Training-induced energy balance mismatch in Standardbred mares

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Submitted 1 October 2005: Accepted 16 March 2006 Research Paper

Abstract

This study tested the hypothesis that exercise training would alter feed intake (FI), body composition (BC) and plasma concentrations of active ghrelin, leptin, cortisol, insulin and glucose. Eight Standardbred mares (12 ± 2 years, 509 ± 36 kg body weight (BW), mean ± SD) were trained (EX) in an equine Equi-ciser (initially 3 days per week at 60% maximal heart rate (HRmax) for 20 min and gradually increased to 5 days per week at 70% HRmax for 30 min, with a 10-min warm-up and 10-min cool-down period at the walk). Six mares (12 ± 2 years, 537 ± 45 kg) served as non-exercise controls (CON). All mares were unfit and had not been subjected to conditioning for 3 years before the experiment. Pre- and post-training incremental exercise tests (GXT) were run to determine HRmax and maximal oxygen uptake (VO2max). A total mixed ration (TMR) of hay cubes was fed free choice for 16 h day−1 with the primary experiment following a 6-week diet adaptation period. Mares’ FI was measured daily and reported in grams per kilogram BW of feed eaten per week. Changes in BC were assessed using BW (electronic scale) and percentage fat calculated using rump fat thickness and the Westervelt equation. Blood samples were taken every 2 weeks at 15:25, before mares were given their allotment of hay cubes on a day when they did not exercise, to measure plasma hormone and glucose concentrations. Gastroscopy for gastric ulcers was performed before, during and after the trial. VO2max increased by 7.0% (P < 0.03) in EX, but did not change (P > 0.05) in CON. FI decreased (P < 0.001) in both groups, but was only different (P < 0.02) between EX and CON at week 3. Digestible energy (DE) intake (Mcal day−1) was initially higher (P < 0.001) than calculated DE requirements in EX. However, over time, DE only matched and then fell below (P < 0.05) the DE intake required for training. In CON horses, DE intake was higher (P < 0.001) than calculated requirements. BW and percentage body fat increased (P < 0.001) over time in EX and CON. Plasma leptin concentration increased (P < 0.001) over time in both groups, but was only 60% higher (P < 0.04) in CON compared to EX at weeks 4–8. There were no differences (P > 0.05) in active ghrelin, glucose, insulin or cortisol between the groups and over time. Five out of seven EX mares developed gastric ulcers. No CON mares developed gastric ulcers. Training was associated with changes in plasma leptin concentration, an increased incidence of gastric ulcers and a disruption of the balance between required DE and actual intake.

Keywords: energy balance; exercise; leptin; ghrelin; equine

Introduction

Many horses go ‘off feed’ after exercise1, yet no studies have examined the effects of exercise on subsequent feed intake in horses and possible mechanisms behind this exercise-induced inappetance. One potential cause could be an interaction between exercise-related factors and hormones involved with the maintenance of energy balance. As reviewed by Meier and Gressner2, these hormones include leptin and ghrelin, which have been correlated with indices of energy sufficiency in humans and rodents and, more recently, horses3–5. Furthermore, leptin is modified by exercise in humans6 and horses5,7, and changes in its concentration may act as a signal to increase or decrease energy intake. Therefore, studies are necessary to determine how leptin, ghrelin and feed intake are concomitantly affected by training in horses.

In addition to any alterations in leptin and ghrelin, the development of gastric ulcers could be another mechanism behind exercise-induced inappetance.
Gastric ulcers are common in racehorses and show horses, and are correlated with ‘picky’ eating. Studies are needed to determine if alterations in feed intake and gastric ulceration can occur independently, and if there is an interactive role between inappetance, gastric ulcers and changes in energy balance hormones.

