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

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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 $2.9 \pm 1.9 \times 10^{-4}$ $L \bullet min^{-1} \bullet mU^{-1}$; this significantly decreased to $0.9 \pm 0.9 \times 10^{-4}$ $L \bullet min^{-1} \bullet mU^{-1}$ 24 hours after treatment (69% reduction) and was $1.5 \pm 0.9 \times 10^{-4}$ $L \bullet min^{-1} \bullet mU^{-1}$ 48 hours after treatment. At baseline, mean ± SD acute insulin response to glucose was 520 ± 196 $mU \bullet min \bullet L^{-1}$; this significantly increased to 938 ± 620 $mU \bullet min \bullet L^{-1}$ (80% increase) and 755 ± 400 $mU \bullet min \bullet L^{-1}$ (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.[1,2] In healthy animals, endotoxin is located within the intestines and is contained there by the mucosal barrier and mucus layer.[2] 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.[1] Clinical signs of endotoxemia develop when body defenses are compromised and blood endotoxin concentration increases. In horses, endotoxemia is most commonly associated with gastrointestinal tract disturbances such as colic and colitis that involve bacterial overgrowth and increased intestinal wall permeability.[3-5] Endotoxemia 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.[3-5] Horses that have retained fetal membranes or that develop pleuropneumonia, wound infections, or gram-negative bacteremia are also at risk for endotoxemia.[1,2]

Abbreviations

LPS	Lipopolysaccharide
IR	Insulin resistance
FSIGT	Frequently sampled IV glucose tolerance
Sg	Glucose effectiveness
SI	Insulin sensitivity
AIRg	Acute insulin response to glucose
DI	Disposition index
TNF-α	Tumor necrosis factor-α
EHC	Euglycemic hyperinsulinemic clamp
IRS-1	Insulin receptor substrate-1

Insulin resistance is a state in which normal concentrations of insulin fail to elicit a normal physiologic response,[6] and it develops as a consequence of endotoxemia in humans[7] and rats.[8] 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.[9,10] Effects of endotoxemia on sensitivity to insulin are relevant because IR is a risk factor for pasture-associated lamini-

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tis in ponies,[11] and there is in vitro evidence that hoof laminar tissues require an adequate supply of glucose to maintain structural integrity.[12] 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.[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,[18,19] although a causal relationship has not been established. Parsons et al[18] retrospectively evaluated horses that had developed acute laminitis during hospitalization and determined that preceding the development of laminitis, those horses were 5 times as likely to have endotoxemia as horses with no clinical signs or evidence of endotoxemia detectable via routine clinicopathologic testing. However, endotoxemia was only inferred from clinical signs and not confirmed via endotoxin assay. Sprouse et al[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.[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.[20] 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.[21] Carbohydrate overload causes an overgrowth of intestinal bacteria that lowers the intraluminal pH and increases intestinal wall permeability.[22-25] Endotoxemia detected during the development of carbohydrate-induced laminitis may reflect this increase in intestinal wall permeability.[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.[26] It provides values for Sg, SI, and AIRg.[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.[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.[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[a] 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[b] (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[27] was used. Briefly, a bolus (300 mg of glucose/kg) of a 50% (wt/vol) dextrose solution[c] 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[d] (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, 150, 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 immediately cooled on ice and then refrigerated. 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[e] on an automated discrete analyzer.[f] Serum insulin concentrations were determined by use of a radioimmunoassay[g] that has been validated for use in horses.[31] 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[32] by use of commercially available software[h,i] and previously described methods.[27,33] 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[j] 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,[34] 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 $2.9 \pm 1.9 \times 10^{-4}$ $L \bullet min^{-1} \bullet mU^{-1}$ at baseline (ie, prior to LPS administration) to $0.9 \pm 0.9 \times 10^{-4}$ $L \bullet min^{-1} \bullet mU^{-1}$ after 24 hours (69% reduction); however, the value at 48 hours did not differ significantly from the pretreatment value. Mean AIRg significantly

Table 1—Mean ± SD physical examination variable and WBC count data in 16 horses that received *Escherichia coli* O55:B5 LPS in 60 mL of sterile physiologic saline (0.9% NaCl) solution (20 ng/kg) administered IV and developed signs of colic and leukopenia (responders) or did not respond to treatment (nonresponders).

