Effects of the addition of endotoxin during perfusion of isolated forelimbs of equine cadavers

Bianca Patan-Zugaj, Dr med vet; Felicia C. Gauff, DVM; Theresia F. Licka, Prof Dr med vet

Objective—To examine the effect of endotoxins on metabolism and histopathologic changes of isolated perfused equine forelimbs.

Sample—Forelimbs (comprising the metacarpus and digit) were collected from cadavers of 12 healthy adult horses after slaughter at an abattoir (14 limbs; 1 forelimb of 10 horses and both forelimbs of 2 horses).

Procedures—Forelimbs were perfused for 10 hours with autologous blood, with and without the addition of endotoxin (80 ng of lipopolysaccharide [LPS]/L). Two limbs of the endotoxin exposure group and 2 nonperfused limbs were loaded to failure of the suspensory apparatus of the pedal bone to evaluate the effect of body weight. Metabolic and histologic variables were evaluated.

Results—Blood pressure increased during the first hour and did not differ between groups. Lactate dehydrogenase activity was similar in both groups and increased significantly during the 10-hour period; glucose consumption at 5 hours and lactate concentration at 8 hours were significantly higher in limbs exposed to endotoxin. The width of secondary epidermal lamellae was greater in LPS limbs. In the primary dermal lamellae of LPS limbs, there were significantly more vessels with an open lumen and aggregates of intravascular neutrophils.

Conclusions and Clinical Relevance—In the blood-perfused isolated forelimbs of equine cadavers, exposure to LPS led to significant changes in the laminar tissue as well as to metabolic changes. Therefore, endotoxin should be considered as a causative factor for laminitis and not merely as a risk factor. (*Am J Vet Res* 2012;73:1462–1468)

Laminitis is a common, severe, and often life-threatening disease in horses.[1] Causes and pathological mechanisms of the disease are not definitively understood.[2] Risk factors (eg, insulin resistance) for and causes (eg, carbohydrate overload) of the development of clinical laminitis are widely variable, and clinical laminitis has been triggered with oral application of a large dose of easily digestible carbohydrates[3,4] or black walnut–heartwood extracts.[5] Also, induced hyperinsulinemia can cause clinical laminitis.[6] Investigators in 1 study[7] found that hepatic portal infusion of 1 mg of LPS/kg/h for 24 hours to healthy horses led to early signs of laminitis, with weight shifting and mild discomfort. Subsequently, endotoxemia was found to increase the risk of developing laminitis in hospitalized horses; however, it was found that an IV bolus application of LPS did not to lead to clinical laminitis in healthy horses.[8,9]

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From the Department for Horses and Small Animals, Veterinary University Vienna, A-1210 Vienna, Austria (Patan-Zugaj, Gauff, Licka); and the Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH25 9RG, Scotland (Licka).
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Address correspondence to Dr. Licka (theresia.licka@vetmeduni.ac.at).

Abbreviations

BM	Basement membrane
GLUT1	Glucose transporter 1
IQR	Interquartile range
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
PAS	Periodic acid–Schiff
PDL	Primary dermal lamellae
SDL	Secondary dermal lamellae
SEL	Secondary epidermal lamellae

The use of isolated perfused tissues is common, especially in drug testing for the assessment of physiologic and pharmacological[10] processes (eg, perfused isolated porcine limbs).[11] Extracorporeal perfusion of equine forelimbs with autologous blood has been successfully performed for 10 hours, without marked changes in metabolism. Histologic and ultrastructural examination of samples of the distal portions of these perfused limbs did not reveal differences from samples obtained from the distal portions of limbs immediately after slaughter.[12]

Dermoepidermal separation has been investigated in hoof lamellar explants.[13] Compared with such explants, distal portions of perfused limbs allow an even more complex assessment of the entire hoof. The pur-

pose of the study reported here was to investigate the effect of exposure to endotoxin on metabolism and histologic changes in laminar tissue of isolated perfused limbs of equine cadavers.