Consequently, the main objective of this study was to test the hypothesis that training would alter feed intake and plasma concentrations of ghrelin and leptin. Plasma concentrations of glucose, insulin and cortisol were also measured to test the hypothesis that there would be relationships between all of these parameters of energy metabolism. The development of gastric ulcers, as well as alterations in body composition and fitness, were also monitored to determine if these factors play a role in exercise-induced inappetance and changes in hormone concentration.

Materials and methods

The experiment was conducted using two groups, an exercise-trained group (EX) of eight mares that exercised up to 5 days per week, and a control group (CON) with six mares that did not exercise. The general protocol for the study included a 6-week adaptation period followed by the 10-week study period, which started and ended with a week of observations with an 8-week training programme between these periods for the EX group. Figure 1 presents a general protocol timeline for the study. The study was performed over the late autumn and winter months in New Jersey, when there was no pasture growth and when temperatures were cold but stable.

Animals

Fourteen healthy, untrained Standardbred mares were evaluated during the 10-week period. Mares were randomly assigned to their respective treatment groups. Eight of the mares (12 ± 2 years, 509 ± 36 kg body weight (BW), mean ± SD) comprised the EX group and six mares (12 ± 2 years, 537 ± 45 kg) served as non-exercise controls (CON). Mares were of similar BW and fitness at the beginning of the trial. All mares were accustomed to the lab, Equi-ciser, treadmill and all tests. All horses were up to date on the administration of vaccinations and deworming medications and all had their teeth floated and confirmed as normal and healthy prior to the start of the study. The horses were housed indoors overnight in 3 x 3 m stalls and turned out into a dry lot pasture during the day. The Rutgers University Institutional Animal Care Review Board approved all methods and procedures used in this experiment.

Ration and feed intake data

The ration consisted of ~ 8 kg day⁻¹ of commercially available total mixed ration (TMR) hay cubes (Eckenberg Farms, Mattawa, WA, USA) that were fed at 15:30 and then removed from the horses at 07:30 the next morning (see Table 1 for the dry matter analysis of the TMR hay cubes). Ingredients included: alfalfa hay, oat hay, rolled corn, timothy hay, rolled barley, soymeal, cane molasses, vegetable oil, plant protein products, monosodium phosphate, monocalcium/dicalcium phosphate, calcium carbonate, magnesium oxide, salt, yeast culture, vitamin A acetate, d-activated animal sterol, vitamin e supplement, vitamin B₁₂ supplement, choline chloride supplement, riboflavin supplement, niacin, d-calcium pantothenate, folic acid supplement, thiamine mononitrate, pyridoxine hydrochloride, biotin, ascorbic acid, menadione dimethylpyrimidinol bisulphite, zinc methionine complex, manganese methionine complex, copper lysine complex, cobalt glucoheptonate, ferrous sulphate, zinc sulphate, manganese sulphate, copper sulphate, potassium iodide, sodium selenite, dried brewer’s yeast, wheat middlings, rice hulls, propionic acid, sodium hydroxide, ammonium hydroxide, glycerine, monosodium glutamate, magnesium chloride, manganese chloride, calcium chloride, calcium...
Table 1  Total mixed ration hay cube feed analysis (dry matter basis, moisture content 9.6%)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy</td>
<td>2.68 Mcal kg⁻¹</td>
</tr>
<tr>
<td>Crude protein</td>
<td>187 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>289 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>359 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Lignin</td>
<td>49 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Non-fibre carbohydrates</td>
<td>330 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Sugar</td>
<td>87 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Crude fat</td>
<td>21 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Ash</td>
<td>128 g kg⁻¹ DM</td>
</tr>
<tr>
<td>TDN</td>
<td>600 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.4 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.8 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.3 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Potassium</td>
<td>15.2 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Sodium</td>
<td>2.9 g kg⁻¹ DM</td>
</tr>
<tr>
<td>Iron</td>
<td>1730 mg kg⁻¹ DM</td>
</tr>
<tr>
<td>Zinc</td>
<td>50 mg kg⁻¹ DM</td>
</tr>
<tr>
<td>Copper</td>
<td>11 mg kg⁻¹ DM</td>
</tr>
<tr>
<td>Manganese</td>
<td>75 mg kg⁻¹ DM</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>1.0 mg kg⁻¹ DM</td>
</tr>
<tr>
<td>Sulphur</td>
<td>2.2 g kg⁻¹ DM</td>
</tr>
</tbody>
</table>

