Effects of intensified training and subsequent reduced training on glucose metabolism rate and peripheral insulin sensitivity in Standardbreds

Ellen de Graaf-Roelfsema, DVM, PhD; Hans A. Keizer, Prof MD, PhD; Eric van Breda, PhD; Inge D. Wijnberg, DVM, PhD; Johannes H. van der Kolk, DVM, PhD

Objective—To determine the influence of intensified training and subsequent reduced training on glucose metabolism rate and peripheral insulin sensitivity in horses and identify potential markers indicative of early overtraining.

Animals—12 Standardbred geldings.

Procedures—Horses underwent 4 phases of treadmill-based training. In phase 1, horses were habituated to the treadmill. In phase 2, endurance training was alternated with high-intensity exercise training. In phase 3, horses were divided into control and intensified training groups. In the intensified training group, training intensity, duration, and frequency were further increased via a protocol to induce overtraining; in the control group, these factors remained unaltered. In phase 4, training intensity was reduced. Standardized exercise tests were performed after each phase and hyperinsulinemic euglycemic clamp (HEC) tests were performed after phases 2, 3, and 4.

Results—10 of 12 horses completed the study. Dissociation between mean glucose metabolism rate and mean glucose metabolism rate-to-plasma insulin concentration ratio (M:I) was evident in the intensified training group during steady state of HEC testing after phases 3 and 4. After phase 4, mean glucose metabolism rate was significantly decreased (from 31.1 ± 6.8 µmol/kg/min to 18.1 ± 3.4 µmol/kg/min), as was M:I (from 1.05 ± 0.31 to 0.62 ± 0.17) during steady state in the intensified training group, compared with phase 3 values for the same horses.

neuroendocrine system (eg, long-term increases in circulating concentrations of stress hormones or exhaustion of hormonal axes). The overtraining syndrome is rarely caused by exercise training alone, and other factors, including transport, feeding, subclinical disease, mental stress, and general management, contribute to its development.1

Symptoms associated with overtraining in humans, such as changes in emotional behavior, sleep disturbances, and hormonal dysregulation, are indicative of changes in the regulatory and coordinative functions of the hypothalamus.1,4,5 However, to our knowledge, no specific variables have been identified that can be used to reliably identify early stages of overtraining.9,8

Overtraining can be experimentally induced in Standardbreds through an intensified training protocol,7 providing an opportunity to study the pathophysiology of early overtraining and search for potential diagnostic biomarkers. The earliest physical sign of overtraining in these horses is the inability to complete intensive training, with evidence of chronic fatigue (eg, increased irritability and reluctance to exercise).7,8 Because glucose is the most important energy substrate for the CNS and the main energy substrate for muscle during increasing workloads, alterations in regulation of glucose metabolism could influence development of central (brain function) and peripheral fatigue. Therefore, this is an interesting variable to investigate in overtraining research in horses.

Hypoglycemia during exercise has been described as a very early symptom of overtraining in non-diabetic humans, possibly caused by a depletion of carbohydrate stores and decreased function of adaptive hormonal mechanisms that normally prevent hypoglycemia.14 In healthy individuals, hypoglycemia counterregulation is a multifactorial process that involves reduction of insulin secretion, increased glucagon secretion, adrenergic activation, and increased secretion of growth hormone and cortisol. Metabolically, this leads to increased glucose production (initially through glycolysis and later through hepatic gluconeogenesis), decreased glucose oxidation and storage in muscles, and increased release and use of alternative fuels, primarily free fatty acids. This also leads to hypoglycemic symptoms and hunger, which increase food intake. These systems maximize glucose availability for use by the brain.15 However, some of these mechanisms fail during overtraining.14 More precisely, impaired secretion of growth hormone and cortisol in response to insulin-induced hypoglycemia and exercise in overtrained athletes has been described.16,17 Whether changes in peripheral insulin sensitivity are also responsible for this phenomenon is not known.

