Effects of continuous or intermittent lipopolysaccharide administration for 48 hours on the systemic inflammatory response in horses

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Objective—To determine whether the method of lipopolysaccharide (LPS) administration (intermittent vs continuous) affects the magnitude and duration of the systemic inflammatory response in horses and whether prolonged (48 hours) endotoxemia induces laminitis.

Animals—12 healthy adult horses (10 mares and 2 geldings).

Procedures—Horses were randomly assigned to receive LPS (total dose, 80 µg; n = 4) or saline (0.9% NaCl solution, 80 mL/h; 4) via constant rate infusion or 8 bolus IV injections of LPS (10 µg, q 6 h; 4) during a 48-hour period. Physical examinations were performed every 4 hours, inflammatory cytokine gene expression was determined for blood samples obtained every 8 hours, and IV glucose tolerance tests were performed.

Results—All LPS-treated horses had signs of depression and mild colic; those signs abated as the study progressed. Administration of LPS increased expression of interleukin-1β, interleukin-6, and interleukin-8, but results were not significantly different between LPS treatment groups. Cytokine expression was significantly higher on the first day versus the second day of LPS treatment. Interleukin-1β expression was positively correlated with rectal temperature and expression of other cytokines. Glucose and insulin dynamics for both LPS groups combined did not differ significantly from those of the saline solution group. Signs of laminitis were not detected in any of the horses.

Conclusions and Clinical Relevance—Horses developed LPS tolerance within approximately 24 hours after administration was started, and the method of LPS administration did not affect the magnitude or duration of systemic inflammation. Laminitis was not induced in horses. (Am J Vet Res 2012;73:1394–1402)
Despite the theoretical involvement of endotoxemia in induction of laminitis in horses, other investigators have found that IV administration of LPS to horses does not cause laminitis. Results of one of those studies indicate that horses develop hoof discomfort following infusion of LPS into the hepatic portal vein for 24 hours, although gross pathological lesions were not identified during necropsy. The discrepancy between results of experimental studies and clinical findings for horses with disease regarding development of laminitis may be attributable to differences between experimentally induced and clinical endotoxemia. Lipopolysaccharide has typically been administered to horses as a single low-dose IV bolus in experimental studies, although administration of long-duration infusions repeated administration of boluses, and high doses of LPS have also been used. To the authors’ knowledge, other investigators have not administered continuous infusions of LPS to horses for >24 hours. In contrast, clinical endotoxemia typically persists for several days before laminitis develops. Anastodetal evidence also suggests that horses with gastrointestinal tract diseases such as bacterial colitis can have intermittent worsening of clinical signs. Such episodes may correspond to translocation of LPS or other factors from the gastrointestinal tract. Repeated exposure to inflammatory stimuli has been implicated in the pathogenesis of organ failure during sepsis; therefore, intermittent exposure to LPS could increase the magnitude of systemic inflammation and be detrimental to digital laminae.

Inflammation causes increased secretion of inflammatory cytokines and hormones that inhibit insulin-regulated cell signaling and induce insulin resistance. Therefore, measures of altered glucose and insulin homeostasis may serve as markers of systemic inflammation. Administration of a single dose of endotoxin decreases insulin sensitivity and alters glucose homeostasis in horses for 24 hours, and decreased insulin sensitivity persists for 48 hours following an 8-hour IV LPS infusion. Measures of glucose and insulin dynamics may therefore indicate the magnitude and duration of systemic inflammation.

The purpose of the study reported here was to evaluate 2 experimental methods for induction of endotoxemia in horses and to determine whether laminitis is caused by administration of LPS for 48 hours. We hypothesized that the method of LPS administration (intermittent vs continuous) would affect the magnitude and duration of the systemic inflammatory response and that prolonged (48 hours) endotoxemia would induce laminitis.