bentonite, natural and artificial flavourings and mineral oil. The mares had gradually become accustomed to the free choice diet regimen over a 6-week acclimatization period and had stabilized their intake of the diet for at least 1 week prior to the start of the experiment. Feed that was not eaten was weighed on a daily basis at 07:30 and subtracted from the feed offered to determine feed intake. Data for feed intake were expressed as gram per kilogram BW eaten over 7 days. A mineralized salt block and fresh water were also available to each horse ad libitum. Digestible energy (DE) requirements for maintenance and exercise were determined utilizing the following equations by Hintz and Pagan12,13:

For maintenance, DE (Mcal day⁻¹)

\[ \text{DE} = 1.4 + 0.03 (\text{kg BW}) \]  

(1)

For exercise, DE (kcal kg⁻¹ h⁻¹)

\[ \text{DE} = e^{3.02 + 0.0065 \times \text{(speed in m min}^{-1})} - 13.92 \times 0.06 \times 0.57 \]  

(2)

Graded exercise test (GXT)

Pre- and post-training fitness parameters were measured during GXTs performed at least 1 week prior to and within 1 week after the 8 weeks of training using previously published methods14. During the GXT, the horses ran on a fixed 6% grade at an initial speed of 4 m s⁻¹ for 60 s. Treadmill speed was then increased to 6 m s⁻¹ for 60 s, followed by 1 m s⁻¹ increases every 60 s until they reached fatigue. Fatigue was defined as the point where the horse could not keep up with the treadmill despite humane encouragement. Prior to walking on the treadmill, horses had a catheter introducer (6 F Argon Medical, Athens, TX, USA) inserted percutaneously into the jugular vein using sterile techniques and local lidocaine anaesthesia. Horses were then led onto the treadmill where a pressure-sensing catheter (Millar Instruments, Houston, TX, USA) was attached and positioned for the measurement of right ventricular pressure. Verification of the position of the catheter was performed before and after each horse using blood pressure waveforms recorded on the physiological recording system (Biopac Systems, Goleta, CA, USA). Heart rate (HR) was calculated using right ventricular pressure waveforms at the last 10 s of each step of the GXT and at 2 min post-exercise. Maximal heart rate (HRmax) was defined as the point where there was no further increase in HR despite an increase in treadmill speed. Oxygen uptake was also measured using an open-flow indirect calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH, USA) and maximal oxygen uptake (VO2max) was defined as the point where there was no further increase in VO2 despite an increase in speed.

Training protocol

Prior to training, the EX horses were grouped based on fitness level with the goal being to keep the horses at a sub-maximal work intensity between 60 and 70% HRmax as determined during the GXT. Groups were based on HR data to keep horses within their target training zones. The training programme consisted of running in the Equi-ciser (Sundre, Alberta, Canada) for 8 weeks. Horses initially started training 5 days per week at 60% HRmax for 20 min and were gradually increased to 5 days per week at 70% HRmax for 30 min, with a 10-min warm-up and 10-min cool-down period at the walk. HR was checked weekly in each group using a HR monitor (Polar, Lake Success, NY, USA). HR was checked by a HR monitor placed on one mare during exercise to make sure the speed was correctly elevating HR into the desired training zone for the group.

Body composition testing procedures

Measurements to assess changes in body composition were made every 2 weeks. Body weights were
measured weekly using an electronic load cell scale. Percentage fat was calculated using ultrasonically measured rump fat thickness (5 cm from midline, at 1/2 of sacral bone) and the Westervelt equation\textsuperscript{15}.