Variable	Nonresponder group (n = 3)	Responder group (n = 13)
Maximal heart rate (beats/min)	49 ± 7	67 ± 17*
Maximal rectal temperature (°C)	38.2 ± 0.6	39.1 ± 0.5*
Decrease in WBC count at 3 hours after treatment, compared with pretreatment value (%)	9.4 ± 5.1	45.1 ± 11.1*
WBC count at 3 hours after treatment (× 10^3 cells/µL)	7.4 ± 1.4	4.0 ± 0.9*
Neutrophil count (× 10^3 cells/µL)	5.5 ± 0.7	2.3 ± 0.8*

Leukopenia was defined as WBC count < 5.6×10^3 WBCs/µL. Reference range for heart rate = 28 to 40 beats/min. Reference range for rectal temperature = 37.8° to 38.8°C. Reference range for neutrophil count = 2.9 to 8.5×10^3 cells/µL.

*For this variable, value was significantly (Mann-Whitney *U* test; $P < 0.05$) different from that of the nonresponder group.

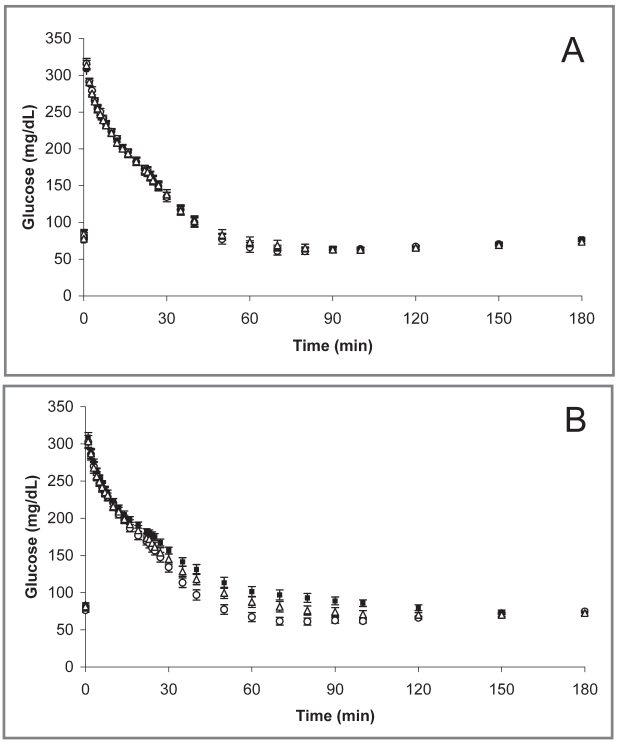

Figure 1—Mean ± SE plasma glucose concentrations during FSIGT tests performed in 16 horses that received an IV infusion of saline (0.9% NaCl) solution (60 mL) or *Escherichia coli* O55:B5 LPS (endotoxin; 20 ng/kg) in 60 mL of saline solution during the first study week and the other treatment during the second week according to a randomized crossover design. Tests were conducted 24 hours before (white circles) and 24 (black squares) and 48 hours (white triangles) after administration of saline solution (panel A) or LPS (panel B). Values derived at 48 hours after LPS infusion are calculated from 15 horses.

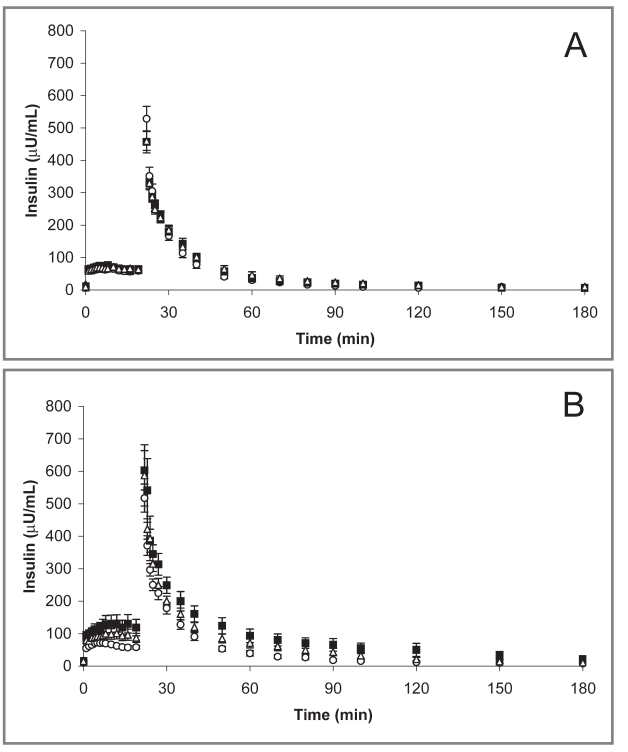

Figure 2—Mean ± SE serum insulin concentrations during FSIGT tests performed in 16 horses that received an IV infusion of saline solution (60 mL) or *E coli* O55:B5 LPS (endotoxin; 20 ng/kg) in 60 mL of saline solution during the first study week and the other treatment during the second week according to a randomized crossover design. Tests were conducted 24 hours before (white circles) and 24 (black squares) and 48 hours (white triangles) after administration of saline solution (panel A) or LPS (panel B). Values derived at 48 hours after LPS infusion are calculated from 15 horses.