Materials and Methods

Sample—Fourteen forelimbs of 12 horses (1 forelimb of 10 horses and both forelimbs of 2 horses) > 1 year of age were obtained from a local abattoir for use in the study. None of the horses (8 mares and 4 geldings; mean ± SD age, 12 ± 6 years; mean body weight, 490 ± 90 kg; mean weight of the distal portion of the forelimbs, 5.6 ± 2.3 kg) had evidence of laminitis or colic at the time of slaughter. Hoof shape and size, coat, and fat distribution were assessed, and phenotypic characteristics of equine metabolic syndrome (eg, abnormal fat distribution and cresty neck) or pituitary pars intermedia dysfunction (eg, hirsutism and lack of trunk musculature) were recorded. Horses were evaluated while walking and assessed for lameness; a short clinical examination was performed to detect obvious signs of systemic disease. Horses with a body condition score between 3 and 8 (scale of 1 to 9) with a cresty neck score ≤ 3 (scale of 1 to 9) were selected.[14,15] Horses were slaughtered by use of a penetrating captive bolt followed by exsanguination, and limbs were disarticulated in the carpometacarpal joint within 7 minutes after stunning via the captive bolt. The resulting limb specimens comprised the metacarpus and digit.

Study design—For the first 10 horses, 10 forelimbs (1 forelimb/horse) were used; 5 limbs were assigned to each of the perfusion groups (control perfusion and endotoxin perfusion). For the final 2 horses, both forelimbs were used; 1 limb of each horse was assigned to the endotoxin perfusion group, and the contralateral limb served as a loading control specimen. Therefore, 7 limbs were exposed to endotoxin during perfusion and 5 were exposed only to physiologic conditions. To assess laminae separation, 2 limbs after endotoxin exposure and their fresh nonperfused contralateral limbs were loaded in a material testing machine to evaluate the effect of body weight.

Preparation for perfusion—During exsanguination, 6 to 8 L of autologous mixed arterial-venous blood was collected and mixed with heparin (5,000 U of heparin/L) to prevent coagulation. The limbs used for perfusion were placed on ice immediately after disarticulation (3 to 5 minutes after exsanguination) and remained on ice during preparation for the perfusion. At this time, the cut end of a 3-mm-diameter polyvinyl chloride tube[a] was inserted in the median artery of each limb and secured with a Chinese-fingertrap suture. The radial artery and palmar branch of the median artery were ligated. Within 10 minutes after exsanguination, limbs were flushed with ice-cold oxygenated preservation solution to remove any remaining blood and decrease the time of ischemia. The preservation solution used was a saline (0.9% NaCl) solution that contained glucose and albumin consistent with the composition of equine plasma.[12]

Blood samples and forelimb specimens were transported on ice to the laboratory. After arrival at the laboratory (90 to 120 minutes after exsanguination), 1,200 mL of plasma was separated, which required 4 to 6 L of blood. The remainder of the blood collected from each horse was stored as whole blood. For perfusion, a mixture of blood and plasma (3:2 [vol/vol]) was used as the perfusate. The perfusate for each limb was placed into 5 reservoirs (600 mL/aliquot). The perfusion was begun within 15 minutes after arrival at the laboratory (ie, 105 to 135 minutes after exsanguination).

Perfusion—Each forelimb was perfused for 10 hours. Temperature of the perfusate was 35°C. Each reservoir was used for 2 hours and then replaced by a new reservoir of perfusate. To balance glucose consumption, 25 mg of glucose/dL was added to each reservoir after the first hour of reservoir use.