**Blood sampling procedures**

Blood samples were taken every 2 weeks at 15:25, before mares were given their allotment of hay cubes. Therefore, mares were without feed for \(\sim 7.5-8\) h prior to sampling. Exercise testing was completed by 09:00. Blood was collected via venipuncture into pre-chilled 10 ml vacutainer tubes. Tubes with heparin anticoagulant were used for glucose, insulin and cortisol samples, while tubes with EDTA anticoagulant were used for active ghrelin and leptin samples (Becton Dickinson Inc., Franklin Lakes, NJ, USA). All blood tubes were centrifuged at 2000 \(\times g\) for 15 min then aliquoted and frozen at \(-80^\circ\)C until analyses. Samples for the measurement of plasma-active ghrelin were treated with 50 \(\mu\)l of 1 N HCl and 10 \(\mu\)l of phenylmethylsulfonyl fluoride (PMSF) per 1 ml of plasma to retard the breakdown of active ghrelin before freezing.

**Gastroendoscopy procedure**

EX and CON horses underwent gastroendoscopy before, at the midway point and after the training period. One mare in the EX group was unable to undergo gastric scoping during the last three tests. Prior to each procedure, horses were denied access to feed for 12 h and water for 8 h. Horses were sedated with 15 mg acepromazine and 150 mg xylazine IV immediately before the procedure and were lightly restrained during the endoscopy. A 3-m video endoscope was used to examine the stomach, including the glandular and non-glandular portions, and the margo plicatus. Prior to examination, the stomach was inflated with air and water was pumped into the stomach to remove any material adhered to the stomach wall. Gastric lesions were characterized by severity using an ulcer scoring system similar to that of Hammond et al.\textsuperscript{16} where:

\[
\begin{align*}
0 &= \text{intact mucosal epithelium (can have reddening/ hyperkeratosis)}; \\
1 &= \text{small single or small multifocal lesions;} \\
2 &= \text{large single or large multifocal lesions; and} \\
3 &= \text{extensive lesions with areas of deep ulceration.}
\end{align*}
\]

**Plasma biochemical and hormone analyses**

Plasma-active ghrelin concentration was determined using a commercial RIA kit (Linco Research, St Louis, MO, USA). The active ghrelin kit was previously validated for use in horses\textsuperscript{3}. In the absence of purified equine active ghrelin, results are expressed as human equivalents of immunoreactive ghrelin. The within-assay CV for active ghrelin was < 10\%. Plasma leptin concentration was determined using a multispecies leptin kit (Linco Research, St Louis, MO, USA), as previously used in horses\textsuperscript{3}, demonstrating a within-assay CV of 8.5\%. Plasma insulin and cortisol concentrations were determined utilizing solid-phase RIA kits (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA, USA) previously validated for use in horses\textsuperscript{17}, and plasma glucose concentration was determined via a hexokinase reaction kit (Diagnostic Products Corp.). The within-assay CVs for the insulin, cortisol and glucose assays were < 3\%. All samples were run in duplicate and within one assay.

**Statistical analysis**

A two-way ANOVA with repeated measures for time and treatment (exercise vs. control) with post hoc analysis of Student–Newman–Keuls (for feed intake data) and Tukey’s test (for active ghrelin, leptin, glucose, insulin, cortisol, BW, body fat percentage and DE data) were used to determine significant changes during the testing period (SigmaStat, SPSS, Inc., Chicago, IL, USA). Alterations in VO\textsubscript{2max} due to training and differences in feed intake based on percentage of BW were tested using a paired \(t\)-test (QuickCalc, GraphPad Software, San Diego, CA, USA). An \textit{a priori} level of statistical significance was set at \(P < 0.05\) for all tests. All data in tables and figures are presented as mean \(\pm\) SEM.

