Effects of high-intensity training on lipid metabolism in Thoroughbreds

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Objective—To investigate the effects of high-intensity training (HIT) on carbohydrate and fat metabolism in Thoroughbreds.

Animals—12 Thoroughbreds (3 to 4 years old; 6 males and 6 females).

Procedures—Horses performed HIT for 18 weeks. They ran at 90% or 110% of maximal oxygen consumption (\(\dot{V}O_{2\text{max}}\)) for 3 minutes (5 d/wk) and were subjected to incremental exercise testing (IET) before and after training. Blood samples were collected during IET, and muscle samples were obtained from the gluteus medius muscle immediately after IET. Phosphofructokinase, citrate synthase, and \(\beta-3\)-hydroxyacyl CoA dehydrogenase (\(\beta\)-HAD) activities were measured to determine glycolytic and oxidative capacities. Peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\)) and fatty acid translocase (FAT/CD36) protein contents were detected via western blotting. Metabolome analysis was performed via capillary electrophoresis–electrospray ionization mass spectrometry to measure substrate concentrations related to carbohydrate metabolism.

Results—Peak speed during IET and \(\dot{V}O_{2\text{max}}\) increased after HIT. Activities of citrate synthase and \(\beta\)-HAD increased after HIT, whereas phosphofructokinase activity remained unchanged. The PGC-1\(\alpha\) and FAT/CD36 protein contents increased after HIT, but plasma lactate concentration and the respiratory exchange ratio decreased after HIT. The plasma free fatty acid concentration increased after HIT, whereas the glucose concentration was not altered. Fructose 1,6-diphosphate, phosphoenolpyruvate, and pyruvate concentrations decreased after HIT.

Conclusions and Clinical Relevance—HIT caused an increase in oxidative capacity in equine muscle, which suggested that there was a decreased reliance on carbohydrate utilization and a concomitant shift toward fatty acid utilization during intensive exercise. (Am J Vet Res 2012;73:1813–1818)

Thoroughbreds are athletes with a high capacity for exercise. Oxidation of carbohydrates and fat provides the energy for their high level of performance. Equine skeletal muscle contains a high amount of energy stored as glycogen. This muscle glycogen is readily available during exercise and is thought to be the most important energy substrate for horses. Fat is stored primarily in adipose tissue and muscle. Although fatty acids cannot provide energy as rapidly as can carbohydrates and are less efficient per unit of oxygen than are carbohydrates, they provide much more energy per gram of wet weight than do carbohydrates. Therefore, the oxidative capacity of fatty acids may also be important for exercise performance in horses.

It is widely accepted that the transfer of fatty acids across the plasma membrane of muscle cells is primarily a protein-mediated process. Three types of membrane-associated proteins have been identified as potential fatty acid transporters: fatty acid binding protein, fatty acid transport protein, and FAT/CD36. The transfer of fatty acids into the muscle tissue of mammals has been attributed to FAT/CD36.
Similar to findings in the skeletal muscle of rodents and humans, FAT/CD36 protein has been detected in the skeletal muscle of horses. However, whether training results in an increase in FAT/CD36 content in equine muscles is not known.

Peroxisome proliferator-activated receptor-γ coactivator-1α is emerging as the master regulator of mitochondrial biogenesis and oxidative capacity in skeletal muscle. The PGC-1α protein content increases in humans and rodents as a result of acute exercise and endurance training. A single bout of exercise can increase PGC-1α mRNA in equine muscle. In another study conducted by our laboratory group, we reported that PGC-1α protein content increases with growth in equine muscle. However, whether PGC-1α protein content increases as a result of training is unknown. Because studies in humans have found that HIT effectively increases oxidative capacity, we considered it appropriate to use training of a higher intensity than that of conventional endurance training.

Metabolomics is an emerging tool used to gain insights into cellular and physiologic responses. Because increases in the maximal enzyme activities and protein contents of transporter proteins do not necessarily reflect energy metabolism during exercise, we considered it appropriate to use a metabolome differential display method based on CE-TOFMS described previously to measure muscle metabolites related to glycolysis substrates in muscle during maximal exercise.

The objective of the study reported here was to investigate changes in PGC-1α and FAT/CD36 protein contents in Thoroughbreds in response to training. We also sought to investigate changes in substrate concentrations related to carbohydrate metabolism, as determined via CE-TOFMS.

**Materials and Methods**

**Animals**—Twelve Thoroughbreds (3 to 4 years old; 6 males and 6 females) were included in the study. The pedigree of each horse was known. Horses had been raised in accordance with standard procedures of the Equine Research Institute of the Japan Racing Association for use in research investigations. All horses received concentrate and hay twice daily, in amounts in accordance with National Research Council feeding standards, and water was available ad libitum. The horses underwent surgery to translocate a carotid artery from the carotid sheath to a subcutaneous location to facilitate arterial catheterization. At least 1 month was allowed to elapse between surgery and any exercise experiments. The study protocols were reviewed and approved by the Animal Welfare and Ethics Committee of the Japan Racing Association Equine Research Institute.