In overtrained horses, very few important differences in muscle concentrations of various substrates and metabolites, buffering capacity, and muscle enzyme activities have been described in comparison with values for trained control horses.11,12,18 Most investigated variables in previous studies (changes in fiber area, number of capillaries per fiber, mitochondrial density, activity of muscle enzymes [eg, 3-hydroxyacyl dehydrogenase, citrate synthase, hexokinase, and creatine kinase], rate of glycogen utilization, and electromyography variables) could not be used to distinguish between control and overtrained horses. In contrast, muscle glycogen concentration was lower in overtrained horses than in control horses in one study,11 and intensified training was associated with increased concentrations of alpha-1-antitrypsin in muscle tissue in another study.19 As a serine protease inhibitor, alpha-1-antitrypsin can protect tissues against enzymes from inflammatory cells, especially elastase. In the latter study,19 it was hypothesized that after a period of normal training, changes in muscle tissue reflect adaptation to physical stress and that increased concentrations of alpha-1-antitrypsin in horses after intensified training are attributable to an extended period of stress. Findings in the previous studies support the assumption that overtraining starts with derangements of the neuroendocrine system, as evidenced by behavioral and hormonal changes detectable before muscle-related clinical signs are evident.

The purpose of the study reported here was to determine the influence of intensified training and subsequent reduced training on glucose metabolism and peripheral insulin sensitivity in young Standardbreds. We evaluated the variables of interest by means of an HEC test before, during, and after intensified training according to a previously described standardized protocol used to induce overtraining in horses14 in an effort to detect potential biomarkers indicative of early stages of overtraining.

Materials and Methods

Animals—Twelve Standardbred geldings (mean ± SD age at the start of the study, 20.2 months; body weight, 368 ± 45 kg) owned by the Faculty of Veterinary Medicine of the University of Utrecht were used in the study. The horses had no known history of illness or exercise problems and had not previously been involved in any kind of organized exercise or training regimen. Horses were individually housed in box stalls and fed grass silage and concentrated feed with additional vitamin supplementation; this diet met nutrient requirements for maintenance and performance (net energy, 58 MJ; range, 54 to 66 MJ). Salt blocks and water were available ad libitum. Prior to the start of experiments, the horses were acclimatized to the exercise facility and accustomed to running on the high-speed treadmill used for the study.8 Blood samples collected from these same horses during SETs and at night-time after each training phase were analyzed for a separate study9 on growth hormone secretory dynamics. Other data from some or all of these horses were also reported in several other studies.16–23 The experiments were approved by the Committee for Animal Welfare of the Utrecht University Faculty of Veterinary Medicine.

Training—Horses were trained in 2 groups of 6; 6 horses/y were trained in pairs that comprised 1 test horse and 1 age-matched control. These pairings were maintained throughout the study, and each of the 6 pairs of horses underwent identical daily routines as previously described.4 Because the inexperience of the horses caused difficulty in determining individual exercise capacities and HRmax, an estimated HRmax of 240 beats/min was used to determine training intensity (speed and inclination)
for all horses on the treadmill. This was the mean HR$_{max}$ reported for Standardbred stallions during treadmill-based training. In the present study, training intensity was adjusted on a weekly basis to achieve targeted heart rates as determined with a telemetric heart rate measuring device during training sessions. Training sessions were performed on a high-speed treadmill. Each session was preceded by a 30-minute warmup walk on a mechanical rotating horse walker followed by an 8-minute warmup on the treadmill, which consisted of 4 minutes of walking at 1.6 m/s and 4 minutes of slow trotting at 3.0 to 4.0 m/s (with no incline). Each session ended with a cool down period that included a 5-minute walk on the treadmill at each horse’s own preferred pace followed by a 30-minute walk on the horse walker. On nontraining (resting) days, horses walked for 60 minutes on the horse walker.

Training during the 4 weeks of phase 1 (habituation) consisted of the following exercise regimens: exercise 3 times/wk at 30% of estimated HR$_{max}$ for 20 to 30 min/session (week 1), exercise 4 times/wk at 30% of estimated HR$_{max}$ for 25 to 45 min/session (week 2), exercise 4 times/wk at 40% of estimated HR$_{max}$ for 30 to 45 min/session (week 3), and exercise 4 times/wk at 50% of estimated HR$_{max}$ for 35 to 45 min/session (week 4). Training in the 18 weeks of phase 2 (normal training) consisted of endurance training and HIT performed on alternate training days. Horses were exercised 4 d/wk throughout this period. Each endurance training session consisted of continuous trotting for 20 to 24 minutes at 60% of estimated HR$_{max}$ or trotting for 16 to 18 minutes at 75% of estimated HR$_{max}$. Each HIT session included three 3-minute periods of exercise or four 2-minute periods of exercise, each at 80% to 85% of estimated HR$_{max}$ interspersed by 3- or 2-minute recovery periods at 60% of estimated HR$_{max}$.