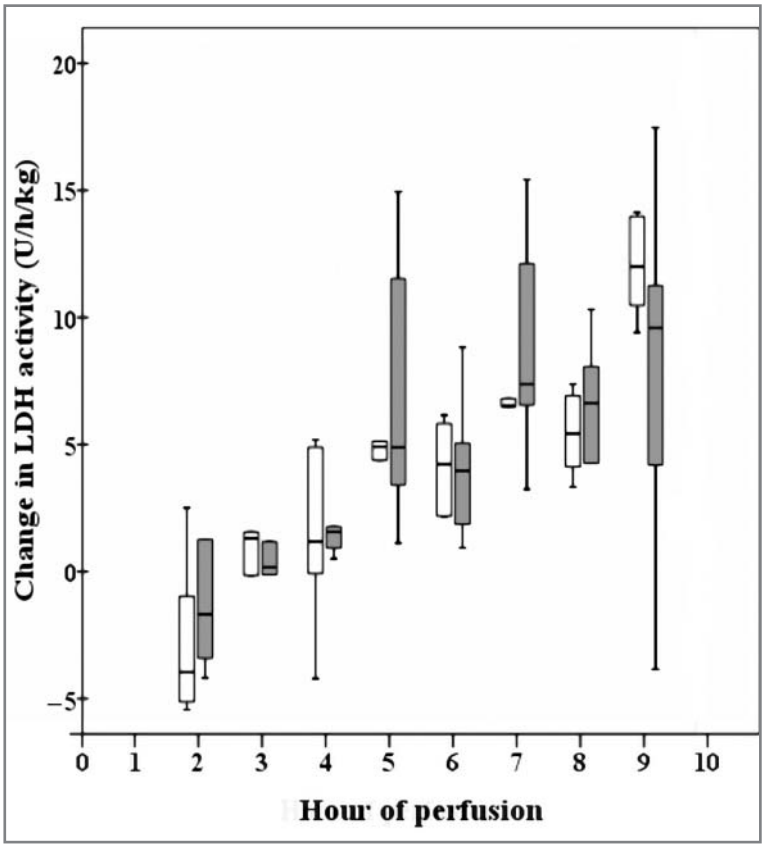

Figure 1—Box-and-whiskers plots of the change in LDH activity in the distal portions of isolated equine forelimbs perfused during a 10-hour period with autologous blood without (n = 5 [control group; white boxes]) and with the addition of 80 ng of LPS/L of perfusate (n = 7 [endotoxin group; gray boxes]). The change in LDH activity was calculated as the LDH activity measured in the plasma of the used perfusate minus the LDH activity measured in the plasma of the unused perfusate at each time point; thus, no value could be determined for the first hour. The change in LDH activity was standardized on the basis of the weight of the limbs. The box represents the second and third quartiles, the horizontal bar in each box represents the median, and the whiskers represent the first and fourth quartiles. There is no significant ($P \geq 0.05$) difference between control and endotoxin groups at any time point.

Immediately after transport, the tube in the median artery was connected to the perfusion system. The system was designed as described.[12] After an equilibration period, perfusion proceeded at a flow rate of 70 to 100 mL of perfusate/min. At this time, 48 ng (ie, 0.24 mL of a 200 ng/mL concentration of *Escherichia coli* O55:B5 endotoxin[b]) was added to the first reservoir for the forelimbs in the endotoxin exposure group. This led to a concentration of 80 ng of endotoxin/L within the perfusate. This endotoxin concentration was maintained in the subsequent 4 reservoirs in the endotoxin exposure group throughout the 2 hours of use for each reservoir. The control forelimbs were perfused for 10 hours in perfusate that did not contain endotoxin.

After samples were perfused for 10 hours, each forelimb was disconnected from the perfusion system and immediately flushed with ice-cold oxygenated preservation solution. The limbs were stored at –18°C until further processing.

Perfusion monitoring—Samples of arterial and venous blood (just prior to flow into the limb and just after flow from the limb, respectively) were collected at the start of the perfusion period (time 0) and at 60 and 120 minutes during perfusion with each reservoir. These samples were used to monitor cell viability during perfusion. Blood gas analysis, including determination of P_{O_2}, P_{CO_2}, oxygen saturation, electrolyte concentrations, pH, PCV, and hemoglobin concentration, was performed on these samples. Oxygen content and oxygen consumption of the samples were calculated as described in a previous study.[12] Glucose, lactate, and serum total protein concentrations and LDH activity were also measured in samples obtained at 0, 60, and 120 minutes for each reservoir. For these measurements, blood samples (5 mL/sample) were collected in tubes that contained lithium heparin and centrifuged at 3,000 × *g* for 5 minutes.[16] The plasma was immediately separated and stored for 12 to 24 hours at 4°C, then analyzed[c] at a certified clinical laboratory. Immediately after supplemental glucose was added to the perfusate at 60 minutes, blood samples were collected and the glucose concentration was measured again. On the basis of these measurements, glucose concentration, lactate concentration, and LDH activity were determined at 20 (glucose) or 15 (lactate and LDH) time points over 10 hours. For comparison, these values were reported in relation to the weight of the limb.

Blood pressure was recorded every 10 minutes with an electronic manometer[d] connected to the perfusion circuit via polyvinyl chloride tubing.[a] Throughout the perfusion period, limbs were positioned on an electronic scale. Mass was recorded every 30 minutes to detect possible edema formation.