Materials and Methods

**Animals**—Twelve healthy horses (10 mares and 2 geldings; age range, 4 to 17 years; weight range, 364 to 559 kg [mean, 502 kg; median, 513 kg]) from the University of Tennessee teaching and research herd were included in the study. Breeds included Thoroughbred (n = 5), Quarter Horse (4), Paint (1), Appaloosa (1), and Arabian (1). Horses with a history of laminitis or evidence of laminitis indicated by results of physical examination were excluded. Horses were housed in stalls in the University of Tennessee Veterinary Medical Center during the study. Grass hay and water were provided ad libitum. The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

**Study design**—Each horse underwent experiments once, and experiments were conducted with horses in groups of 3. Horses were transported to the Veterinary Medical Center and allowed to acclimate to the environment for 24 hours before experimental procedures were initiated. Physical examinations were performed and body weight measurements obtained at the time horses arrived at the Veterinary Medical Center. A 14-gauge IV catheter was placed in each jugular vein of each horse, and baseline (–24-hour) IVGTTs were performed between 9:30 AM and 11:00 AM the morning after horses arrived. One of the IV catheters in each horse was used for administration of treatments (LPS or saline [0.9% NaCl] solution), and the other was used for collection of blood samples. Administration of LPS or saline solution was initiated the following day between 9:00 AM and 9:30 AM (0 hours). Horses were assigned by means of a completely randomized design to receive a CRI of LPS (n = 4) or saline solution (control horses; 4) or 8 bolus injections of LPS (n = 4) IV for 48 hours. A crossover study design could not be used because horses develop antibodies to core LPS. Blood samples (20 mL) were collected immediately prior to LPS or saline solution administration and used for a CBC, serum biochemical analysis, and assessment of whole blood inflammatory cytokine gene expression. Blood samples were obtained at 3 and 24 hours for performance of CBCs. Physical examinations were performed every 4 hours during administration of treatments, at which times horses were assessed for the development of laminitis via the Obel scoring method. Blood samples were collected every 8 hours during administration of treatments for assessment of whole blood inflammatory cytokine gene expression. Additional IVGTTs were performed at 8, 24, 32, and 48 hours. Intravenous catheters were removed at the end of the study period.

**LPS and saline solution administration**—Horses in the LPS CRI group received commercially available *Escherichia coli* O55:B5 LPS solution diluted to a final concentration of 0.021 µg/mL, which was infused via one of the IV catheters at a rate of 80 mL/h for 48 hours. Horses in the LPS bolus group received 10 µg of LPS diluted in 60 mL of sterile saline solution IV every 6 hours; LPS boluses were administered slowly during a 30-minute period. Horses in the control group received a CRI of sterile saline solution IV at a rate of 80 mL/h for 48 hours.

**IVGTT procedures**—At the start of each IVGTT, a blood sample (20 mL) was collected from each horse via one of the IV catheters into serum tubes and tubes containing sodium fluoride and potassium oxalate, and a bolus of 50% dextrose solution (100 mg/kg) was then administered IV via the other IV catheter. Additional blood samples were collected at 13, 30, 45, 60, 75, 90, 105, 120, and 150 minutes after administration of dextrose for measurement of blood glucose and insulin concentrations. Tubes containing sodium fluoride and...
potassium oxalate were immediately cooled on ice, and serum tubes were allowed to clot at room temperature (approx 22°C) for 1 hour. Tubes were centrifuged at 1,000 x g for 10 minutes, and plasma and serum were harvested and stored at −20°C until analysis.

**Measurement of blood variables**—Blood samples for CBC, fibrinogen, and biochemical analyses were collected into appropriate anticoagulant or serum tubes. Blood samples were analyzed by personnel of the University of Tennessee Clinical Pathology laboratory.

**Whole blood inflammatory cytokine gene expression**—Blood samples for quantitation of IL-1β, IL-6, IL-8, IL-10, TNF-α, and β-glucuronidase gene expression were collected into whole blood RNA collection tubes and allowed to incubate at room temperature for 8 hours, then stored at −20°C until analyzed.

Total RNA was extracted from thawed whole blood samples with a blood RNA extraction kit in accordance with the manufacturer’s protocol. Concentration and purity of RNA in each sample were determined via spectrophotometry. For each RNA sample, reverse transcription was performed with 1 µg of RNA diluted in 41.5 µL of nuclease-free water, which was added to 38.5 µL of a master mixture that contained 0.5 µL of avian myeloblastosis virus reverse transcriptase (22 U/µL), 1 L of oligo dT primer (0.5 µg/µL), 1 µL of ribonuclease inhibitor (40 U/µL), 4 µL of deoxyribonuclease triphosphate mixture (10 mM), 16 µL of magnesium chloride (25 mM), and 16 µL of avian myeloblastosis virus reverse transcription 5X buffer. Reverse transcription reactions were performed by use of a thermal cycler at 42°C for 15 minutes followed by 95°C for 5 minutes.