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.

		Time point		
Variable	**Treatment**	**Before treatment**	**24 h**	**48 h***
SI ($\times 10^{-4}$; L•min^{-1}•mU^{-1})	Control	3.0 ± 1.9^{a}	2.7 ± 1.5^{a,b}	2.7 ± 1.3^{a,b}
	LPS	2.9 ± 1.9^{a,b}	0.9 ± 0.9^{c}	1.5 ± 0.9^{b,c}
Sg ($\times 10^{-2}$; min^{-1})	Control	2.3 ± 0.6^{a}	2.5 ± 0.8^{a}	2.4 ± 0.8^{a}
	LPS	2.3 ± 0.6^{a}	1.9 ± 0.6^{a}	2.1 ± 0.7^{a}
AIRg (mU•min•L^{-1})	Control	503 ± 260^{a}	594 ± 319^{a,b}	537 ± 275^{a,b}
	LPS	520 ± 196^{a,b}	938 ± 620^{c}	712 ± 400^{c,b}
DI ($\times 10^{-2}$)	Control	1.3 ± 0.8^{a}	1.4 ± 0.6^{a}	1.3 ± 0.6^{a}
	LPS	1.4 ± 1.0^{a}	0.6 ± 0.5^{b}	1.0 ± 0.9^{a,b}

*Mean ± SD values for the endotoxin group are calculated from data derived from 15 horses.

$^{a-c}$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 via 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 ($P = 0.024$).

increased from 520 ± 196 mU•min•L^{-1}at –24 hours to 938 ± 620 mU•min•L^{-1} and 712 ± 400 mU•min•L^{-1} at 24 and 48 hours after LPS treatment, respectively. After LPS treatment, mean DI significantly (time × treatment; $P = 0.024$) decreased from the baseline value of 1.4 ± 1.0×10^{-2} to 0.6 ± 0.5×10^{-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

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 < 5.6×10^3 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.[13-17] In 1 study,[17] IV administration of *E coli* O55:B5 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,[16] *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.[35,36] Toll-like receptor-4, myeloid differentiation factor-2, and cluster differentiation factor-14 can be affected.[35] In humans, missense mutations affecting the extracellular domain of the toll-like receptor-4 are associated with a diminished response to inhaled LPS.[35] However, a common mutation was not identified when this hypothesis was tested in horses that had blunted responses to LPS.[37]

Glucose and insulin dynamics were assessed in horses by use of FSIGT test and minimal model analysis procedures in the present study. These methods have been previously used in horses and are preferred over the EHC technique, which is an alternative method of quantifying SI.[26,27,38] One advantage of the minimal model is that it provides measures of the pancreatic response (AIRg) and ability of glucose to mediate its own disposal (Sg), as well as SI.[29] The DI, which represents the ability of pancreatic beta cells to compensate for decreased insulin sensitivity, can also be calculated.[27] Measures of AIRg, Sg, and DI are not provided by use of the EHC technique. The EHC technique is also more difficult to perform from a technical standpoint because an infusion line must remain connected to each horse throughout the 2- to 3-hour study period,[k] which provides more opportunity for equipment failure and stress.

