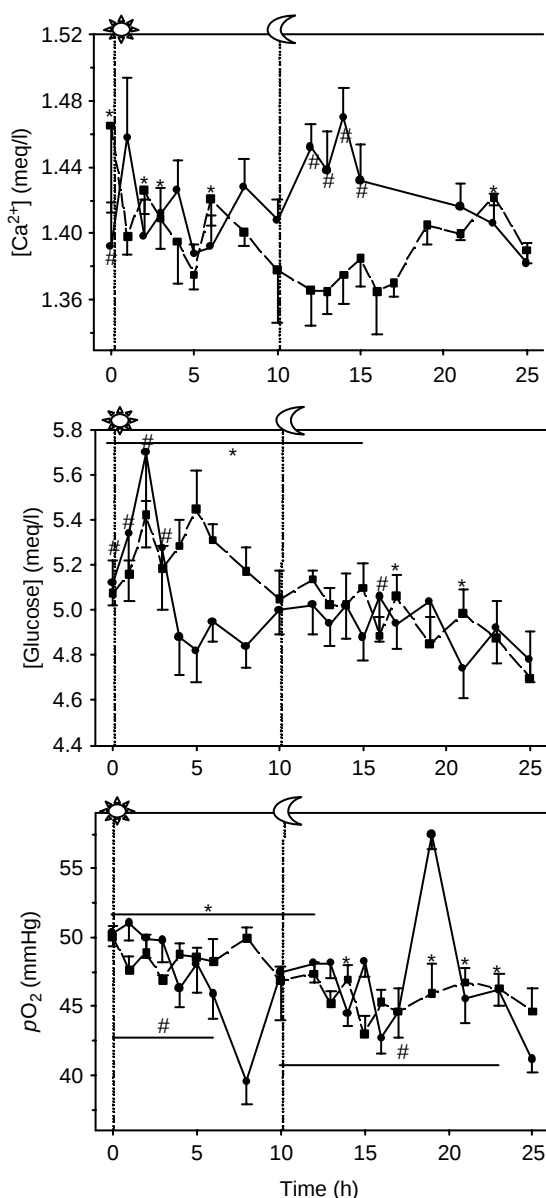


FIG. 4 The time course of plasma (a) $[Ca^{2+}]$, (b) $[Glucose]$ and (c) pO_2 . Feed was withheld for the 25 h sampling period. ■ Group 1 horses; ● Group 2 horses; ☼ denotes sunrise (~6:50 am Group 1, ~7:48 am Group 2). ☾ denotes sunset (~5:13 pm Group 1 horses, ~4:45 pm Group 2 horses). Values are mean $\pm$ SE for four horses (Group 1) or five horses (Group 2); *, #: significantly greater than the minimum value (a: 16–17, 25 h), (b: 25, 21 h), (c: 15, 8 h) for Groups 1 and 2, respectively

no change⁵ or unspecified changes¹; however, these studies were also confounded by feeding influences. Jansson and Dahlborn⁶ found that plasma $[Na^+]$ decreased during the night-time when horses were fed twice daily, but not when fed six times daily. When the same two groups of horses from the present study were fed twice daily⁸, plasma $[Na^+]$ was decreased for up to 8 h post feeding before recovering. However, although it is difficult to discern any variations in $[Na^+]$ from studies where feeding occurred, based on the second group of horses in the present study, it

does appear that plasma $[Na^+]$ may exhibit nocturnal decreases in the absence of feeding influences.

Plasma $[Cl^-]$ was increased during the night-time in the present study (significant only for Group 2). Slocombe *et al.*⁵ determined that plasma $[Cl^-]$ was increased during the afternoon compared to the morning; however, Yashiki *et al.*¹ found no changes in $[Cl^-]$ over a 24 h period. Since plasma $[Cl^-]$ for Group 2 in the present study was increased during the night-time period and did not return to baseline values by the next morning, it is possible that the cause of the increased $[Cl^-]$ was a loss of plasma volume (PV) as the 25 h sampling period progressed.