Real-time PCR assays were performed with validated equine-specific intron-spanning primers and probes. Briefly, cDNA samples were diluted 1:1 with 80 µL of nuclease-free water. The PCR assay mixtures contained 4.5 µL of diluted cDNA, 5 µL of reaction master mixture, and 0.5 µL of 20X primer-probe assay mixture. Samples were loaded onto plates by use of an automated system and assayed in duplicate with a real-time PCR thermal cycler. Thermal cycling parameters were 95°C for 10 minutes followed by 40 cycles of 95°C for 10 seconds and 60°C for 15 seconds. Amplification efficiencies of individual PCR reactions were between 80% and 120% as determined with computer software. The comparative cycle threshold (ΔΔCt) method was used to determine relative changes in inflammatory cytokine gene expression. Cytokine expression was normalized relative to expression of the housekeeping gene β-glucuronidase, and expression values were expressed relative to values for blood samples obtained at 0 hours; therefore, the gene expression value for each cytokine in blood samples obtained at 0 hours was 1.

**Plasma glucose and serum insulin concentrations**—Plasma glucose concentrations were measured in duplicate with a colorimetric assay via an automated discrete analyzer. Serum insulin concentrations were measured in duplicate with a radioimmunoassay that had been validated for use with equine plasma by personnel in our laboratory and by other investigators. An intra-assay coefficient of variation < 5% was required for acceptance of glucose assay results, and a value < 10% was required for acceptance of insulin assay results. The AUCg and AUCi were calculated by use of the trapezoidal method via computer software.

**Statistical analysis**—A mixed-model ANOVA for repeated measures was performed with computer software to determine effects of treatment and time on rectal temperature, heart rate, and respiratory rate. The autoregressive correlation parameter was excluded from the model for analysis of AUCg, AUCi, total WBC count, neutrophil count, lymphocyte count, and fold changes in whole blood inflammatory cytokine gene expression. Mean separation was performed with Fisher protected least significant difference test, and results were considered significant for values of P < 0.05. Gene expression data were logarithmically transformed to meet ANOVA normal distribution assumptions, and data were reported as geometric means with 95% CIs. One-sample t tests with Bonferroni corrections for multiple comparisons were used to compare mean cytokine expression at each time to the pretreatment (0-hour) value. Values of P < 0.003 were considered significant for Bonferroni corrections. Single degree of freedom contrasts were used to compare mean cytokine expression for all times on the first day of treatment versus mean cytokine expression for all times on the second day of treatment within each treatment group. Single degree of freedom contrasts were also used to compare gene expression at corresponding times (eg, 8 and 32 hours) on the first and second days of treatment within each treatment group. Body weight was initially included in the model as a covariate term but was excluded from the statistical model because results were not significant. The decision to exclude body weight was further supported by results of Spearman rank correlation coefficients and Pearson correlation coefficients, which indicated weak associations between weight and other dependent variables. Associations between cytokine expression, rectal temperature, heart rate, and respiratory rate were assessed with Spearman rank correlation coefficients. Correlation analysis was performed at the level of individual horses. Results were considered significant for values of P < 0.05 for all correlations. Data for both LPS groups of horses (CRI and bolus administration) were pooled and compared with data for the control group of horses for analysis of AUCg and AUCi values. Values of AUCg and AUCi required logarithmic transformation to meet normality requirements and were reported as geometric means with 95% CIs. All other data were reported as least squares mean ± SEM.