**Treadmill measurements**—After translocation of the carotid artery, horses were acclimated to running on a treadmill. Horses were subjected to IET on a treadmill at an incline of 6% to identify each horse’s \( V_{\text{O2max}} \) and the speed required to attain it. Horses were fed 3 hours before IET. The IET protocol involved walking (1.7 m/s) for 2 minutes and trotting (3.5 m/s) for 5 minutes. The speed of the treadmill was then increased to 6, 8, and 10 m/s (in increments of 1 m/s at 1-minute intervals) until the horse was too exhausted to maintain its position at the front of the treadmill. During this procedure, each horse was fitted with an open-flow mask for \( V_{\text{O2max}} \) measurement. Briefly, oxygen and carbon dioxide concentrations and ambient temperature and relative humidity were measured continuously. All instrument signals were stored on a computer with an analogue-to-digital converter, and calculations were performed with a software analysis package. The mean \( V_{\text{O2}} \) for the last 15 seconds of each treadmill speed was considered the \( V_{\text{O2}} \) for that speed, and \( V_{\text{O2max}} \) was determined at a plateau point via linear regression analysis.

A catheter inserted in a jugular vein was used for collection of a venous blood sample (10 mL) during the IET; the samples were used for measurement of plasma lactate and glucose concentrations, as described elsewhere. Blood samples were centrifuged at 1,800 \( \times \) g for 10 minutes at 4°C. Plasma was harvested, and plasma lactate concentrations were measured with an automated blood lactate analyzer. Plasma free fatty acid concentration was measured via an enzymatic colorimetric technique by use of a kit as described by the manufacturer. Lactate, glucose, and free fatty acid concentrations were measured in duplicate.

**Training procedure**—Horses were subjected to HIT for 18 weeks. During the initial 10 weeks, horses ran at 90% of \( V_{\text{O2max}} \) for 3 minutes. During weeks 11 to 18, horses ran at 110% of \( V_{\text{O2max}} \) for 3 minutes. The training program consisted of a warmup period (walking at 1.7 m/s for 1 minute and trotting at 3.5 m/s for 3 minutes), cantering at 90% (or 110%) of \( V_{\text{O2max}} \) for 3 minutes, and a cooldown period (walking at 1.7 m/s for 3 minutes). Training was conducted 5 days/week on a treadmill at an incline of 6%. Horses were subjected to IET before and after the 18-week training period. The final IET was performed at least 24 hours after the last training exercise. Intensity of training was determined on the basis of the pretraining \( V_{\text{O2max}} \). Veterinarians monitored health of the horses during the training period. All horses completed the 18 weeks of training and both IETs. On days horses did not train, they were kept in a pasture.

**Muscle biopsy specimens**—Muscle biopsy specimens were obtained at rest under local anaesthesia (2% lidocaine) with a 2-mm-diameter needle from all horses from the same portion of the gluteus medius muscle and at the same depth (5 cm from the skin surface) before and after the training period. Samples for CE-TOFMS were obtained immediately after both IETs. Specimens were frozen in melting isopentane cooled by liquid nitrogen and stored at −80°C until analyzed.

**Western blotting**—Muscle specimens were homogenized, and proteins were separated via SDS-PAGE as described elsewhere. The anti–PGC-1α antibody against amino acids 777 to 797 in the carboxyl terminus of the human sequence was used as in a previous study. The anti–CD36 antibody against the human sequence near the N-terminus of CD36 was obtained; it was predicted that the anti–CD36 antibody would react with other mammalian species (ie, equine) because of sequence homology. Also, expres
Densitometric analyses of the recorded images subsequently quantified with a chemiluminescent imaging system. Personnel at a metabolomics analysis lab conducted the experiments. Activities of PFK, CS, and β-HAD were measured via standard procedures. Activities of PFK, CS, and β-HAD were measured via standard procedures. The fluorescence of the band in the final spectrum, and single bands (5 to 20 µg) for electrophoresis and the chemiluminescence were developed via enhanced chemiluminescence and subsequently quantified with a chemiluminescent imaging system. Densitometric analyses of the recorded images were performed with commercially available software. In a preliminary experiment, we confirmed there was a linear relationship between the amount of protein loaded (5 to 20 µg) for electrophoresis and the chemiluminescence of the band in the final spectrum, and single clear bands for PGC-1α and CD36 were observed.

Enzyme activities—Activities of PFK, CS, and β-HAD were measured via standard procedures. The activities were evaluated as described in other studies (PFK, CS, and β-HAD).