**Results**

**Exercise training fitness assessment**

At the end of the training period, horses in the EX group increased (\(P < 0.001\)) their VO\textsubscript{2max} as measured in a GXT, by 6.5\% (from 121 \(\pm\) 4 to 129 \(\pm\) 3 ml kg\(^{-1}\) min\(^{-1}\)). Horses in the control group had no change (\(P > 0.05\)) in VO\textsubscript{2max} over the testing period (121 \(\pm\) 3 \textit{vs.} 121 \(\pm\) 5 ml kg\(^{-1}\) min\(^{-1}\)).

**Feed intake**

Feed intake decreased (\(P < 0.001\), Fig. 2) in both groups throughout the trial; however, a difference (\(P < 0.02\)) between the EX and CON groups was only evident at week 3 of training. There was a 17.5\% decrease in feed intake in the EX group and a 17.0\% decrease in feed intake in the CON group during the 8 weeks of the experiment. As a percentage of BW, both the EX and CON groups ate a lower percentage (\(P < 0.05\)) of their BW by week 8 compared to week 1 of the training trial. The exercise group started out eating 2.6 \(\pm\) 0.1\% of their BW and decreased to 2.3 \(\pm\) 0.1\% of their BW. The control group ate 2.6 \(\pm\) 0.1\% of their BW at the start of the trial and decreased to 2.39 \(\pm\) 0.1\% of their BW (data
not shown). When the data were examined in terms of calculated DE required per day compared to the actual DE intake, remarkable results were found. As the DE requirement for the EX mares increased due to training, the EX mares' overall DE intake was decreasing as they decreased their feed intake. In the first 4 weeks of training, the EX mares' DE intake was substantially higher \((P < 0.001, \text{Fig. 3A})\) than their DE requirements. However, during weeks 5 and 6 of the trial, there was a match between predicted DE requirements and DE intake. During weeks 7 and 8 of the training trial, the EX mares' DE intake was lower \((P < 0.03, \text{Fig. 3A})\) than the calculated DE requirement. On the other hand, in the CON group, DE intake was higher \((P < 0.001, \text{Fig. 3B})\) than calculated DE requirements at all time points during the study.

**Body composition**

Body weight and body fat percentage increased \((P < 0.001, \text{Fig. 4A and 4B})\) in both groups over time throughout the study; however, there was no difference \((P > 0.05)\) between the EX and CON groups at any time point for either parameter.

**Plasma analyses**

Plasma leptin concentration increased \((P < 0.001, \text{Fig. 5A})\) in both the EX and CON groups over time. At week 4, the CON group had a 60.4% higher \((P < 0.04, \text{Fig. 5A})\) plasma leptin concentration than the EX group. There were no differences \((P > 0.05, \text{Figs 5B, 6A–6C})\) between EX and CON groups or over time for active ghrelin, glucose, insulin or cortisol during the testing period.

**Gastric mucosal lesions**

Low-grade gastric mucosal lesions (score of 1 on scale of 0–3) became evident in one horse in the EX group halfway through the training period. By the end of 8 weeks of training, five of the seven EX horses exhibited low-grade gastric mucosal lesions (score of 1–2). All ulcers appeared on the squamous epithelial mucosa adjacent to the margo plicatus. No horses in the CON group exhibited gastric ulcers or mucosal lesions at any time point (Table 2).