In phase 3 (normal or intensified training), 1 horse of each pair was randomly selected to undergo intensified training for 6 weeks; the other horse continued training as described for phase 2 for 6 weeks. The intensified training regimen consisted of alternating days of endurance training and HIT for 6 d/wk during the first 3 weeks. In the last 3 weeks, horses were exercised 7 d/wk, with HIT on 4 days and endurance training on 3 days. For horses undergoing intensified training, endurance training was gradually increased to 24 to 35 minutes at 60% to 75% of estimated HR$_{max}$; HIT was also gradually increased to five 3-minute periods of exercise at 80% to 85% of estimated HR$_{max}$ interspersed with 2-minute recovery periods at 60% of estimated HR$_{max}$ or to six 2-minute periods of exercise at 80% to 85% of estimated HR$_{max}$ interspersed with 1-minute recovery periods at 60% of estimated HR$_{max}$.

Training during the 4 weeks of phase 4 (reduced training) was the same for all horses. This regimen consisted of endurance training for 20 minutes at 60% of estimated HR$_{max}$ for 3 d/wk and at 70% of estimated HR$_{max}$ for 1 d/wk.

**SETs**—On the final day of phases 1, 2, 3, and 4, all horses underwent an SET. This test took place approximately 24 hours after the final exercise period. Prior to the test, horses walked for 30 minutes in the horse walker. A further warmup was provided on the treadmill that included 4 minutes of walking (1.6 m/s), 4 minutes of trotting (4.5 m/s), and 1 minute of walking (1.6 m/s). The SET then started with trotting for 20 minutes at approximately 80% of estimated HR$_{max}$ followed by a 5-minute walk at 1.5 m/s as a cool down. Heart rate was monitored constantly with the same meter used during training and continuous ECG monitoring. As described elsewhere, blood lactate concentrations and total trotting time to fatigue were also monitored. Fatigue was defined as breaking stride (from trot to canter or from trot to standing or striking) and was evaluated at a later time on videotapes made during the SET for accurate measurements of time spent on the treadmill at a trot. Speed and inclination were not modified for the different training phases. At the end of phases 3 and 4, horses in the intensified training group had signs of overtraining according to the results of studies reported elsewhere and were considered to be overtrained for purposes of the present study.

**HEC**—The HEC procedure was performed on the morning of the third day after SETs at the end of phases 2, 3, and 4 to assess glucose metabolism and peripheral insulin sensitivity. The procedure was performed as reported in earlier studies with previously described modifications. Food was withheld for 12 hours, and an IV catheter was aseptically placed in each jugular vein. One of the catheters was used for infusion of a 50% glucose solution and for insulin administration, and the other was used for blood sample collection. A priming dose of 45 mU of insulin/kg, dissolved in 30 mL of saline (0.9% NaCl) solution, was administered IV over a period of 10 minutes to induce hyperinsulinemia. Immediately after administration of the priming dose, an insulin infusion was started at a constant infusion rate of 6 mU/kg/min, and glucose infusion was started simultaneously at a constant infusion rate of 8.6 µmol/kg/min. During the insulin and glucose infusions, heparinized blood samples (3 mL) were collected every 10 minutes. Blood glucose concentration in these samples was assayed ≤ 2 minutes after collection by use of an automated analyzer, and the glucose infusion rate was adjusted when the blood glucose concentration was not within the laboratory reference range (3.9 to 5.6 mmol/L). Glucose and insulin infusions were discontinued after maintaining a steady state of blood glucose concentration for 30 minutes. Plasma insulin concentration was determined in 3 samples that were obtained during the steady state of blood glucose concentration at 10-minute intervals and transferred into lithium heparin tubes. Plasma was separated and stored at –20°C until insulin concentrations were measured by means of a radioimmunoassay kit valid for use in horses.