**Results**

**Clinical signs**—Horses in each treatment group did not differ significantly with respect to body weight or age. All LPS-treated horses had signs of depression, anorexia, and mild colic including pawing, yawning, stretching, muscle fasciculations, and lateral recumbency. Three of 4 horses in the LPS bolus group had few clinical signs attributable to LPS administration after 24 hours, although 1 of these horses had signs of lethargy until 32 hours. The other horse in the LPS bolus group
developed mild signs of colic after administration of the first 7 doses of LPS; that horse did not develop signs of colic after administration of the final dose of LPS. Signs of depression, anorexia, and colic abated in all horses in the LPS CRI group by 12 hours, with the exception of 1 horse that had a dull attitude until 32 hours. Signs of laminitis were not detected in any of the horses. All LPS-treated horses became febrile (rectal temperature > 38.5°C), and transient fever was detected at 40 hours in 1 control group horse that developed cellulitis at one of the IV catheter sites. Time (P < 0.001) and treatment × time (P < 0.001) effects were significant (Figure 1). Mean LPS CRI group rectal temperature was significantly higher from 4 to 16 hours than it was at 0 hours; maximum rectal temperature (38.9 ± 0.2°C) was detected at 8 hours. Mean LPS bolus group rectal temperature was significantly higher at 4, 8, and 20 hours than it was at 0 hour; maximum rectal temperature in these horses (39.0 ± 0.1°C) was detected at 8 hours. No increase in rectal temperature was detected for horses in the LPS CRI group after 16 hours or in the LPS bolus group after 20 hours. The mean value of the highest rectal temperature did not differ significantly between the 2 LPS treatment groups. Six of 8 horses that received LPS developed tachycardia (heart rate ≥ 48 beats/min); however, neither treatment nor time effects were significant for this variable. No significant differences in respiratory rate were detected following LPS administration.

Hematologic evaluations—Leukocyte counts were not affected by the method of LPS administration (data not shown). Leukopenia (reference range, 4.6 × 10^3 WBCs/µL to 12 × 10^3 WBCs/µL) was detected in 1 of 4 horses in the LPS CRI group and in 2 of 4 horses in the LPS bolus group. Pretreatment serum biochemical analysis results were within reference intervals for all horses.

Inflammatory cytokine gene expression—Significant effects of time were detected for expression of IL-1β (P < 0.001; Figure 2), IL-6 (P = 0.006), IL-8 (P = 0.005), and IL-10 (P = 0.009). Significant treatment × time effects were detected for IL-1β (P < 0.001), IL-8 (P = 0.047), and TNF-α (P = 0.044) expression. A significant (P = 0.029) effect of treatment was detected for expression of IL-8, but no differences were detected between results for the LPS treatment groups. Expression of IL-1β, IL-6, and IL-8 was significantly higher for both LPS treatment groups at ≥ 1 times, compared with pretreatment values (determined with 1-sample t tests), whereas IL-10 and TNF-α expression was not higher at any time compared with pretreatment values for either LPS treatment group. Except for expression of TNF-α in horses in the LPS CRI group, peak expression of cytokines was detected at 8 hours in horses in both LPS groups. Comparison of results for corresponding times on the first and second days of treatment revealed significant differences between those times for expression of IL-1β and IL-10 in horses of both LPS groups and for expression of IL-6, IL-8, and TNF-α in horses of the LPS bolus group (Table 1). Differences in IL-10 and TNF-α expression were detected between days 1 and 2 of treatment, although values were not different from pretreatment values. Comparison of mean gene expression values for all times for each day indicated expression of IL-1β (horses of both LPS groups) and IL-8, and IL-10 (horses of the LPS bolus group) was significantly higher on day 1 than it was on day 2. Expression of IL-1β and TNF-α differed significantly for the control group between days 1 and 2; however, values did not increase significantly relative to pretreatment values.

Correlations among gene expression and physical examination variables—Interleukin-1β expression was positively correlated with rectal temperature (Spearman ρ = 0.49; P < 0.001), IL-8 expression (Spearman ρ = 0.57; P < 0.001), and IL-10 expression (Spearman ρ = 0.53; P < 0.001). Other correlations with Spearman ρ coefficients between −0.37 and 0.37 were identified but were not reported. When only maximum cytokine expression values, rectal temperatures, heart rates, and respiratory rates were included in analyses, peak IL-1β expression was positively correlated with peak rectal temperature (Spearman ρ = 0.69; P < 0.013).