**Discussion**

**Body composition, feed intake and fitness**

This is the first study, to our knowledge, that has concomitantly examined the effects of training on feed intake, body composition, fitness, energy balance hormones and the development of gastric ulcers. The data from this study are very important since the athletic horse undergoing heavy training often has difficulty maintaining feed intake. This study also has relevance for the large number of horses that are not actively or intensely trained that may over-compensate their energy intake and become obese on high-energy
diets. Despite gaining both body mass and increasing body fat percentage, the mares in the EX group did increase both their VO\textsubscript{2max} (7.0%) and their endurance capacity, as evidenced by the increase in duration tolerated during the work-outs during the training period. Therefore, the training protocol was successful in increasing the fitness capacity of the EX mares. However, even though the magnitude of the training response was similar to that reported in a previous study\textsuperscript{18}, the training volume and intensity did not provide enough energy expenditure to counterbalance the effects of their high-energy intake. This resulted in the unpredicted finding of an increase in BW and body fat percentage in all of the mares used in the present study. We speculate that this change in body composition was most likely attributable to the free choice, hay cube-feeding regimen, which was required for the experimental design. Care was taken to fully adapt the horses to the ration and it appeared that intake had stabilized. However, while it is not uncommon under many management regimens for horses to receive *ad libitum* hay as a major portion of their ration, the horses in the present study were fed a very energy-dense, total mixed ration hay cube containing 2.68 Mcal kg\textsuperscript{-1} of DE (DM basis). The high energy density of this feed was an important factor considered in the design of the study so that horses could meet their dietary energy requirements fairly easily. Therefore, if the horses did decrease their intake due to exercise or some other factor, the study would not be confounded by gross deficiencies in dietary energy and/or other nutrients. Still, the body fat percentage seen in the horses of the present study is similar to that reported for unfit Standardbreds but higher than the 11% reported for fit racehorses\textsuperscript{4}.

Interestingly, the energy intake of the CON mares was well above the intake level calculated for maintenance of their BW, placing this group of horses in a state of positive energy balance. In the EX mares, on the other hand, energy intake was initially higher than their calculated energy requirements. However, unlike the CON mares, the EX mares soon only matched and, later in training, had energy intake that was lower than their computed energy expenditure. Nevertheless, there were no differences between the EX and CON groups for BW or body fat percentage throughout the trial. Therefore, one major conclusion is that the training programme designed for the EX
group was not sufficient to combat the positive energy balance brought about by the hay cube-feeding regimen. This does not invalidate the training protocol utilized as it was of a duration and intensity that was sufficient to induce physiological adaptations without producing lameness or other problems. Another point to consider in this analysis is that we used equations to calculate the energy density of the ration and nutrient requirements of the horses. These equations only give estimates that are not as precise as actual calorimetry measurements. Notwithstanding these limitations, however, are the obvious observations that (1) the ration was very dense in energy, as can be seen by the ingredient list and guaranteed analysis, and (2) there was a mismatch in energy intake/expenditure due to the weight gain of the horses. Furthermore, J. Pagan (Equine Science Society Symposium, ESS 2005), in his new research on the energy equations, argues that the energy equations during exercise underestimate energy requirements, which would make our data even more outstanding with even larger discrepancies between intake and expenditure. Thus, the message appears to be that it is very hard to match energy intake with energy demands in the equine athlete. There are very few studies in other species that have examined the effects of exercise with free-choice feed intake. For example, most studies of humans are designed with the subjects on restricted or steady-state diets, primarily because the goal of many of those experiments has been to examine specific factors related to weight loss or exercise training itself19. However, in two separate studies, both men and women who were on ad libitum diets and exercising at low, medium and high intensities demonstrated an inability to increase energy intake enough to match energy expenditure due to exercise20,21. Interestingly, those results contrast with the well-accepted theory that humans will increase their energy intake in the face of increased energy expenditure22. Thus, it appears that humans are actually poor at matching energy intake and energy expenditure when specific exercise regimens are prescribed and the subjects have ad libitum access to food. It is of interest to note that in humans the mismatch between energy intake and energy expenditure is in a negative direction, with a lack of compensation of energy intake for increased energy expenditure. This is similar in some respects to the results found in the horses of the present study; however, the mismatch between energy intake and energy expenditure in the horses occurred mostly in the direction of positive energy balance, with energy intake exceeding energy expenditure. The observation that there was a decrease in feed intake in both the EX and CON groups could be representative of a regulation in energy balance that occurs only after horses reach a certain excessive positive energy balance.

