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The effect of training and cessation of training on plasma total carbon dioxide in Standardbred horses

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Abstract

Twenty-seven mature Standardbred mares (9–27 years, ~522 kg) were used to test the hypothesis that training and short-term cessation of training would alter total plasma total carbon dioxide (tCO_2) concentrations. Plasma tCO_2 concentrations were measured in blood samples (20 ml) collected at rest from the same ten unfit mares that were used in two separate studies that were conducted 1 month apart. Comparisons between the samples obtained from the two trials were made to demonstrate the consistency of plasma tCO_2 concentrations in the untrained population. Another set of resting blood samples was collected from an additional 17 mares that were being utilized in a training study that was under way during the same period. All the mares were housed in groups on dry lots, and were fed approximately 12 kg of mixed alfalfa-grass hay divided into two feedings per 24 h period. During the 'detraining' period, the 17 horses were housed in 5×5 m stalls, and were fed the same hay ration. Water and trace-mineral blocks were available *ad libitum*. Blood samples were collected from the mares prior to the last session of their 12 weeks of training at 60% HR_{max} (maximum heart rate), as well as on the third day following 2 days of detraining (simulated quarantine). Plasma tCO_2 concentrations were measured in duplicate using a Beckman EL-ISE analyser. For data analysis, *t*-tests with the *a priori* level of statistical significance set at $P < 0.05$ were used. Resting plasma tCO_2 concentrations were lower ($P < 0.05$) in the trained horses (31.4 ± 1.9 mMol l^{-1}) than in the untrained horses (34.4 ± 0.9 mMol l^{-1}). There were no effects ($P > 0.05$) due to cessation of training. It was concluded that training causes a decrease in plasma tCO_2 concentrations that is not reversed by limited cessation of training.

Keywords: plasma tCO_2 concentration; training; detraining

Introduction

Administering sodium bicarbonate or other alkalinizing agents via a nasogastric tube prior to a competition is a well-known (and illegal) abuse of the buffering capabilities of these compounds. This practice, referred to as 'milkshaking', is a threat to the health and welfare of the horse as well as to the integrity of the entire racing industry[1,2]. To counter this threat, many racing jurisdictions measure total plasma carbon dioxide (tCO_2) concentrations.

Plasma tCO_2 concentration is an important physiological factor that is controlled so as to ensure the tight regulation of blood pH[3–5]. Animals transport >90–95% of all CO_2 produced in metabolically active tissue to the lungs in the form of carbonic acid, which rapidly dissociates to bicarbonate and hydrogen ions in the presence of an aqueous environment. This renders tCO_2 an effective and reliable measure of bicarbonate concentration within blood plasma[3–5]. Multiple investigations have reported ~30 mMol l^{-1} to be the average plasma tCO_2 concentration for racing horses[1–9]. Thus, most racing jurisdictions have set the threshold for a positive test at 37 mMol l^{-1} to avoid false positives[1–9], which is approximately four standard deviations from the mean.

One area of controversy arises when one compares the data obtained from populations of very fit racehorses with those obtained from the unfit horses used at many research institutions. Training can affect

the buffering capacity of humans[10]. Regrettably, no information has been published on the effect of training on plasma tCO_2 concentrations in normal horses. Another area of concern in New Jersey and elsewhere has been the effect of the limited amount of exercise performed by horses during quarantine. Some horse owners have suggested that the limited exercise and stall rest associated with a few days of quarantine may have an effect on plasma tCO_2 concentrations. Strong speculation regarding this issue has been made in court cases[11]. Unfortunately, as with the effect of training, there have been no horizontally collected data published in the scientific literature to support or rebuke this speculation. Therefore, the purpose of this study was to test the hypothesis that training and short-term cessation of training would alter tCO_2 concentrations in Standardbred horses.

Materials and methods

All the methods and procedures used in this experiment were reviewed and approved by the Rutgers University Institutional Review Board for the Care and Use of Animals. Twenty-seven mature Standardbred mares (9–27 years, ~522 kg) were used in this experiment. Resting plasma tCO_2 concentrations were measured on two occasions in the same ten unfit mares used in two separate studies[5,12] that were conducted 1 month apart. These two studies were conducted at the same time during which another experiment, with the objective to examine the effect of training and ageing on a variety of parameters, was in progress[13]. The analysis of plasma tCO_2 concentrations in the samples that were collected from the later study revealed that there was no effect of age, so all data are included in the present paper; therefore, data obtained from all three experiments and from both groups of horses were utilized in the present analysis. All mares were housed in groups on 2–3-acre dry lots, and were fed approximately 12 kg of mixed alfalfa-grass hay divided into two feedings per 24 h period. During the detraining period, the 17 horses that were part of the training study were housed in 5 × 5 m stalls, and were fed the same hay ration. Water and trace-mineral blocks were available *ad libitum*.

