Effects of an intravenous endotoxin challenge on glucose and insulin dynamics in horses

Ferenc Tóth, DVM; Nicholas Frank, DVM, PhD; Sarah B. Elliott, BSc; Raymond J. Geor, BVSc, PhD; Raymond C. Boston, PhD

Objective—To evaluate the effects of endotoxin administered IV on glucose and insulin dynamics in horses.

Animals—16 healthy adult mares.

Procedures—Each week of a 2-week randomized crossover study, each horse received an IV injection (duration, 30 minutes) of Escherichia coli O55:B5 lipopolysaccharide (LPS) in 60 mL of sterile saline (0.9% NaCl) solution (20 ng/kg) or sterile saline solution alone (control treatment). Frequently sampled IV glucose tolerance test procedures were performed at 24 hours before (baseline) and 24 and 48 hours after injection; glucose and insulin dynamics were assessed via minimal model analysis.

Results—13 of 16 horses had a clinical response to LPS, which was characterized by mild colic and leukopenia. Before treatment, mean ± SD insulin sensitivity was 2.9 ± 1.9 × 10⁻⁴ L·min⁻¹·mU⁻¹; this significantly decreased to 0.9 ± 0.9 × 10⁻⁴ L·min⁻¹·mU⁻¹ 24 hours after treatment (69% reduction) and was 1.5 ± 0.9 × 10⁻⁴ L·min⁻¹·mU⁻¹ 48 hours after treatment. At baseline, mean ± SD acute insulin response to glucose was 520 ± 196 mU·min⁻¹·L⁻¹; this significantly increased to 938 ± 620 mU·min⁻¹·L⁻¹ (80% increase) and 755 ± 400 mU·min⁻¹·L⁻¹ (45% increase) at 24 and 48 hours after LPS treatment, respectively.

Conclusions and Clinical Relevance—Compared with baseline values, insulin sensitivity was decreased for 24 hours after IV injection of LPS, and affected horses had a compensatory pancreatic response. These disturbances in glucose and insulin dynamics may contribute to development of laminitis in horses. (Am J Vet Res 2008;69:82–88)

Endotoxin is a heat-stable LPS located within the outer membrane of gram-negative bacteria that can be released as a result of rapid bacterial growth or cell death. In healthy animals, endotoxin is located within the intestines and is contained there by the mucosal barrier and mucus layer. If endotoxin enters the portal blood system via active transport or passive diffusion from the intestinal tract, LPS molecules are removed from circulation by the Kupffer cells of the liver. Clinical signs of endotoxia develop when body defenses are compromised and blood endotoxin concentration increases. In horses, endotoxia is most commonly associated with gastrointestinal tract disturbances such as colic and colitis that involve bacterial overgrowth and increased intestinal wall permeability. Endotoxia develops when the mucosal barrier is compromised and there is movement of endotoxin into the blood and lymphatic system or when endotoxin passes through the serosa into the peritoneal cavity. Horses that have retained fetal membranes or that develop pleuropneumonia, wound infections, or gram-negative bacteremia are also at risk for endotoxia.

Insulin resistance is a state in which normal concentrations of insulin fail to elicit a normal physiologic response, and it develops as a consequence of endotoxia in humans and rats. This disturbance in glucose metabolism in horses is a concern because of its putative link with laminitis. Administration of a high dose of LPS (125 µg/kg) to ponies induces hyperglycemia within the first hour, followed by significant hypoglycemia, compared with blood glucose concentrations before endotoxin administration; however, plasma insulin concentrations remained unchanged. Effects of endotoxia on sensitivity to insulin are relevant because IR is a risk factor for pasture-associated lamini-
tis in ponies,\textsuperscript{11} and there is in vitro evidence that hoof laminar tissues require an adequate supply of glucose to maintain structural integrity.\textsuperscript{13} If IR is an important risk factor for laminitis, then endotoxemia may exacerbate this disturbance in glucose metabolism and further increase the risk of disease.