To determine the effect of body weight, 2 limbs from the endotoxin exposure group were loaded immediately after perfusion; the contralateral forelimb from each of these horses was loaded immediately after transport to the laboratory and served as the control specimen. The limbs were disarticulated at the distal interphalangeal joint, the hoof was positioned with the dorsal hoof wall perpendicular to the ground, and the force transducer (3 cm × 5 mm) of a material testing machine[e] was positioned parallel to the dorsal hoof wall in the center of the articular surface of the distal phalanx. A distance of 20 mm at a speed of 0.5 mm/s was used for force measurements, and the forces exerted were recorded. After loading, the hooves were frozen at –18°C and processed in a manner similar to that of the nonloaded limbs.

Lamellar histologic examination—For the histologic examination, 2 samples of the dorsal hoof wall were collected from each frozen hoof, as described elsewhere.[12] The fixed samples were embedded in paraffin, sectioned at a thickness of 5 µm, mounted on slides, and stained with H&E and PAS stains. Slides were evaluated qualitatively, specifically to assess the appearance of the BM, fiber alignment in the SDL, and presence of neutrophils within the vessels. In the loaded samples, localization of loss of tissue cohesion was also defined. For semiquantitative evaluation, vessels with an open lumen in the axis of the PDL were counted in 5 fields of view at 4× magnification in the proximal and the distal hoof wall samples, and the mean

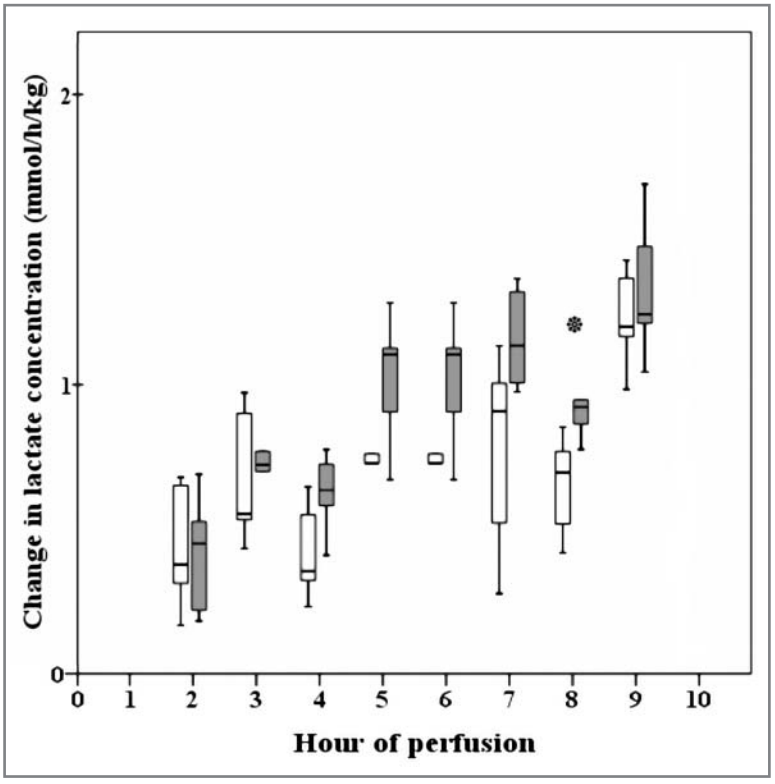

Figure 2—Box-and-whiskers plots of the change in lactate concentration in the distal portions of isolated equine forelimbs perfused during a 10-hour period with autologous blood without and with the addition of 80 ng of LPS/L of perfusate. The change in lactate concentration was calculated as the lactate concentration measured in the plasma of the used perfusate minus the lactate concentration measured in the plasma of the unused perfusate at each time point; thus, no value could be determined for the first hour. The increase in lactate concentration was standardized on the basis of the weight of the limbs. *Within a time point, the values differ significantly ($P = 0.026$) between the endotoxemia group and control group. *See* Figure 1 for remainder of key.

and SD of each sample were calculated. Additionally, width of the SEL at the midpoint between the lamellar apex and the lamellar base, defined as the minimum distance between the BMs, was measured at 20× magnification with a computer program.[f] For the proximal and distal segments, 20 SEL in the middle of a primary epidermal lamellae were counted.