Insulin sensitivity values prior to LPS infusion and those determined in horses during the week that they received the control treatment compared favorably with results of previous studies.[27,39] A mean SI value of 1.9×10^{-4} $L \bullet min^{-1} \bullet mU^{-1}$ was reported for nonobese Thoroughbred geldings,[27] and the mean value for a group of 46 horses was 2.1×10^{-4} $L \bullet min^{-1} \bullet mU^{-1}$.[39] In contrast, lower mean SI values of 0.39×10^{-4} $L \bullet min^{-1} \bullet mU^{-1}$ and 0.08×10^{-4} $L \bullet min^{-1} \bullet mU^{-1}$ have been detected in healthy ponies and ponies that had previously had laminitis,[40] which suggests that insulin sensitivity is generally lower in ponies than in horses.[41,42] Mean SI significantly decreased following LPS administration in the horses of the present study. At 24 hours after treatment, mean SI was significantly lower than the mean value before LPS infusion; at 48 hours after treatment, mean SI was still lower than the mean value before LPS infusion, but this difference was not significant. A biphasic glucose response has been detected after LPS administration in humans.[7] Glucose infusion rates during EHC procedures were significantly higher 120 minutes after LPS was administered, compared with rates in persons receiving saline solution, but then progressively decreased and were significantly lower than rates in persons receiving saline solution at 420 minutes after LPS administration. Endotoxemia also induces IR in rats, as evidenced by a 37% decrease in glucose disposal following LPS administration.[8] At the tissue level, continuous LPS infusion significantly decreased insulin receptor abundance, inhibited insulin-stimulated tyrosine phosphorylation of insulin receptors, and decreased the number of IRS-1 molecules within liver tissues collected from treated rats.[43] In contrast, only tyrosine phosphorylation of IRS-1 molecules was significantly affected in rat skeletal muscle tissue. Results of those studies suggest that IR can be rapidly induced through interference with tissue insulin signaling pathways; however, in those experiments, rats received markedly larger doses of LPS than those administered to horses in the study reported here.

Increases in serum TNF-α activity have been detected in the plasma of horses following the experimental induction of endotoxemia, and this cytokine may mediate the development of IR in equids.[16,17,44] Results of a study[44] involving rodents indicated that TNF-α induces serine phosphorylation of IRS-1, which disrupts the interaction of IRS-1 with the catalytic domain of the insulin receptor and inhibits insulin-stimulated activation of the phosphatidylinositol 3–kinase cascade. Alternatively, IR may develop in horses after LPS administration because of increases in circulating cortisol

concentrations. Administration of LPS significantly increases cortisol concentrations in ponies, and pretreatment of rat adipocytes with glucocorticoids inhibits insulin-mediated glucose uptake in vitro.[9,10,45] Increased catecholamine concentrations have also been detected in humans after injection of LPS, and this response may lower SI.[46] In an in vitro study[47] 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.[48]

Glucose effectiveness in horses was not altered by LPS administration in the present study, and values were consistent with those previously reported. In nonobese and moderately obese Thoroughbred geldings, mean ± SE Sg values of $1.43 \pm 0.16 \times 10^{-2}$min^{-1} and $1.59 \pm 0.19 \times 10^{-2}$min^{-1} have been detected, respectively, and for 46 healthy horses, a 95% confidence interval for Sg of 0.12 to 2.95×10^{-2}min^{-1} has been reported.[27,39] 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 mU•min•L^{-1} detected in 46 healthy horses, but were within the 95% reference interval of 67 to 805 mU•min•L^{-1} previously reported for this variable.[39] 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.[11] However, in the horses of the present study, mean DI decreased by 57% over 24 hours in response to LPS, which suggests that this compensatory response was inadequate. More time may be required for the pancreatic beta cells to fully respond to the decrease in SI, or alternatively, endotoxin may directly inhibit pancreatic function.

The data obtained in the present study may be relevant to the pathogenesis of laminitis because laminar keratinocytes appear to have a high requirement for glucose.[12] The presence of insulin-sensitive glucose transporter 4 protein within equine hoof tissues suggests that insulin plays an important role in glucose uptake.[49] 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.[12] 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.[50] It has previously been shown that endothelin-1 concentrations are higher in laminar connective tissues from horses with laminitis.[51,52] In a recent study in horses, Eades et al[52] 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.

a. Abbocath-T 14G × 140 mm, Abbott Laboratories, North Chicago, Ill.
b. Butterfly, Abbott Laboratories, North Chicago, Ill.
c. Dextrose 50% injection, Abbott Laboratories, North Chicago, Ill.
d. Humulin R, Eli Lilly & Co, Indianapolis, Ind.
e. Glucose, Roche Diagnostic Systems Inc, Somerville, NJ.
f. Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
g. Coat-A-Count insulin, Diagnostic Products Corp, Los Angeles, Calif.
h. MinMod Millenium, version 6.10, Raymond Boston, University of Pennsylvania, Kennet Square, Pa.
i. Stata 9.2, Stata Corp, College Station, Tex.
j. PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.
k. Pratt S, Geor RJ, McCutcheon LJ. Effects of diets differing in starch and fat content on insulin sensitivity during a euglycemic-hyperinsulinemic clamp (abstr). *J Vet Intern Med* 2004;18:456.

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