**Plasma hormones**

The increase in plasma leptin concentration over time in both the EX and CON groups was most likely reflective
of the increases in body mass and body fat percentage of the mares during the experimental period. Previous studies have shown that plasma leptin concentration is positively correlated to body fat percentage and fat mass\textsuperscript{4,25,24}. Interestingly, in the present study, the mares in the EX group exhibited lower plasma leptin concentrations than the CON group. That difference became even more pronounced by week 4 of the training period and continued until the completion of the study. This difference was surprising since the EX and CON groups did not differ in BW, body fat percentage, feed intake or any other parameter measured after week 4. Thus, the effect of training exercise on the concentration of plasma leptin appears to be independent of body composition. This in turn suggests that factors associated with exercise \textit{per se} rather than total number of adipocytes had a greater role in the modulation of plasma leptin concentration. Additional support for an independent role of exercise training in the modulation of plasma leptin concentration can be found in a study that used obese humans\textsuperscript{25}. In that study, 16 months of exercise training was associated with decreases in plasma leptin concentration, and the association was independent of changes in insulin and body fat percentage. It was concluded that the number of hours of exercise appeared to be responsible for this alteration in plasma leptin. Interestingly, those results from humans are consistent with a previous study conducted using Standardbred mares where a difference in leptin concentration was observed in fit vs. unfit horses\textsuperscript{4,24}. Although much of that difference was attributed to variation in body composition, it was also hypothesized that the difference could have been due to the exercise training of the horses in the fit group\textsuperscript{4,24}. Moreover, in another study, horses performing a short-term, high-intensity exercise test had lower plasma leptin concentrations 24 h post-exercise\textsuperscript{4,24}. Therefore, it appears as though high-intensity exercise may have the ability to alter plasma leptin concentration in horses and that exercise training may also modulate the hormone’s concentration. More studies are required to further elucidate potential mechanisms of action associated with this phenomenon.

Even though training appears to have had an independent effect on plasma leptin concentration, plasma leptin concentration did increase in both groups over time. This progressive increase in plasma leptin concentration was concurrent with a steady decrease in feed intake. While only descriptive, this observation would suggest that the increase in plasma leptin concentration could have played a role in the mechanisms associated with the decrease in feed intake that occurred in all of the horses in this study. We can only speculate about the responses seen in the present study but, in a recent review by Caro \textit{et al.}\textsuperscript{26}, a similar argument is made, based on studies\textsuperscript{27} where it was shown that central administration of leptin to rodents and rhesus monkeys causes a decrease in food intake. Other studies\textsuperscript{28} have elucidated neuronal pathways in the brain through which leptin exerts this anorexic effect. The question remains, however, if peripheral changes in leptin concentration actually cause alterations in feed intake. In obese humans, leptin ‘resistance’ appears to be a problem, with an inability of leptin to exert its appetite-reducing effects\textsuperscript{29}. Although there was a parallelism between increasing leptin concentration and decreases in feed intake in the horses of the present study, no cause and effect relationship can be determined. Further research is needed to establish if and how increased leptin concentration affects feed intake in horses.

Other intriguing findings of the present study was the lack of an observed difference between the EX and CON groups as well as a lack of a difference over time for plasma concentration of ghrelin. Ghrelin is negatively correlated with percentage body fat in humans\textsuperscript{30} and horses\textsuperscript{4,24}. Thus, one would have expected to see a decrease in plasma ghrelin concentration in response to the apparent increase in body fat percentage in both the EX and CON groups. This prediction is supported by two studies, one where plasma ghrelin concentration was decreased in unfit Standardbred mares compared with a leaner comparison group\textsuperscript{4,24}, and a second study that reported lower plasma ghrelin concentrations in obese humans compared with their leaner counterparts\textsuperscript{30}. Further support for the prediction that there would be a difference between EX and CON horses comes from the observation that anorexic humans have higher plasma ghrelin concentrations\textsuperscript{31,32} than normal controls that can be returned to normal with weight gain\textsuperscript{31}. Combined human studies suggest that ghrelin fluctuates with alterations in body composition. However, another study of humans demonstrated that an alteration in body composition via a 5% weight loss was not sufficient to return ghrelin to normal fasting concentrations\textsuperscript{33}. It is possible, therefore, that the horses of the present study did not exceed a threshold that must be reached before changes in body composition influence the secretion and action of ghrelin.