During the steady state of blood glucose concentration, the glucose infusion rate equals the glucose metabolism rate, provided that endogenous glucose production is completely suppressed by hyperinsulinemia. The glucose metabolism rate was computed as follows:

\[
M (\text{mmol/kg/min}) = \text{INF (mmol/kg/min)} - \text{SC (mmol/kg/min)}
\]

where \(M\) is the glucose metabolism rate, \(\text{INF}\) is the glucose infusion rate, and \(\text{SC}\) is the so-called space correction.
factor (required to adjust for glucose that has been added or removed from the extracellular volume because the plasma glucose concentration is not kept perfectly constant during HEC tests). Plasma glucose concentrations at the beginning and end of each 10-minute period were evaluated, and space correction was calculated as follows:

\[ SC \ (\text{mmol/kg/min}) = (G_2 - G_1) \times 0.019 \]

where \( G_1 \) is the plasma glucose concentration at the beginning of the 10-minute period and \( G_2 \) is the plasma glucose concentration at the end of the 10-minute period.

**Statistical analysis**—Linear regression was performed with computer software. Scatterplots were created, and strength of the linear association between the glucose metabolism rate and M:I was assessed by determining the correlation coefficient (\( r \)) and testing whether it was different from zero by use of the Pearson product moment correlation test (2 tailed). Differences in glucose metabolism rate and M:I were analyzed by means of a linear mixed-effects model with a 1-step autoregressive process. Fixed factors included horse, pair, group, and training phase. Interaction for group and training phase was calculated. Differences in trotting time during the SET between the intensified training and control groups at the end of training phases were assessed via linear mixed effect model as well. Phase 3 group assignments (intensified training and control) were applied for analysis of data for all phases. IT = Intensified training.

Values with different superscripts within a row are significantly (\( P < 0.05 \)) different. Significance of correlations was determined on the basis of the Pearson product moment correlation testing.

Table 1—Mean glucose metabolism rate, mean plasma insulin concentration, M:I, and correlation between mean glucose metabolism rate and M:I during steady state in 10 (1.5-year-old) Standardbreds at the end of 3 training phases (phases 2, 3, and 4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean glucose metabolism rate ((\mu\text{mol/kg/min}))</td>
<td>24.6 ± 7.5</td>
<td>24.9 ± 5.9</td>
<td>26.8 ± 9.2</td>
</tr>
<tr>
<td>Mean plasma insulin concentration ((\mu\text{mol/L}))</td>
<td>2.97 ± 0.47</td>
<td>3.166 ± 0.659</td>
<td>3.273 ± 0.259</td>
</tr>
<tr>
<td>M:I</td>
<td>0.88 ± 0.37</td>
<td>0.83 ± 0.28</td>
<td>0.84 ± 0.31</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.991</td>
<td>0.937</td>
<td>0.987</td>
</tr>
<tr>
<td>P value</td>
<td>0.009</td>
<td>0.019</td>
<td>0.002</td>
</tr>
</tbody>
</table>

In phase 1 (4 weeks), horses were habituated to the treadmill. In phase 2 (18 weeks), endurance training was alternated with high-intensity exercise training for all horses. In phase 3 (6 weeks), horses were divided into control (\( n = 5 \)) and intensified training (5) groups. In the intensified training group, training intensity, duration, and frequency were further increased, whereas in the control group, these remained unaltered. In phase 4 (4 weeks), training intensity was reduced to the same level for all horses. Phase 3 group assignments were applied for analysis of data for all phases.

**Results**

Training was discontinued for 1 pair of horses because of injury, and these horses were removed from the study. Data were analyzed for the remaining 10 horses that completed all phases of the 32-week training exercise program (phase 1 = habituation, 2 = normal training, 3 = normal [control group] or intensified training [intensified training group], and 4 = reduced training).

**SETs**—Results of SETs at the end of phases 3 and 4 were previously described. In the present study, these same data were reanalyzed via mixed effect modeling.
Figure 2—Correlation between the mean rate of glucose metabolism and M:I during steady state in the same 10 horses in Figure 1 following various training phases (A through F). Correlation between the 2 variables was significant for control group horses at the end of phases 2 (panel A), 3 (B), and 4 (C). Although this correlation was also significant for horses of the intensified training group at the end of phase 2 (panel D), it was nonsignificant at the end of phases 3 (E) and 4 (F). See Figure 1 for remainder of key.
resulting in different significant P values than previously reported. Horses of the intensified training group maintained trotting at high speeds (7.5 to 8.5 m/s with a treadmill inclination of 1% to 4%) during SETs at the end of phase 3 for 16.1 ± 2.3 minutes, a significantly (P = 0.004) shorter time than the 19.8 ± 0.4 minutes for control horses that continued normal training as in phase 2. At the beginning of SETs at the end of phase 3, horses in the intensified training group frequently broke stride. In addition, after 4 weeks of reduced training in phase 4, horses of the intensified training group still had a significantly (P = 0.007) shorter mean trotting time to fatigue during SETs (16.2 ± 2.3 minutes) than did horses of the control group (19.7 ± 0.76 minutes).