Statistical analysis—Data were analyzed with a commercial software program.[g] All data were evaluated for a normal distribution via the Kolmogorov-Smirnov test. Descriptive statistics used to summarize results were expressed as mean ± SD. Nonparametric data were reported as median and IQR. Parametric data were further evaluated by means of a paired *t* test. The LDH activity, lactate concentration, and glucose concentration were analyzed by use of a repeated-measures ANOVA. Nonparametric data (vessel count and SEL width of the nonloaded samples) were compared by means of the Mann-Whitney *U* test. Correlation between changes in lactate concentration and LDH activity was tested by calculating the Pearson's correlation coefficient. Values of $P < 0.05$ were considered significant.

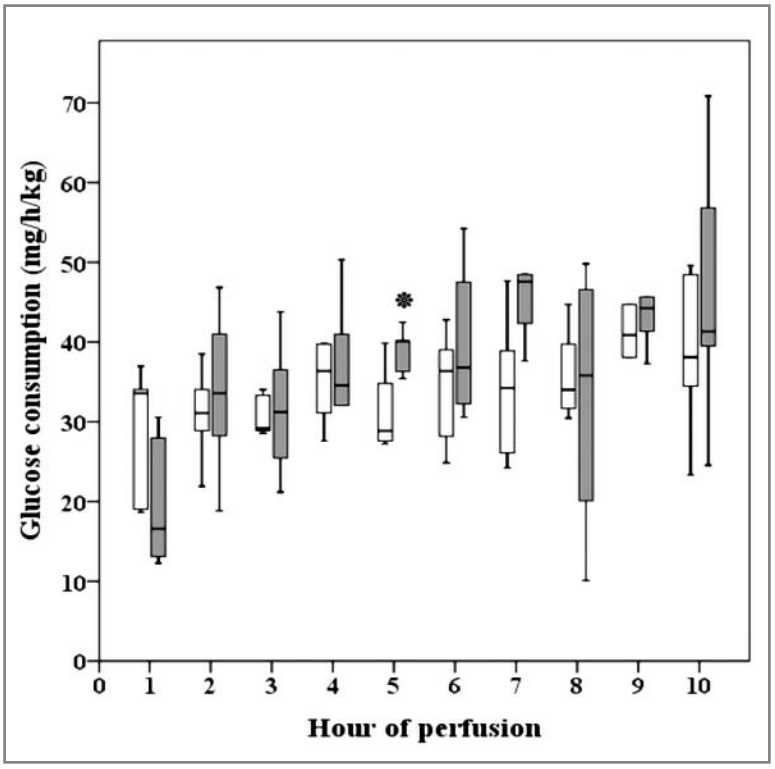

Figure 3—Box-and-whiskers plots of glucose consumption in the distal portions of isolated equine forelimbs perfused during a 10-hour period with autologous blood without and with the addition of 80 ng of LPS/L of perfusate. The glucose consumption was calculated as the glucose concentration measured in the plasma of the used perfusate minus the glucose concentration in the plasma of the unused perfusate at each time point; the glucose consumption was standardized on the basis of the weight of the forelimbs. *See* Figures 1 and 2 for key.

Results

Hemodynamic variables—Blood pressure during the first hour of perfusion was significantly ($P = 0.025$) higher in both the endotoxin exposure group (160 mm Hg; IQR, 97 mm Hg) and physiologic conditions group (148 mm Hg; IQR, 80 mm Hg) than during all subsequent hours, except for hour 7 ($P = 0.071$). After the equilibration period, median arterial blood pressure during the entire perfusion period was 101 mm Hg (IQR, 57 mm Hg) in the physiologic conditions group and 114 mm Hg (IQR, 97 mm Hg) in the endotoxin exposure group.

Metabolic variables—The LDH activity increased significantly ($P < 0.001$) during the hours of perfusion in both groups. However, the mean ± SD increase in LDH activity was not significantly higher in the endotoxin exposure group (4.5 ± 6.1 U/h/kg) than in the physiologic conditions group (3.4 ± 4.9 U/h/kg). The change in LDH activity at each hour during perfusion of the limbs did not differ significantly between the groups (**Figure 1**).

The lactate concentration increased significantly ($P = 0.019$) during the use of each reservoir. The mean ± SD increase in lactate concentration in the physiologic conditions group (0.6 ± 0.4 mmol/h/kg) was significantly ($P = 0.045$) less than that in the endotoxin exposure group (0.8 ± 0.4 mmol/h/kg). The lactate concentration was higher during the first hour of perfusion for each reservoir, compared with that during the second hour of perfusion. At 8 hours, a significant difference in lactate concentration was detected between the groups (**Figure 2**). There was a significant positive correlation between the increase in lactate concentration and the increase in LDH activity ($r = 0.709$; $P < 0.001$).