AUCg and AUCi—A significant (P = 0.028) effect of time was detected for AUCg (Figure 3). Values of AUCg for horses that received LPS did not differ from those for horses that received saline solution. Neither treatment nor time effects were significant for AUCi.
Discussion

Exposure to LPS induced systemic inflammation in horses in the present study, but our hypothesis was not supported because the method of LPS administration did not affect the magnitude or duration of the inflammatory response and neither method of LPS administration induced laminitis. Clinical signs attributable to LPS administration abated over time in all horses, indicating development of tolerance to endotoxin. Inflammatory cytokine expression results were comparable between LPS treatments; positive correlations were detected between IL-1β expression and rectal temperature and among expression values for IL-1β, IL-8, and IL-10.

Rectal temperature significantly increased following LPS administration to horses in the present study; this finding was consistent with results of other studies. Rectal temperature only increased in response to LPS administration on the first day of treatment, indicating horses became refractory to the effects of LPS. No increase in rectal temperature was detected in horses in the LPS CRI and LPS bolus groups after 16
When rectal temperatures ≥38.5°C were used to define clinically relevant fever, fever was detected in horses of the LPS bolus group at 4 and 8 hours and in horses of the LPS CRI group from 4 through 12 hours. Mean rectal temperatures for the control group did not indicate clinically relevant fever at any time, and the increases in rectal temperature detected in these horses at 16 and 48 hours were likely attributable to diurnal variation.30 Transient fever was detected at 40 hours in 1 horse in the control group, and this affected the mean temperature value for that group.

Subjective observations of investigators in the present study also indicated horses of both LPS groups developed tolerance to endotoxin. Attitudes of most horses were similar to pretreatment attitudes by 12 and 24 hours after initiation of treatment in the LPS CRI and LPS bolus groups, respectively. One horse in the LPS bolus group continued to have mild signs of colic and depression following administration of the first 7 of 8 LPS doses, but such signs became milder as the study progressed and were not observed after the final dose of LPS was administered. Endotoxin tolerance has been detected in horses19,31 and is attributable to a decrease in certain clinical and biochemical responses after repeated exposure to LPS.

Inflammatory mediators investigated in this study included the proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α and the anti-inflammatory cytokine IL-10, which has a regulatory role during sepsis.32 Interleukin-1β, IL-6, IL-8, and TNF-α are regulators of innate immunity,33,34 and expression of these cytokines and other cytokines involved in adaptive immune responses is altered in digital laminae, lungs, and the liver.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Group</th>
<th>Day 1 (8 h)</th>
<th>Day 2 (32 h)</th>
<th>24-h mean day 1 vs 2† Treatment × time interaction‡</th>
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<tbody>
<tr>
<td>IL-1β</td>
<td>LPS bolus</td>
<td>26 (13–50)a</td>
<td>1.9 (1.3–2.7)b</td>
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<td>LPS CRI</td>
<td>18 (5.5–62)a</td>
<td>2.9 (2.0–4.3)b</td>
<td>0.001 0.088</td>
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<td>1.1 (0.62–2.0)b</td>
<td>3.2 (1.1–4.8)c</td>
<td>0.220 0.054</td>
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<tr>
<td>IL-6</td>
<td>LPS bolus</td>
<td>5.3 (2.3–10)b</td>
<td>0.63 (0.34–2.9)d</td>
<td>0.039 0.007</td>
</tr>
<tr>
<td></td>
<td>LPS CRI</td>
<td>4.2 (1.2–15)b</td>
<td>1.8 (1.1–2.3)c</td>
<td>0.091 0.155</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.8 (1.1–2.9)b</td>
<td>2.6 (1.6–4.4)c</td>
<td>0.193 0.047</td>
</tr>
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<td>IL-8</td>
<td>LPS bolus</td>
<td>69 (21–232)b</td>
<td>10 (1.9–50)b</td>
<td>0.002 0.208</td>
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<td></td>
<td>LPS CRI</td>
<td>14 (7.0–30)c</td>
<td>6.6 (3.2–14)c</td>
<td>0.404 0.155</td>
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<td>Control</td>
<td>1.0 (0.28–4.7)c</td>
<td>1.2 (0.41–3.5)c</td>
<td>0.003 0.278</td>
</tr>
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<td>IL-10</td>
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<td>0.37 (0.10–1.3)c</td>
<td>0.002 0.208</td>
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<td>LPS CRI</td>
<td>4.9 (1.1–21)c</td>
<td>1.2 (0.43–3.1)c</td>
<td>0.278</td>
</tr>
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<td>1.4 (0.88–2.3)c</td>
<td>0.752 0.044</td>
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<td>TNF-α</td>
<td>LPS bolus</td>
<td>2.5 (0.83–7.5)c</td>
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<td>1.0 (0.20–3.6)c</td>
<td>0.990 0.074</td>
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<td>Control</td>
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<td>0.66 (0.29–1.5)c</td>
<td>0.074</td>
</tr>
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</table>

Values are geometric mean ± 95% CI.