HEC—Overall, the HEC was stopped after 189 ± 63 minutes, associated with a mean space correction of −0.00279 ± 0.01235 L. The mean ± SD rate of glucose metabolism, plasma insulin concentration, M:1, and correlations between the mean rate of glucose metabolism and M:1 during steady state in horses at the end of training phases 2 through 4 were summarized (Table 1). In the intensified training group, mean glucose metabolism rate decreased significantly (from 31.1 ± 6.8 µmol/kg/min to 18.1 ± 3.4 µmol/kg/min; P = 0.043), as did the M:1 (from 1.05 ± 0.31 to 0.62 ± 0.17; P = 0.038), between the end of phase 3 and the end of phase 4. These variables were not significantly different between the same 2 phases for horses of the control group.

Overall correlation between mean glucose metabolism rate and M:1 at the end of phase 2 (control group and intensified training group combined; Figure 1) was 0.964 (P < 0.001). The mean rate of glucose metabolism was significantly correlated with M:1 for control horses at the end of training phases 2 through 4. This significant correlation was also found for intensified training group horses at the end of phase 2, but the correlation was nonsignificant at the end of phases 3 and 4 (Table 1, Figure 2).

Discussion

In the present study, we evaluated glucose metabolism rates and peripheral insulin sensitivity via the HEC method in healthy Standardbreds that underwent a previously described4-7 4-part training protocol (phase 1 = habituation, 2 = normal training, 3 = normal [control group] or intensified training [intensified training group], and 4 = reduced training). Phase 2 was termed normal training because we considered the horses to be exercising without excessive effort. Healthy Standardbreds that underwent intensified training in phase 3 had significant changes in glucose metabolism and peripheral insulin sensitivity as well as dissociation between the mean rate of glucose metabolism and M:1, and these changes persisted after phase 4.

As reported elsewhere,4,28 horses in the intensified training group in the present study had a 19% shorter mean treadmill trotting time to fatigue at the end of phase 3 than did control horses. Similar changes were evident at the end of phase 4.28 The decrease in performance during SETs at the end of training phases 3 and 4 was considered to fit the definition of overtraining as decreased performance with no recovery within several weeks described by Rivero et al3 and was consistent with the results of other studies.3,8,9,10,15,28-30 that supported a diagnosis of overtraining in these same horses. These included studies of the same horses, performed simultaneously with the present study and reported elsewhere, that indicated the behavior of intensified training group horses changed significantly between phases 2 and 3, and was also significantly different from that of control horses during phase 3, as measured objectively with a standardized, novel horse test. These changes were still present after phase 4. The behavioral changes observed (e.g., less interest in unfamiliar horses and a low degree of environmental responsiveness) were considered indicative of mental stress.16,28

In the overtrained state, multiple factors may cause increases or decreases in glucose metabolism, and outcomes may vary because of the complex interaction of these factors. For instance, in human subjects undergoing acute mental stress testing (5 minutes of intense mathematical calculations) when insulin-mediated glucose uptake is already stimulated, sympathetic overactivity (with associated increased plasma concentrations of adrenalin and noradrenalin) is initially accompanied by increased glucose uptake in muscle tissue.26 Chronic stress causes hyperglycemia and insulin resistance due to chronic activation of the sympathetic system and hypothalamic-pituitary-adrenal axis.28 Chronic mental stress is associated with increased uptake of glucose by the brain; this activity is mediated by blood glucose concentrations and independent of circulating insulin concentrations. Physical training is reported to improve insulin sensitivity in humans.28,31 In addition, overtraining is associated with a neuroendocrine imbalance (e.g., excessive secretion of hormones or exhaustion of hormonal axes), which hampers overall ability to predict increases or decreases in insulin sensitivity even more.