There was no difference between glucose, insulin and cortisol between the groups or over time in the present study. The consistent plasma cortisol concentrations simply reveal that there were no overt stressors affecting either group.

**Gastric ulceration**

With studies demonstrating a strong correlation between exercise training and the development of gastric ulcers\textsuperscript{8,9}, and recent findings of potential mechanisms linking the two\textsuperscript{34}, it was not surprising that many of the mares in the EX group developed...
low-grade gastric ulcers (score of 1–2 on a scale of 0–3) after the half-way point of the training period. By the end of 8 weeks of training, five of seven EX horses exhibited low-grade gastric ulcers, while no horses in the CON group exhibited gastric ulcers at any time point. This finding was not surprising, yet was still intriguing as many steps were employed in the present study to help deter the onset of gastric ulcers (for example, 16 h of available feed, turn-out with other horses, low-stress environment, etc.). However, even low-intensity exercise can produce increases in gastric pressure, volume and pH concurrently with contraction of the abdomen in horses. Lorenzo-Figueras and Merritt suggest that these alterations in gastric physiology can expose the proximal portion of the stomach to acidic gastric contents, causing excessive exposure that leads to squamous mucosal lesions, such as those that were seen in the horses of the present study. It must be noted, however, that, even with the presence of gastric ulcers, the decrease in feed intake in the EX mares did not differ from the ulcer-free CON mares. Furthermore, the EX mares did not demonstrate any signs of distress, such as colic, poor body condition or diarrhoea, that are commonly reported along with gastric ulceration. One interesting point to consider, however, is the fact that, during the last 2 weeks of the study, the calculated DE requirements were higher than actual DE intake in the EX horses. One could speculate that the gastric ulceration interfered with the physical ability of the EX mares to increase their feed intake to meet their calculated requirements. On the other hand, one should take note that the mares were weight stable during the last 2 weeks of the training trial and hence did not appear to be in negative energy balance.

In conclusion, 8 weeks of exercise training coupled with a TMR, high-energy diet resulted in an increase in fitness, BW and body fat percentage with lower plasma leptin concentrations in the exercise group compared with a non-exercising control group. While both EX and CON horses decreased their feed intake during the trial, the EX mares initially ate more, but then subsequently ate less than their calculated requirements for exercise training. This mismatch in energy intake and energy expenditure may have been due to the development of gastric ulcers that occurred during training or may simply demonstrate how horses do not regulate energy balance well. The fact that the CON horses consistently ate more than their calculated requirements throughout the entire trial further strengthens the latter argument. While one cannot rule out the influence of endocrine factors, however, the inadequate attempt to more closely match energy intake with energy expenditure suggests that the increase in plasma leptin concentration is only part of a more complex mechanism contributing to the control of feed intake. Finally, the lack of an association between plasma ghrelin concentration and the alterations in feed intake and body composition in the present study lead to new questions regarding ghrelin’s role in the horse. Ghrelin did not appear to be sensitive to relatively small changes in body composition, leading to the speculation that this hormone’s role lies more in the anticipation and initiation of feed intake rather than in the long-term control of energy stores. The present study only begins to examine the complex relationship between the many factors that may affect the balance between energy intake and energy expenditure.

Acknowledgements

The authors thank D. Todd Wilkinson and the many undergraduate and graduate students who helped conduct this study. The authors also thank Jennifer McKeever for helping to prepare the manuscript. Support for this project was provided by the New Jersey State Equine Initiative and the New Jersey Agricultural Experimentation Station.

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