*Data for corresponding times on days 1 (8 hours after initiation of treatment) and 2 (32 hours after initiation of treatment) were compared. †Single degree of freedom contrasts were used to compare mean cytokine expression for all times on the first day of treatment (hours 0 through 24) versus mean cytokine expression for all times on the second day of treatment (hours 32 through 48) within each treatment group. Interaction of treatment × time effects was determined via ANOVA.

# within a cytokine and treatment group, values with different superscript letters are significantly (P < 0.05) different.

Table 1—Relative expression of various cytokines in blood samples obtained from horses receiving 80 µg of LPS administered IV via CRI (n = 4), 10-µg boluses of LPS administered IV every 6 hours (4), or saline (0.9% NaCl) solution administered IV via CRI at a rate of 80 mL/h (control horses; 4) for 48 hours.
of laminitic horses.6,12 In the present study, peripheral leukocyte responses to LPS administration were assessed via real-time PCR assay of RNA extracted from whole blood samples. This method was chosen because isolation of RNA from whole blood minimizes RNA degradation and alterations in the gene expression profile during sample collection and storage versus other methods.13 One finding of the present study was that administration of LPS to horses increased IL-1β, IL-6, and IL-8 expression. Surprisingly, IL-10 and TNF-α expression did not increase significantly in horses in either LPS group, compared with pretreatment values. This finding may be attributable, in part, to the conservative nature of the Bonferroni correction used in the statistical analysis.36 Alternatively, TNF-α expression may have peaked and decreased prior to collection of blood samples at 8 hours. Expression of TNF-α can peak early during an inflammatory response and can decrease rapidly after 1 to 2 hours, even during continuous infusion of LPS and persistence of fever.13

Two potential weaknesses of our study design were identified. All horses received the same total dose of LPS, rather than doses calculated on the basis of body weights. This approach was deliberately selected to ensure that all horses received the same challenge exposure because the amount of endotoxin horses are exposed to during clinical disease does not vary with body weight. An LPS bolus dose of 10 μg was chosen because it approximates a commonly used LPS dose of 20 ng/kg for a 500-kg horse.24 Therefore, the total dose administered to each horse in the LPS bolus group was 80 μg, and the same total dose was administered to horses in the LPS CRI group. The impact of this approach on results was determined via inclusion of body weight as a covariate in all analyses; no significant effects of body weight were detected. Another weakness of the study was that blood samples were collected 2, 4, or 6 hours following LPS administration to horses in the LPS bolus group. The finding of decreased cytokine expression during the course of 1 day might therefore be attributable to longer times between LPS administration and blood sample collection. However, comparisons between results for the first and second days of treatment provided meaningful results because blood sample collection schedules were the same for both days. For example, gene expression was significantly higher for all cytokines at 8 hours on day 1 in the LPS bolus group, compared with the corresponding time (32 hours) relative to LPS administration on day 2. Direct comparisons between results for the 2 LPS treatment groups were also made for values at 24 and 48 hours of treatment; horses in each group had received the same cumulative doses of LPS at each of these times. Mean cytokine expression values for all times during each day were also calculated, and values for day 1 were compared with those for day 2 for each LPS treatment group. The finding that inflammatory cytokine expression was markedly decreased or undetectable in samples obtained from horses in both LPS treatment groups on day 2, compared with that in blood samples obtained on day 1, supported the conclusion that horses developed endotoxin tolerance.