Administration of exogenous LPS is used to experimentally induce endotoxemia but does not represent development of the disease clinically, which may be why laminitis has not developed in LPS-treated horses.\textsuperscript{13-17} However, endotoxemia may still play a role in the development of laminitis, and this method allows physiologic responses to LPS administration to be isolated and studied. Endotoxemia has been associated with acute laminitis in 2 studies,\textsuperscript{18,19} although a causal relationship has not been established. Parsons et al\textsuperscript{18} retrospectively evaluated horses that had developed acute laminitis during hospitalization and determined that preceding the development of laminitis, those horses were 3 times as likely to have endotoxemia as horses with no clinical signs or evidence of endotoxemia detectable via routine clinico-pathologic testing. However, endotoxemia was only inferred from clinical signs and not confirmed via endotoxin assay. Sprouse et al\textsuperscript{19} also reported that plasma endotoxin concentrations significantly increased in 11 of 13 horses that developed Obel grade 3 laminitis after alimentary carbohydrate overload.

Clinical signs consistent with endotoxemia have been observed in horses that developed laminitis after administration of oligofructose.\textsuperscript{20} Horses that received 10 g of oligofructose/kg via intragastric administration developed diarrhea within 12 hours and had signs of depression, inappetence, fever, and tachycardia within the 48-hour period following treatment.\textsuperscript{21} Mean WBC and neutrophil counts initially decreased but then increased within 48 hours of oligofructose administration. These findings suggest that endotoxemia contributes to the development of laminitis associated with carbohydrate overload in horses.\textsuperscript{21} Carbohydrate overload causes an overgrowth of intestinal bacteria that lowers the intraluminal pH and increases intestinal wall permeability.\textsuperscript{22-25} Endotoxemia detected during the development of carbohydrate-induced laminitis may reflect this increase in intestinal wall permeability.\textsuperscript{19}

The purpose of the study reported here was to evaluate the effects of endotoxin administered IV on glucose and insulin dynamics in horses. We hypothesized that endotoxemia would induce alterations in glucose and insulin dynamics in horses. The FSIGT test was selected to evaluate this hypothesis because FSIGT test data can be assessed via minimal model analysis. The minimal model of glucose and insulin dynamics is a nonlinear model that uses data obtained from the FSIGT test to partition the glucose disposal into glucose- and insulin-mediated fractions.\textsuperscript{26} It provides values for Sg, SI, and AIRg.\textsuperscript{26-28} Glucose effectiveness describes the capacity of glucose to mediate its own disposal, whereas SI represents the ability of insulin to promote glucose disposal and decrease endogenous glucose production. The acute insulin response to glucose is a measure of initial-phase endogenous insulin secretion in response to exogenous glucose. The ability of pancreatic beta cells to increase insulin secretion in response to decreased insulin sensitivity is represented by the DI.\textsuperscript{29}

**Materials and Methods**

**Horses**—Sixteen healthy mares from the University of Tennessee teaching and research herd were evaluated during the study period (February to June 2006). Horses were admitted to the University of Tennessee Large Animal Hospital in pairs, and each pair of horses remained hospitalized for 14 days. Only mares were selected to eliminate differences attributable to sex. The horses were 4 to 12 years old (mean age, 9.1 years; median, 9.5 years); breeds included mixed (n = 8), Quarter Horse (3), Tennessee Walking Horse (1), Appaloosa (1), and Paint (3). Horses were weighed at the time of admission; weights ranged from 436 to 563 kg (mean weight, 493.6 kg; median, 498.6 kg). Body condition score (on a scale of 1 to 9) ranged from 4 to 6.\textsuperscript{30} The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