Plasma $[lactate^-]$ exhibited small yet significant fluctuations of ~ 0.1 – 0.3 meq l^{-1} during the 25 h period of the present study. In contrast, when the same horses were fed twice daily, plasma $[lactate^-]$ exhibited a prolonged, cyclical increase of up to 0.8 meq l^{-1} and recovery post-feeding⁸. In human subjects, although plasma $[lactate^-]$ increased up to ~ 1.4 meq l^{-1} postprandially, it also exhibits periodic fluctuations of 0.1 – 0.4 meq l^{-1} in post-absorptive and fasted states¹⁴. Most $lactate^-$ circulating in the resting state is derived from glycolysis in erythrocytes, adipocytes and myocytes^{15,16}. In turn, $lactate^-$ serves as a substrate for either gluconeogenesis and is oxidized (via decarboxylation to acetyl-CoA) or may enter a lipogenesis pathway¹⁴. Hence, the periodic fluctuations of blood lactate observed by Feneberg *et al.*¹⁴ and in the present study may reflect variations of glycolytic lactate production, lactate use or both. Plasma $[glucose]$ in the present study was maximally increased for the first few hours of sampling, maximally decreased for the last few hours and did not appear to be linked to $[lactate^-]$. Indeed, Feneberg *et al.*¹⁴ found that during night-time fasting periods, plasma $[glucose]$ and $[lactate^-]$ are not coupled. Alternatively, a role for intrinsic oscillating insulin pulses has been proposed to regulate the daily variation of plasma $[lactate^-]$ ¹⁴; however, more research is needed to verify this suggestion.

Plasma $[A_{tot}]$ (based on [PP]) during the night-time period increased for both groups of horses in the present study (suggesting a decrease in PV), and did not recover to baseline values by the end of the 25 h sampling period. We suggest that the increase in $[A_{tot}]$ represents a mild dehydration due to reduced water intake despite free access to water at all times; horses substantially decrease water intake when no food is available¹⁷. In contrast to the present results, when horses were fed twice daily, $[A_{tot}]$ exhibited a cyclical increase and recovery post-feeding as seen previously^{3,6,13}, but not a specific night-time increase. Jansson and Dahlborn⁶ attempted to minimize the feeding-dependent influences on [PP] by feeding their horses small meals every 4 h for a total of six feedings per day. These frequent meals resulted in increased [PP] for 4 h

during the night-time period, but not when the same horses were fed only twice daily. In contrast, Clarke *et al.*³ did not find any changes in [PP] or packed cell volume with a similar feeding protocol. Nevertheless, a daytime decrease in [PP] and increase in PV has been demonstrated in other animals^{18,19} and could be associated with increased activity during the daytime, requiring increased cardiac output to supply tissue oxygen, corresponding to a decreased pO_2 for Group 2 during the sedentary night-time period in the present study.

Dependent variables

The evening and night-time period was associated with an increased plasma acidosis, marked by an increased $[H^+]$ and decreased TCO_2 , compared with the morning hours. The timing and magnitude of the acidosis differed between the two groups of horses. Plasma $[H^+]$ for Group 2 reached its peak during the overnight period and was significantly increased from baseline from 3 h (10 am) until the end of sampling. Plasma $[H^+]$ for Group 1 was decreased from baseline during the early daytime hours (8 am–1 pm) and then increased back to baseline for the duration of sampling. The magnitude of fluctuation in the dependent variables also differed between the two groups. The amplitude of changes in plasma $[H^+]$ and TCO_2 were 9 neq l^{-1} and 10 meq l^{-1} , respectively, for Group 1. In Group 2, however, the amplitudes of $[H^+]$ and TCO_2 were 3.5 neq l^{-1} and 5 meq l^{-1} , respectively. The reasons for the altered $[H^+]$ and TCO_2 time course and amplitude responses between groups cannot be determined from this study. It is suggested that individual variation between horses and seasonal factors may affect daily variation of equine acid-base parameters.

We attempted to determine the physicochemical contributions of each of the independent variables to plasma $[H^+]$ and TCO_2 . However, the changes in $[H^+]$, TCO_2 and the independent variables were relatively small compared to those seen with exercise^{20,21} and feeding⁸. Thus, the contributions of [SID], pCO_2 or $[A_{\text{tot}}]$ to diurnal changes in $[H^+]$ and TCO_2 were difficult to ascertain over the course of the entire 25 h period. It was determined, however, that the primary contributor to the increased $[H^+]$ during the evening and night-time for both groups of horses was the decrease in plasma [SID] (due to increases in $[Cl^-]$ and decreases in $[Na^+]$) combined with the increase in $[A_{\text{tot}}]$ (due to increased [PP]); there were no significant changes in pCO_2 during the sampling periods.