Mean ± SD glucose consumption during the 10 hours of perfusion was 33.5 ± 7.8 mg/h/kg in the physiologic conditions group and 35.7 ± 12.7 mg/h/kg in the endotoxin exposure group. Comparison of glucose consumption between the groups for each hour of perfusion revealed significantly higher glucose consumption for the endotoxin exposure group than for the physiologic conditions group at hour 5 (**Figure 3**).

Limbs obtained from 2 horses (body weight, 406 and 320 kg) required forces of 14,000 and 9,000 N, respectively, to dislocate the pedal bone from the hoof wall in the material testing machine. Forces equivalent to 3.4 and 2.8 times body weight were necessary to disrupt the suspension of the pedal bone in the limbs of the 406- and 320-kg horse, respectively, in both groups.

Histologic evaluation—Semiquantitative evaluation of the histologic pattern revealed a marked difference between the 2 groups. Mean ± SD width of the SEL in segments of the dorsal hoof wall was significantly ($P < 0.001$) greater in all specimens of the endotoxin exposure group (proximal segment, 14 ± 3 μm; distal segment, 18 ± 6 μm) than in the specimens of the physiologic conditions group (proximal segment, 10 ± 2 μm; distal segment, 13 ± 3 μm).

Mean ± SD number of vessels with an open lumen per visual field at 4× magnification in the PDL did not differ between the proximal and distal segments of the hoof wall in the physiologic conditions group (proximal segment, 2 ± 2; distal segment, 2 ± 2) and

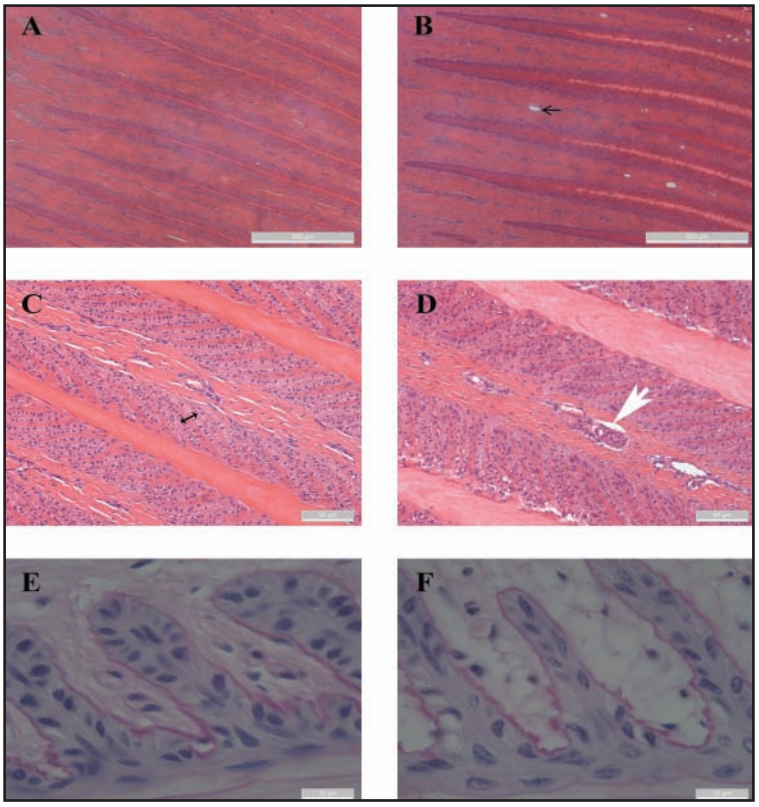

Figure 4—Photomicrographs of H&E-stained (A–D) and PAS-stained (E and F) sections of laminar tissues in the dorsal hoof wall of isolated equine forelimbs perfused during a 10-hour period with autologous blood without (A, C, and E) and with the addition of 80 ng LPS/L of perfusate (B, D, and F). Panels A–D are sections from unloaded hooves, whereas panels E and F are sections from hooves loaded to disruption of the suspensory apparatus of the pedal bone. In panels A and B, notice the difference in the number of vessels with an open lumen. A vessel lumen within a primary dermal lamella is indicated (black arrow). In panels C and D, notice the difference in the width of the SEL. The width of 1 secondary epidermal lamella is indicated (double-headed arrow). An aggregation of neutrophils (white arrow) can be seen in a vessel in the PDL in the center of panel D. In panels E and F, notice the disruption of the connective tissue within the SDL in loaded hooves, with a marked decrease in the density of connective tissue in the SDL and elongation of the SEL. Bar = 500 µm for A and B, 50 µm for C and D, and 10 µm for E and F.