**Experimental design**—A randomized crossover study design with repeated measures was used; each horse received the LPS and control treatments. Eight horses were randomly selected to receive an injection of LPS during the first of the 2-week period and then an injection of sterile saline (0.9% NaCl) solution (control treatment) alone during the second week; the remaining 8 horses received the LPS and control treatments in the reverse order. Horses were weighed and physical examinations were performed on the first day (Friday), and then each horse was housed separately in 3.7 × 3.7-m stalls within the veterinary teaching hospital. Grass hay and water were provided ad libitum, and each horse was acclimated to its new environment for approximately 72 hours. Horses were evaluated during each of the 2 study weeks; each week, procedures were performed according to the same schedule. On the first day of the week (Monday), an IV catheter was placed and a sham FSIGT test was performed. An FSIGT test was performed 24 hours before treatment (Tuesday; baseline [–24 hours]), and the infusion of LPS or saline solution was administered between 12:00 and 12:30 PM on Wednesday (designated time = 0). Frequently sampled IV glucose tolerance test procedures were performed 24 (Thursday) and 48 hours (Friday) after treatment. All FSIGT tests were performed between 12:00 and 3:00 PM. Intravenous catheters were removed at the end of each study week. After the completion of the study, horses were returned to the University of Tennessee teaching and research herd.

**Lipopolysaccharide administration—**Escherichia coli O55:B5 LPS was mixed with 60 mL of sterile saline solution under a fume hood by a person (SBE) who wore gloves and used a respirator to minimize exposure. The LPS solution (20 ng/kg) or 60 mL of saline solution alone was infused via the IV catheter during a 30-minute period. Horses were observed for signs of colic and physical examination variables, including rectal temperature, heart rate, respiratory rate, mucous membrane color, and capillary refill time, were recorded every 15 minutes for the first 3 hours, then every
30 minutes for the following 3 hours, and then every 2 hours for 18 hours.

**CBC analysis**—Blood was collected from the indwelling jugular catheter into tubes containing EDTA before the LPS or saline solution infusion was initiated (commenced at time = 0) and 3 hours later (ie, 2.5 hours after completing the LPS infusion). Samples were immediately transported to the clinical pathology laboratory for CBC analysis.

**FSIGT test procedure**—On the first day of each study week, each horse was weighed and a 14-gauge polypropylene catheter was inserted into the left jugular vein. During tests, the horse was allowed access to grass hay and water ad libitum. Patency of the IV catheter was maintained between tests by injection of 5 mL of saline solution containing heparin into the catheter every 6 hours. An injection cap and infusion set were (length, 30 cm; internal diameter, 0.014 cm) were attached to the catheter. The FSIGT test procedure first described for use in horses by Hoffman et al was used. Briefly, a bolus (300 mg of glucose/kg) of a 50% (wt/vol) dextrose solution was administered to each horse via the infusion line and catheter, followed by injection of saline solution containing heparin. Blood samples were collected via the catheter immediately before and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 minutes after infusion of dextrose. At 20 minutes, regular insulin (30 mU/kg) was administered followed by another infusion of saline solution containing heparin. Blood samples were subsequently collected via the catheter at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 130, and 180 minutes after the dextrose infusion. At each time point, 3 mL of blood was withdrawn from the infusion line and discarded. A 6-mL blood sample was then collected, followed by infusion of 5 mL of saline solution containing heparin. Half the volume of the blood sample was transferred to a tube containing sodium heparin, which was discarded. The remaining blood volume was transferred to a tube containing no anticoagulant. Those samples were allowed to clot at 22°C for 1 hour, and then serum was harvested via low-speed (1,000 g) centrifugation. Plasma and serum samples were stored at −20°C until further analyzed.

**Plasma glucose and serum insulin concentrations**—Plasma glucose concentrations were measured by use of a colorimetric assay on an automated discrete analyzer. Serum insulin concentrations were determined by use of a radioimmunoassay that has been validated for use in horses. Each sample was assayed in duplicate, and intra-assay coefficients of variation < 5% or < 10% were required for acceptance of glucose and insulin assay results, respectively.

**Interpretation of FSIGT test data by use of the minimal model**—Values of SI, Sg, AIRg, and DI were calculated for each FSIGT test in accordance with the minimal model by use of commercially available software and previously described methods. Disposition index was calculated via multiplication of AIRg by SI.