Early-phase endotoxin tolerance develops within hours to days after exposure to LPS and is partly attributable to downregulation of factors involved in Toll-like receptor 4 signaling cascades.37,38 During development of endotoxin tolerance, secretion of proinflammatory cytokines decreases but anti-inflammatory mediator secretion and certain antimicrobial functions in cells remain unchanged. This mechanism protects cells from damage attributable to severe inflammation. The focus of the present study was determination of inflammatory cytokine expression in blood samples; gene expression in tissue samples was not evaluated. Systemic inflammation could contribute to the development of laminitis if cells in tissues do not develop endotoxin tolerance. Fulminant inflammation can persist in organs even after endotoxin tolerance develops in circulating leukocytes,36,40 and repeated exposure of leukocytes to inflammatory stimuli aggravates inflammation and worsens organ damage.41 Exposure of macrophages to xanthine oxidase–derived free radicals45 or LPS43 during early inflammation may enhance the strength of signaling via Toll-like receptor 4 during subsequent inflammatory stimulation.21 Additionally, some cells with endotoxin tolerance may retain the ability to produce proinflammatory mediators. For example, neutrophils with endotoxin tolerance can produce IL-8 in response to LPS stimulation46; IL-8 is a potent neutrophil chemotactant, so that finding is relevant to neutrophil emigration during laminitis.13 Further studies are warranted to determine whether digital laminar tissues in horses respond to multiple inflammatory events by becoming refractory or whether such events increase tissue damage attributable to inflammation.

Laminitis did not develop in any of the horses that received LPS in the present study, and this finding is consistent with results of other studies.24,45–47 Direct evidence linking endotoxemia with laminitis has not been found, to the authors’ knowledge. The magnitude and duration of endotoxemia during clinical disease is greater than it has been in experimental studies. Horses with clinical endotoxemia have circulating concentrations of toxins other than LPS that originate from the gastrointestinal tract. Gastrointestinal tracts in horses contain a diverse population of gram-negative bacteria and multiple species of gram-positive bacteria; the LPS used in experimental studies is typically derived from a single bacterial strain and is therefore only 1 component of the complex mixture of factors involved in clinical endotoxemia. Factors other than endotoxins and exotoxins might have a role in clinical disease. Vasoactive protein metabolites have been detected in the distal aspects of gastrointestinal tracts of horses,49 and concentrations of these factors increase in response to excess dietary carbohydrate intake.50 Therefore, combinations of multiple of toxins are likely responsible for development of laminitis, and LPS is likely only one of these contributing factors. Lipopolysaccharide-induced activation of platelets51 and alteration of vascular responses52,53 might increase the susceptibility of digital laminar tissues to damage caused by other factors. Tissue samples were not evaluated in the present study; LPS may induce mild inflammation in digital tissues that increases susceptibility of those tissues to damage.
caused by other factors circulating during clinical sepsis. Other investigators determined that LPS does not induce leukocyte emigration or activate matrix metalloproteases in laminar tissues collected 12 hours after administration, but vascular function and endothelial activation were not directly assessed in that study. Digital laminar biopsy specimens should be collected after administration of LPS to horses in future studies to determine expression of markers of endothelial cell dysfunction.

Lipopolysaccharide administration did not induce significant changes in measures of glucose and insulin dynamics in horses in the present study, and this finding differed from results of previous studies conducted by personnel in our laboratory. This discrepancy in results may be attributable to administration of LPS for 48 hours in the present study versus administration of a single bolus of LPS in one of the other studies or to the small number of horses used in each of those studies. The AUCg was lowest for all horses at 8 hours after initiation of treatments, compared with subsequent time points in the present study, although values at that time were not significantly lower than those at 0 hours. This finding indicated that glycemic control decreased over time in these horses. Lower insulin sensitivity has been detected in healthy horses after prolonged stall confinement, and this finding is attributed to stress. Horses in the present study were confined to stalls and underwent procedures that could have induced stress and affected glucose homeostasis.

Results of the present study indicated endotoxin tolerance developed in horses to which LPS was administered continuously or intermittently. Inflammatory cytokine expression was only determined for peripheral leukocytes; further studies would be required to determine whether similar changes in gene expression develop in tissues. Results of this study did not indicate that endotoxin has a direct causal role in the pathogenesis of laminitis, but results did indicate that LPS induced systemic inflammation in horses. Therefore, LPS may act with other factors to cause disease. Future studies are warranted to determine whether LPS predisposes digital laminar tissues to damage from other factors, potentially via effects on vascular endothelium.

References

19. Duncan SG, Meyers KM, Reed SM, et al. Alterations in coagula-

References


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