endotoxin exposure group (proximal segment, 6 ± 3; distal segment, 4 ± 4). However, a significantly greater number of vessels had an open lumen in the proximal segment ($P < 0.001$) and distal segment ($P = 0.024$) of the endotoxin exposure group than in the physiologic conditions group.

Aggregates of neutrophils within vessels were evident in all specimens of the endotoxin exposure group (**Figure 4**). None of the specimens of the physiologic conditions group had aggregates of neutrophils. In the PAS-stained slides, fiber alignment of the connective tissue of the SDL was parallel and no detachment was observed in specimens of both the endotoxin exposure group and physiologic conditions group. In PAS-stained slides, the BM was evident as a well-contoured, thin, dark magenta line in specimens of the physiologic conditions group and as a blurred, light pink line in specimens of the endotoxin exposure group.

Considerable disruption of connective tissue was observed in the specimens of the loaded endotoxin exposure group. The remaining connective tissue was detached from the BM (Figure 4). In these samples, the BM formed small bubbles at the tips of the SDL, and a line of retraction of the BM at some SDL tips could be detected.

Discussion

On the basis of analysis of results of the present study, it appears likely that a persistently high concentration of endotoxins may lead to marked disturbances in metabolism of the hoof, and the potential role of endotoxin in the induction of laminitis should be investigated further. A recent in vivo study[9] of endotoxins as a potential cause of laminitis did not reveal such an effect; however, this may have been attributable to the single dose of endotoxins applied and the resulting short-term endotoxemia, considering that a continuous infusion of a much larger dose can induce some laminitic changes.[7] Rapid elimination of endotoxins has been reported in live horses after bolus administration of 3,000 ng of LPS/kg; at 3 hours after administration, only 10% of the peak value was still detectable.[17] This is markedly different from horses with colic that require hospitalization. As long as 40 hours after surgery, a mean ± SD concentration of 38.16 ± 11.99 ng of LPS/L has been measured.[18] After the initiation of carbohydrate overload–induced laminitis, endotoxin concentrations up to 81.5 ng/L were detected within 8 hours.[19] As indicated by results of the present study, a similar level of endotoxemia over a similar time frame leads to obvious changes in digital tissues.[19] This differs from oligofructose-induced laminitis in which much lower mean ± SD endotoxin concentrations of 2.4 ± 1.0 ng/L have been reported.[20] In anesthetized rats, a single dose of endotoxin (10 mg/kg, IV) leads to a reduction in blood pressure (which returns to baseline values after 2 hours), with subsequent development of hypertension; however, in studies[10,21] with anesthetized horses, the blood pressure increased > 30 minutes after a bolus injection of 20 mg of LPS/kg followed by a constant rate infusion. Those studies[10,21] involved the use of extremely high concentrations of LPS to mimic endotoxemic shock, which is a situation that cannot be compared directly with horses clinically affected with colic or perfused limbs of equine cadavers in the present study.

In the present study, addition of endotoxin (80 ng/L or 6.5 ng/kg) to the perfusate did not change the blood pressure. Sensitivity of the blood pressure measurements with regard to capillary blood flow remains unclear. In all perfused limbs in the present study, blood pressure was increased after the first hour of perfusion, independent of LPS; this was also reported in limbs perfused under physiologic conditions by other investigators.[12] In a study[22] on blood perfusion of isolated porcine limbs, total organ resistance was calculated by dividing perfusion pressure by the perfusion flow, and no increase in blood pressure was detected

after the first hour of perfusion. This may have been attributable to the fact that overall blood pressure was reduced by the vasculature in the flaccid muscles that were included with those limbs. In the distal portions of muscle-free equine limbs, an effect of acclimation of the vascular system of the limbs is possible but has not yet been proven.