**Statistical analysis**—Horses were classified as responders or nonresponders according to their response to endotoxin, and groups were compared by use of the nonparametric Mann-Whitney U test. Mixed-model ANOVA for repeated measures was performed by use of statistical software to determine the effects of treatment (LPS vs saline solution) and time (−24, 24, or 48 hours) on Sg, SI, AIRg, and DI. When a significant treatment-time effect was detected, the Bonferroni test for multiple comparisons was used to identify significant differences between least squares means. Significance was defined at a value of P < 0.05.

**Results**

The response to endotoxin was determined via observation of signs of colic and detection of leukopenia, which was defined as WBC count < 5.6 × 10^3 WBCs/µL at 3 hours after LPS administration. Compared with horses that were classified as nonresponders (n = 3), rectal temperature, heart rate, WBC count, percentage decrease (from baseline) in WBC count, and neutrophil count differed significantly in horses that were identified as responders (n = 13; Table 1). In responders, signs of colic detected within 3 hours of LPS administration included pawing, rolling, and stretching. Nonresponders did not develop signs of colic. Frequently sampled intravenous glucose tolerance tests were successfully performed, and mean ± SE plasma glucose and serum insulin concentrations were calculated (Figures 1 and 2). Data from a single FSIGT test performed 48 hours after LPS administration in 1 horse were excluded from the statistical analysis because the DI value was clearly an outlier and had a studentized residual (adjusted by dividing it by an estimate of its SD) of 7.44.

Compared with the control treatment, injection of LPS significantly decreased SI (time × treatment; P = 0.04) and increased AIRg (time × treatment; P = 0.006) over time (Table 2). Mean ± SD SI significantly decreased from 2.9 ± 1.9 × 10^{-1} Lmin^{-1} mU^{-1} at baseline (ie, prior to LPS administration) to 0.9 ± 0.9 × 10^{-4} Lmin^{-1} mU^{-1} after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly decreased from 2.9 ± 0.7 × 10^{-1} mU/L to 0.6 ± 0.5 × 10^{-2} mU/L at baseline to 3.0 ± 0.9 × 10^{-2} mU/L after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly decreased from 2.9 ± 0.7 × 10^{-1} mU/L to 0.6 ± 0.5 × 10^{-2} mU/L at baseline to 3.0 ± 0.9 × 10^{-2} mU/L after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly decreased from 2.9 ± 0.7 × 10^{-1} mU/L to 0.6 ± 0.5 × 10^{-2} mU/L at baseline to 3.0 ± 0.9 × 10^{-2} mU/L after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly decreased from 2.9 ± 0.7 × 10^{-1} mU/L to 0.6 ± 0.5 × 10^{-2} mU/L at baseline to 3.0 ± 0.9 × 10^{-2} mU/L after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly decreased from 2.9 ± 0.7 × 10^{-1} mU/L to 0.6 ± 0.5 × 10^{-2} mU/L at baseline to 3.0 ± 0.9 × 10^{-2} mU/L after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value.
increased from $520 \pm 196 \text{ mU min}^{-1} \text{L}^{-1}$ at 24 hours to $938 \pm 620 \text{ mU min}^{-1} \text{L}^{-1}$ and $712 \pm 400 \text{ mU min}^{-1} \text{L}^{-1}$ at 24 and 48 hours after LPS treatment, respectively. After LPS treatment, mean DI significantly increased from $1.4 \pm 1.0 \times 10^{-2}$ to $0.6 \pm 0.5 \times 10^{-2}$ at 24 hours after endotoxin administration. No significant treatment-time effects were detected for values of SG.