CE-TOFMS—All CE-TOFMS experiments were performed with a capillary electrophoresis system by personnel at a metabolomics analysis lab. The experiments were performed as previously reported. Briefly, muscle biopsy specimens (50 mg) were added to 500 µL of ice-cold methanol containing internal standards and homogenized at 4,000 rpm for 60 seconds at 4°C. Extracts then were transferred to a separate tube, mixed with 200 µL of water and 500 µL of chloroform, and centrifuged at 2,300 rpm for 5 minutes at 4°C. The aqueous layer was centrifugally filtered through a 5-kDa cutoff membrane to remove proteins. The filtrate was lyophilized, dissolved in 50 µL of water, and subjected to CE-TOFMS analysis. Separations were conducted in a fused silica capillary (inner diameter, 50 µm; length, 80 cm). Sample solutions were injected at 5 kPa for 25 seconds, and voltage of 30 kV was applied. Electrospray ionization–time-of-flight mass spectrometry was conducted in the negative ion mode; the capillary voltage was set at 3,500 V. The scan range was 50 to 1,000 m/z.

### Results

Mean ± SEM body weight was not altered by HIT (495.5 ± 9.8 kg before HIT and 500.9 ± 9.5 kg after HIT). Peak speed and VO2max during IET increased significantly after HIT. The RER at 10 m/s during IET decreased significantly; however, at exhaustion during IET, the RER was not altered after HIT (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>10 m/s</th>
<th>Exhaustion</th>
<th>Basal</th>
<th>10 m/s</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td>5.7 ± 0.1</td>
<td>6.2 ± 0.2</td>
<td>6.7 ± 0.3</td>
<td>5.7 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>0.80 ± 0.1</td>
<td>15.90 ± 2.5</td>
<td>22.30 ± 2.9</td>
<td>0.83 ± 0.1</td>
<td>6.60 ± 1.1*</td>
<td>20.50 ± 2.3</td>
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<tr>
<td>Free fatty acids (mM)</td>
<td>0.24 ± 0.02</td>
<td>ND</td>
<td>0.29 ± 0.03</td>
<td>0.26 ± 0.04</td>
<td>ND</td>
<td>0.40 ± 0.03*</td>
</tr>
</tbody>
</table>

Values reported are nmol/g of muscle tissue. See Table 1 for remainder of key.

Statistical analysis—Data were expressed as mean ± SEM. Paired t tests were used to analyze the data. Values of P < 0.05 were considered significant.

### Metabolite

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 1-phosphate</td>
<td>185 ± 22</td>
<td>108 ± 38</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>4,238 ± 462</td>
<td>2,299 ± 748</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>584 ± 70</td>
<td>359 ± 125</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate</td>
<td>1,652 ± 229</td>
<td>802 ± 196*</td>
</tr>
<tr>
<td>3-phosphoglycerate</td>
<td>343 ± 25</td>
<td>283 ± 44</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>68.0 ± 3.2</td>
<td>50.0 ± 6.0*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>192.0 ± 8.5</td>
<td>145.0 ± 9.5*</td>
</tr>
<tr>
<td>Lactate</td>
<td>46,396 ± 11,046</td>
<td>61,397 ± 7,591</td>
</tr>
</tbody>
</table>

Values reported are nmol/g of muscle tissue. See Table 1 for remainder of key.

Table 1—Mean ± SEM values for body weight, VO2max, peak speed, and RER during an IET in 12 Thoroughbreds before (untrained) and after HIT for 18 weeks (trained).

<table>
<thead>
<tr>
<th>Horses</th>
<th>Body weight (kg)</th>
<th>VO2max (mL/kg/min)</th>
<th>Peak speed (m/s)</th>
<th>10 m/s</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained</td>
<td>495.5 ± 9.8</td>
<td>162.5 ± 3.5</td>
<td>11.1 ± 0.1</td>
<td>1.08 ± 0.04</td>
<td>1.21 ± 0.03</td>
</tr>
<tr>
<td>Trained</td>
<td>500.9 ± 9.5</td>
<td>190.5 ± 2.9*</td>
<td>12.6 ± 0.2*</td>
<td>0.99 ± 0.02*</td>
<td>1.29 ± 0.02*</td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.05) from the corresponding value for the untrained horses.

Table 2—Mean ± SEM plasma concentrations of glucose, lactate, and free fatty acids during an IET in 12 Thoroughbreds before (untrained) and after HIT for 18 weeks (trained).