One of the most interesting findings of the present study was the loss of significant correlation between the mean rate of glucose metabolism and insulin-mediated glucose uptake (as assessed via the M:1) in horses of the intensified training group, but not the control group, at the end of training phases 3 and 4. To our knowledge, no other reports have been published regarding the correlation between the mean rate of glucose metabolism and insulin-mediated glucose uptake in overtrained horses. In our opinion, dissociation between these variables does not simply reflect changes in insulin sensitivity, and in the present study, it appeared to be associated with uncorrelated increases in glucose metabolism and insulin sensitivity during intensified training in phase 3, followed by significant decreases during reduced training in phase 4. We hypothesized that this may reflect an increased heterogeneity in glucose-insulin dose-response relationships in horses of the intensified training group, likely caused by differences in adaptation to overtraining among the horses or by differences in the degree of overtraining among horses. Another explanation might be that overtraining not only stimulates insulin-dependent glucose uptake in muscle but also leads to an insulin-independent increase in glucose transport. For instance, enhanced expression of mitochondrial uncoupling proteins can cause an insulin-independent increase in glucose uptake.32,33 In mice, it has been shown that uncoupling protein-1 expression in muscle increases glucose turnover via adenosine mo-
nophosphate–activated protein kinase uptake and metabolism. Further research is needed to investigate the causes of dissociation between glucose metabolism and insulin sensitivity in horses.

In the study reported here, intensified training of horses in phase 3 appeared to increase glucose metabolism and insulin sensitivity (M:I), but this value (1.05 ± 0.31) differed significantly only from that obtained at the end of reduced training in phase 4 (0.62 ± 0.17). Our findings are comparable to results of studies in humans, in which termination or reduction of training resulted in decreased insulin sensitivity as reflected in higher insulin concentrations after an oral glucose tolerance test. In the present study, the mean rate of glucose metabolism was significantly decreased after phase 4 in horses of the intensified training group, compared with phase 3 values. The decreased insulin sensitivity in horses of the intensified training group between these same time points was probably not fully responsible for the significant decrease in mean glucose metabolism because the correlation between mean glucose metabolism rate and M:I appeared to be weaker in phase 4 (r = 0.701; P = 0.187) than that in phase 3 (r = 0.812; P = 0.095), possibly indicating that the horses were not yet recovering. If the horses had been recovering, one would expect correlation between these values to improve. The fact that the findings suggested no improvement might reflect a time lag of the effects of intensified training in these horses.

Physical training has been shown to improve glucose tolerance and insulin action in humans, and horses. However, cessation of training seems to rapidly reverse the training-associated increase in insulin sensitivity in humans. Investigators of several studies were unable to detect effects of long-term moderate training in horses on glucose metabolism or peripheral insulin sensitivity at 48 to 72 hours, respectively, after the last period of exercise, which might indicate that training-associated increases in insulin sensitivity reverse rapidly in horses also. In the present study, no significant increase in glucose metabolism or insulin sensitivity was detected in horses of the control group 72 hours after the last exercise period at the end of phase 3. The fact that we did not detect training effects 3 days after the last training might support the idea described by Borghouts and Keizer that training potentiates the acute effect of exercise on insulin sensitivity, instead of prolonging the effect. Further studies are necessary to investigate this hypothesis in horses because muscle glycogen synthesis after glycogen-depleting exercise takes much longer in horses than in humans. In humans, glycogen synthesis in muscle occurs in 2 stages after exercise. During the first hour of recovery, there is a rapid, insulin-independent stage stimulated by glycogen depletion and muscle contraction–stimulated glucose transporter type 4 translocation, followed by a slower, insulin-dependent stage with markedly increased muscle insulin sensitivity for up to 48 hours. Increased insulin sensitivity after glycogen-depleting exercise facilitates rapid synthesis of glycogen in muscle in humans. However, in horses, no increase in insulin sensitivity was detected 24 hours after a single period of exercise in trained horses. In addition, no increase in crude muscle membrane glucose transporter type 4 content was found after exercise inducing 40% to 50% glycogen depletion, which is expected given that a horse needs up to 72 hours for complete replenishment of muscle glycogen content.

To the best of our knowledge, dissociation between the mean rate of glucose metabolism and M:I during steady state in the HEC procedure has not been previously reported in association with overtraining. Interestingly, this dissociation persisted after 4 weeks of reduced training that followed intensified training of horses in the present study, which supports a diagnosis of overtraining. Furthermore, the reduced training period of phase 4 was essential for the detection of significant differences in glucose metabolism rate and M:I during steady state in these horses after intensified training in phase 3.

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