Blood samples and analysis

Blood samples (20 ml) were obtained from the unfit horses via jugular venipuncture while they stood quietly prior to dosing and feeding[5,12]. Samples (20 ml) were collected from the trained mares[13] prior to their last session of 12 weeks of training at 60% HR_{max}. Following the completion of their last exercise session, the trained mares were placed in stalls, and a second set of samples was collected on the third day following 2 days of 'detraining' that was used as simulated quarantine. Samples were collected using an 18-gauge needle and two tubes (Vacutainer; Becton Dickinson, Parsippany, NJ, USA) containing lithium heparin. These samples were placed in crushed ice for analysis later in the day. Each tube was spun in a refrigerated centrifuge (TJA-6; Beckman-Coulter, Fullerton, CA, USA) at a rate of $1500 \times g$ and at a temperature of 4°C for a period of 10 min. The plasma fraction was then decanted and analysed in duplicate using an ion-sensitive electrolyte analyser (Synchron EL-ISE; Beckman-Coulter) to determine the tCO_2 concentrations.

Statistical analysis

The data were analysed using *t*-tests with the *a priori* level of statistical significance set at $P < 0.05$ (Sigma Stat 2.0; SPSS, Inc., Chicago, IL, USA).

Results and discussion

The results presented in the figures are expressed as means ± standard error. Training had an effect ($P < 0.05$) on resting plasma tCO_2 concentrations, with lower values being present in the trained horses ($31.4 \pm 1.9\ mMol\,l^{-1}$) than in the untrained horses ($34.4 \pm 0.9\ mMol\,l^{-1}$). There were no effects ($P > 0.05$) due to cessation of training (Figs 1 and 2). Thus, a major finding of the present study was that there was a substantial decrease in plasma tCO_2 concentrations with training that was not reversed by short-term cessation of training. This finding is important for two reasons. Many published studies of management and other factors affecting plasma tCO_2 concentrations have been done by universities where unfit horses that were part of a research teaching and research herd were utilized[14–16]. While these populations are appropriate for testing the effects of various factors on plasma tCO_2 concentrations, the present study has demonstrated that even moderate training affects

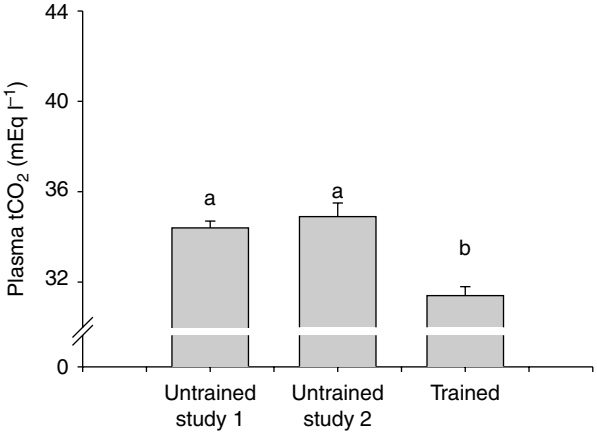

Fig. 1 Mean ± SE plasma tCO_2 concentration measured in untrained horses ($n = 10$) on two occasions and in trained horses ($n = 17$) following 12 weeks of moderate-endurance exercise training. Means with different letters are different ($P < 0.05$)

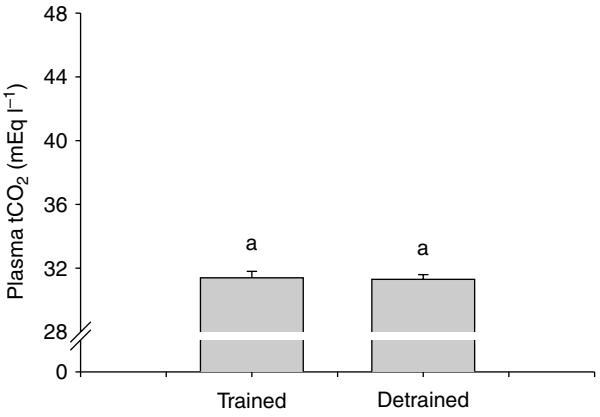

FIG. 2 Mean ± SE plasma tCO_2 concentration measured in horses ($n = 17$) at rest before their last training session following 12 weeks of moderate-endurance exercise training and on the third day following 2 days of detraining (simulated quarantine). Means with different letters are different ($P < 0.05$)

resting plasma tCO_2 concentrations. As with humans[10], higher-intensity conditioning appears to lower resting plasma tCO_2 concentrations further towards the concentrations observed in fit racehorses (Dr Ric Birks, 2002, personal communication, University of Pennsylvania). Thus, it would be inappropriate to make a simple comparison of unfit horses with the population of fit racehorses to attempt to challenge the resting values obtained from tightly controlled studies[3,6,8,9] of populations of racehorses.

Another major finding of the present study was that detraining for a short period analogous to the quarantine period used in many racing jurisdictions does not affect plasma tCO_2 concentrations. In theory and practice, these jurisdictions offer a short quarantine period to allow a trainer to demonstrate that a horse that exceeds the posted threshold has done so because it had a naturally high plasma tCO_2 concentration. One issue that has been raised regarding the quarantine period has been that the lack of exercise or 'detraining' could potentially affect plasma tCO_2 concentrations. The present study has demonstrated that there is no effect of limited cessation of training on plasma tCO_2 concentrations.

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