Perspectives

The present study demonstrated significant daily fluctuations in plasma $[A_{\text{tot}}]$ and [SID], $[Cl^-]$, $[K^+]$, $[Na^+]$ and $[lactate^-]$, which contributed to significant changes in $[H^+]$ and TCO_2 . The amplitude of change in plasma TCO_2 was $5\text{--}10 \text{ meq l}^{-1}$ during the sampling period,

and some horses naturally exhibited TCO_2 levels of $35\text{--}37 \text{ meq l}^{-1}$ at several time points throughout the day. Within the horse racing community, the concern is that some horses may approach or exceed the TCO_2 testing threshold (37 meq l^{-1}) for determination of whether a horse was administered an alkalinizing agent for the purpose of enhancing exercise performance⁹. This clearly occurs when no alkalinizing substances have been given. It appears that daily variation should certainly be taken into consideration as a contributor to elevated TCO_2 when using TCO_2 threshold tests.

An interesting and novel finding of the present study appears to be the differences in the daily fluctuations of many measured parameters between the two groups of horses, despite the use of identical diets, methods, protocols, location and researchers. Although the internal barn lighting was kept constant between the studies, the dissimilarities between the two sampling groups could be due to the difference in natural daylight hours or the horses' metabolism between the autumn period when the first group of horses was studied and the winter period when the second group was studied. There were 7 weeks between when the first and second groups of horses were studied, and the second group of horses was sampled in late December, right around the time of winter solstice when daylight hours are the shortest of the entire year. Thus, it appears that, not only diurnal variation, but also seasonal variation should be taken into account when interpreting any equine blood samples.

Clearly, the daily variation of equine acid-base status deserves more research. There is a need for more studies into the fluctuations in acid-base state over the course of a day, looking at different types of diets, breeds, gender the interactions of exercise and feeding, and even individual differences. As individual horses may have a daily rhythm that does not exactly coincide with other horses, it would be worthwhile for a future study to include repeated measurements on the same horses over several 25 h periods. Additionally, it is difficult to establish an 'ideal' protocol for studying daily variation in horses, specifically without feeding influences. Previous studies^{3,6} have fed horses small, frequent meals in order to determine whether certain plasma parameters exhibit daily variation. However, although this protocol would minimize large, acute feeding responses, there would certainly still be feeding influences on the fluctuations in plasma parameters. The present study withheld food for the duration of sampling in order to obtain a true daily variation response; however, it is possible that some food from the previous evening's meal was still in the GI tract during the beginning of the study, which could have affected the results. As well, maintaining hydration during a non-feed trial is an important consideration. The horses in the present study

may not have consumed sufficient water to maintain hydration. Thus, a progressive mild (from plasma [protein] results) dehydration contributed to the $[A_{\text{tot}}]$ and a mild acidosis. While differences in water intake were not measured, they were observed to be between 2 and 81 l (less than half a bucket) per horse over the duration of the experiment. Such modest differences between individuals among sampling groups cannot realistically be attributed to differences in water consumption. In future studies, it may be beneficial to maintain hydration, or even an assured steady state of feeding, by intravenous methods.

Summary and conclusions

The present study investigated and detailed the time course of daily variation in key dependent and independent acid-base variables in Standardbred horses over a 25 h period. The night-time period was associated with an increased acidosis, marked by increases in plasma $[H^+]$ and decreases in TCO_2 , compared with the morning hours. The primary contributors to the night-time acidosis appeared to be an increased $[A_{\text{tot}}]$ (due to increased $[PP]$), and a decreased $[SID]$ (due to increases in $[Cl^-]$ and decreases in $[Na^+]$), as there were no differences in pCO_2 during the sampling period. The circadian responses differed in some respects between the two groups of horses studied, suggesting that subtle differences in environment and season may manifest in acid-base status. It was concluded that many equine plasma electrolyte and acid-base parameters exhibit fluctuations in the absence of feeding and exercise and it is likely that some of these changes are due to daily variation.

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