Table 3—Mean ± SEM values for muscle metabolites measured via CE-TOFMS metabolomics in specimens obtained at exhaustion during an IET in 4 Thoroughbreds before (untrained) and after HIT for 18 weeks (trained).
Discussion

In the study reported here, we determined that training can induce a shift from carbohydrate oxidation to fatty acid oxidation during IET in Thoroughbreds. The novel observations of this study are that after HIT for 18 weeks, skeletal muscle PGC-1α and FAT/CD36 protein expression increased; activities of CS and β-HAD increased, whereas PFK activity remained unchanged; and concentrations of muscle metabolites related to glycolysis (fructose 1,6-diphosphate, phosphoenolpyruvate, and pyruvate) measured via metabolomics analysis decreased at exhaustion during IET. Thus, these results suggested that training would induce a shift from carbohydrate oxidation to fatty acid oxidation with increases in FAT/CD36 protein content and β-HAD activity. Less glycolysis occurred during exercise, but maximal activity of PFK was not altered.

Training increases the skeletal muscle mitochondrial content and maximal capacity for carbohydrate and fat oxidation, and it permits the exercise at higher absolute power outputs. Endurance training in horses results in increases in \( V_{\text{O}_2\text{max}} \) and the oxidative enzyme activity of muscles. The present study revealed that HIT for 18 weeks resulted in an 11% increase in \( V_{\text{O}_2\text{max}} \) and 50% and 59% increases in the maximal activities of key mitochondrial enzymes (CS and β-HAD, respectively), but not a glycolytic enzyme (PFK), which was in accordance with results of earlier studies that used conventional race training in Thoroughbreds. Moreover, we found a significant increase in FAT/CD36 protein content after HIT in association with an increase in β-HAD activity in Thoroughbreds.

The capacity for increased fatty acid oxidation induced by exercise training is considered to be most closely associated with fatty acid uptake across the plasma membrane by FAT/CD36. Analysis of these data suggested that training increased the reliance on lipids as an energy source as a result of an increase in plasma free fatty acid uptake. Investigators in another study reported that FAT/CD36 protein expression is higher in red oxidative skeletal muscle than in white glycolytic skeletal muscle in horses. Because training induces a change in muscle fiber composition from fast glycolytic fibers to fast and slow oxidative fibers, the change in FAT/CD36 content in the present study may have been associated with a transition in fiber type. Regarding other metabolite transporters, investigators in a study reported an increase in glucose transporter 4 secondary to training in horses. In addition, in another study conducted by our laboratory group, we reported that monocarboxylate transporters 1 and 4, which are lactate transporters, increased after HIT. Thus, HIT increases the capacity for oxidation of both fat and carbohydrates (including lactate).

In the present study, we also found an increase in PGC-1α content in equine skeletal muscle after training. Recent studies have indicated that PGC-1α has a pivotal role in mitochondrial biogenesis in skeletal muscle. Combined with our previous result of increases in PGC-1α content with growth, the results for the present study suggest that PGC-1α plays a role as a master regulator in oxidative adaptation in horses, as has been reported in rodents and humans.

In the present study, we used metabolomics analysis (CE-TOFMS) and found that although PFK activity was not altered, concentrations of muscle metabolites related to glycolysis (fructose 1,6-diphosphate, phosphoenolpyruvate, and pyruvate) decreased at exhaustion during IET after training. In addition, fructose 6-phosphate and glucose 6-phosphate concentrations decreased slightly. It must be acknowledged that these results for CE-TOFMS were based on a relatively small sample size. However, despite this, we were able to detect significant changes in muscle metabolites related to glycolysis.

Furthermore, we detected a decrease in plasma lactate concentration at submaximal exercise intensities. The RER decreases during both high- and moderate-intensity exercise because of an increase in oxidative capacity secondary to training. Similar to results in these reports, RER during submaximal exercise intensities decreased slightly.

![Figure 1](image1.png)

**Figure 1**—Mean ± SEM PGC-1α (A) and FAT/CD36 protein (B) content in the gluteus medius muscle of 12 Thoroughbreds before (untrained) and after HIT for 18 weeks (trained). *Value differs significantly (P < 0.05) from the value for the untrained horses. AU = Arbitrary units.

![Figure 2](image2.png)

**Figure 2**—Mean ± SEM activities of PFK (A), CS (B), and β-HAD (C) in the gluteus medius muscle of 12 Thoroughbreds before (untrained) and after HIT for 18 weeks (trained). See Figure 1 for remainder of key.
ties decreased significantly after training in the present study. This decrease in RER may be attributable to the decrease in lactate production in muscles because a portion of expired carbon dioxide is derived from the buffering of lactic acid. However, decreases in plasma lactate concentrations during exercise might be attributable to increased lactate oxidation and glycogenolysis or gluconeogenesis from lactate in the liver.  

Collectively, the present study indicated that there can be significant increases in the capacity for oxidation of fatty acids in Thoroughbreds after training. These changes may contribute to increases in exercise performance of Thoroughbreds.

References

Thoroughbreds to conventional race training and detraining. 