In the 3 horses that did not develop a clinical response to endotoxin administration, mean SI increased

Table 2—Mean ± SD minimal model analysis values obtained from FSIGT test data derived from 16 horses that received an IV infusion of physiologic saline solution (60 mL; control treatment) or E coli O55:B5 LPS in 60 mL of sterile saline solution (20 ng/kg) during each week of a 2-week study period according to a randomized crossover design. The FSIGT tests were conducted 24 hours before and 24 and 48 hours after administration of the injection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Time point</th>
<th>Before treatment</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI ($10^{-4}$; U min$^{-1}$ mU$^{-1}$)</td>
<td>Control</td>
<td>$3.0 \pm 1.9a$</td>
<td>$2.7 \pm 1.5a,b$</td>
<td>$2.7 \pm 1.3a$</td>
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</tr>
<tr>
<td></td>
<td>LPS</td>
<td>$2.9 \pm 1.9a,b$</td>
<td>$0.9 \pm 0.9a,b$</td>
<td>$1.5 \pm 0.9a,b$</td>
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</tr>
<tr>
<td>SG ($10^{-3}$; min$^{-1}$)</td>
<td>Control</td>
<td>$2.3 \pm 0.6a$</td>
<td>$2.5 \pm 0.8a$</td>
<td>$2.4 \pm 0.8a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>$2.3 \pm 0.6a$</td>
<td>$1.9 \pm 0.6a$</td>
<td>$2.1 \pm 0.7a$</td>
<td></td>
</tr>
<tr>
<td>AIRg (mU min$^{-1}$ L$^{-1}$)</td>
<td>Control</td>
<td>$503 \pm 260a$</td>
<td>$594 \pm 319a,b$</td>
<td>$537 \pm 275a,b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>$520 \pm 196a,b$</td>
<td>$938 \pm 620a,b$</td>
<td>$712 \pm 400a,b$</td>
<td></td>
</tr>
<tr>
<td>DI ($10^{-2}$)</td>
<td>Control</td>
<td>$1.2 \pm 0.9a$</td>
<td>$1.4 \pm 0.9a$</td>
<td>$1.3 \pm 0.9a$</td>
<td></td>
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<tr>
<td></td>
<td>LPS</td>
<td>$1.4 \pm 1.0a$</td>
<td>$0.6 \pm 0.5a$</td>
<td>$1.0 \pm 0.9a$</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean ± SD. Values with different superscripts differ significantly, as determined by use of an ANOVA for repeated measures and comparison of least squares mean values by use of a Bonferroni adjustment.*

**For variables with significant ($P < 0.05$) treatment-time effects, mean values with different superscripts differ significantly, as determined by use of an ANOVA for repeated measures and comparison of least squares mean values by use of a Bonferroni adjustment. Treatment-time effects were detected for SI ($P = 0.041$), AIRg ($P = 0.006$), and DI ($P = 0.024$).
by 12% and then decreased by 27% at 24 and 48 hours following LPS infusion, respectively, compared with the value before treatment. Compared with the pretreatment value, mean AIRg was decreased by 2% and increased by 7% at 24 and 48 hours after injection, respectively; mean Sg was decreased by 36% and increased by 14% at 24 and 48 hours after injection, respectively. The DI values did not increase or decrease from baseline by >1% after LPS administration.

Discussion

In the present study, 13 of 16 healthy horses that received E coli O55:B5 LPS via IV infusion at a dose of 20 ng/kg developed signs of mild colic and leukopenia (ie, WBC count < 5.6 X 10³ WBCs/µL). At 24 hours after LPS administration, insulin sensitivity was significantly lower than the value prior to treatment and AIRg increased to compensate for this alteration in glucose dynamics.

Signs of colic, fever, tachycardia, and leukopenia were detected in the horses that responded to LPS in the present study, and these findings are consistent with those of previous reports. In study 17 IV administration of E coli O35:B3 LPS at a dose of 30 ng/kg decreased the WBC count, compared with baseline values, and the nadir occurred 2 hours after infusion. Horses developed mild restlessness, sweating, increased respiratory effort, and tachypnea in response to LPS; mean rectal temperature increased over time, and a peak value >39°C was detected 4 hours after initiation of the endotoxin infusion. Plasma interleukin-6 and TNF-α activities were also increased in treated horses. In another study, E coli O26:B5 LPS was administered to horses at the dose used in the present study (20 ng/kg). In those horses, mean rectal temperature was approximately 39°C at 3 hours after initiation of the LPS infusion; mean heart rate also increased significantly from baseline values and peaked at approximately 50 beats/min after 1.5 hours. In that same study, neutrophil counts were assessed every 30 minutes and the lowest mean value (<3,000 cells/µL) was detected 1 hour after LPS infusion. However, similar to the finding in the responder group of the present study, the mean neutrophil count remained less than the pretreatment value for 3 hours after LPS was infused.

In the study reported here, 3 horses failed to develop signs of colic or leukopenia. This lack of response may be attributable to circulating anti-endotoxin antibodies or genetic polymorphism within intracellular LPS signal transduction pathways. Toll-like receptor-4, myeloid differentiation factor-2, and cluster differentiation factor-14 can be affected.

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concentrations. Administration of LPS significantly increases cortisol concentrations in ponies, and pretreatment of rat adipocytes with glucocorticoids inhibits insulin-mediated glucose uptake in vitro. Increased catecholamine concentrations have also been detected in humans after injection of LPS, and this response may lower SI. In an in vitro study of isolated rat muscle, physiologic concentrations of epinephrine inhibited insulin-mediated glucose uptake into tissues by modulating activation of IRS-1-associated phosphatidylinositol 3-kinase. Intravenous administration of epinephrine has also been shown to delay the ability of insulin to inhibit endogenous glucose production in humans.

Glucose effectiveness in horses was not altered by LPS administration in the present study, and values were consistent with those previously reported. In non-obese and moderately obese Thoroughbred geldings, mean ± SE Sg values of 1.43 ± 0.16 × 10^−3 min−1 and 1.59 ± 0.19 × 10^−3 min−1 have been detected, respectively, and for 46 healthy horses, a 95% confidence interval for Sg of 0.12 to 2.95 × 10^−3 min−1 has been reported. In the study reported here, mean AIRg was 83% and 45% higher than the pretreatment value at 24 and 48 hours after LPS administration, respectively. Before LPS infusion and during the week that horses received the control treatment, AIRg values were higher than the mean value of 270 μU min^-1 L^-1 detected in 46 healthy horses, but were within the 95% reference interval of 67 to 805 μU min^-1 L^-1 previously reported for this variable. Minimal model analysis values may vary between study populations because of differences in evaluation techniques and breed, sex, age, or diet of the study horses.

The results of our study have indicated that pancreatic beta cells of horses respond to endotoxin-induced IR by secreting more insulin. Compensated IR has been previously described in ponies and is recognized by the presence of hyperinsulinemia in chronically insulin-resistant animals. However, in the horses of the present study, mean DI decreased by 57% over 24 hours in resistant animals.

The presence of insulin-sensitive glucose transporter 4 protein within equine hoof tissues suggests that insulin plays an important role in glucose uptake. In vitro experiments performed with freshly isolated hoof explants have also revealed that laminae separate at the dermoepidermal junction when glucose concentrations are decreased within the tissue culture medium. Insulin resistance might also predispose horses to laminitis by inducing endothelial cell dysfunction. This may increase endothelin-1 synthesis and cause a concurrent decrease in nitric oxide production in the endothelium, resulting in vasoconstriction. It has previously been shown that endothelin-1 concentrations are higher in laminae of LPS-resistant tissue from horses with laminitis. In a recent study in horses, Eades et al. determined that digital venous blood endothelin-1 concentrations were significantly higher than baseline values at 11 hours after carbohydrate was administered to induce laminitis.

In the present study, administration of 20 ng of E coli O55:B5 LPS/kg decreased insulin sensitivity while enhancing the acute insulin response to glucose and did not alter glucose effectiveness in horses. Results suggest that endotoxemia will further compromise glucose and insulin dynamics in chronically insulin-resistant horses, which may place them at higher risk for development of laminitis.

References