However, limbs exposed to endotoxin had metabolic changes in the present study. A significant increase of glucose consumption was detected at hour 5, which was 4 hours after the onset of LPS administration.[23] A similar increase in glucose uptake was detected in a study[23] on suckling rats. Similar to results for tissues of the distal portions of equine limbs, GLUT1 is the main glucose transporter in suckling rats. Three hours after administration of *Salmonella enteritidis* LPS to suckling rats, glucose consumption increased (2.6-fold increase) significantly.[23] Future studies with the addition of endotoxin at various time points throughout a perfusion will be needed to investigate these findings and relate them to the perfusion or the addition of LPS. The subsequent increase in lactate concentration may have been a consequence of the increased glucose consumption at hour 5.

Similar to the physiologic processes in the brain, glucose in a foot is primarily metabolized to lactate, and the increases in lactate concentrations indicate that the overall metabolic situation of the distal portion of the limb is dependent on the corium. Activity of LDH in the corium is much higher than that in other tissues, which emphasizes the importance of lactate metabolism in this tissue.[24] The significant change in LDH activity over time represented an increase of LDH activity within the dying cells or an increase in the number of LDH-containing cells that were dying, or both.[25] In live horses, a bolus injection of 10,000 ng of LPS/kg leads to an increase in venous lactate concentration from 10 to 40 mg/dL at 180 minutes after injection without subsequent development of laminitis.[26] These findings could be reproduced in the distal aspects of isolated equine limbs in the present study, and addition of endotoxin led to a significant increase in lactate concentration at 8 hours of perfusion. However, changes in lactate concentrations in response to LPS cannot be compared between an isolated limb and the entire horse. In the method used in the present study, we could not mimic the complex process of in vivo endotoxemia, but we were able to investigate the toxic effect of endotoxemia on the laminar tissues and the increase in metabolism after LPS administration. At 7 to 22 hours after LPS administration to rats, GLUT1 mRNA increased 10 to 25 times,[27] which is an effect that explains the significant increase in glucose consumption for the GLUT1-mediated glucose transport system of the corium.[25]

In the control limbs, the histologic findings are similar to those of healthy hooves and to those of perfused limbs in another study.[12] In horses with clinical laminitis, the width of the SEL is reduced and the SEL is elongated[28]; however, in the forelimbs of the endotoxin exposure group in the present study, the width of the SEL was increased. These contrary findings can be explained by the absence of mechanical forces attributable to body weight that would typically cause elongation and a consequent decrease in width of the SEL. In the loaded samples, the described elongation was evident. Loads on a single forelimb in an acutely laminitic horse during the phase of weight shifting were detected at approximately 28% of body weight,[29] which gradually wears down the suspensory apparatus of the pedal bone. In the present study, much higher loads were needed to dislodge the pedal bones in a single loading cycle, as expected. The single loading cycle was chosen over repeated loading with 28% of body weight over several hours; this allowed us to obtain a measurement within 10 minutes after ending the perfusion but before there was marked tissue degeneration.

In forelimbs of the endotoxin exposure group, there was a greater number of vessels with an open lumen, which indicated vasodilatation. This is not necessarily in disagreement with the ultrasonographically determined vasoconstriction in the much larger palmar digital arteries and veins at 75 minutes after LPS bolus injection (30 ng/kg) in live horses,[30] given that those vessels may have been contracting and relaxing independently. It has been proposed[4] that there is vasodilatation during the development of laminitis, which reduces lamellar perfusion by widening arteriovenous shunts. This is supported in part by the findings of the present study in that an increase in the number of vessels with an open lumen in the PDL was seen in the endotoxin exposure group and epidermal lamellae samples. However, perfusion of capillaries and arteriovenous shunts was not assessed.

Another important finding in the histologic evaluation was the presence of neutrophil aggregates. This has been reported in the onset phase of clinical laminitis (32 hours after carbohydrate overload) in H&E-stained slices of the laminar epidermis.[31] More recently, studies[20,32] have revealed activation of platelets and neutrophils in the developmental stage of induced clinical laminitis. Activity of equine neutrophil elastase in the laminar tissue increases significantly as early as 3 hours after induction of laminitis with black walnut–heartwood extract.[32] Activation of platelets via p38 mitogen-activated protein kinase has been detected 12 hours after induction of laminitis with oligofructose, which is 8 to 18 hours before the first clinical signs were observed.[20] Although the exact mechanism of the involvement of neutrophils in the pathogenesis of laminitis remains unclear, the findings of the present study indicated that they play a relevant role during the early stages of laminitis.

In the study reported here, isolated equine forelimbs were successfully used to investigate endotoxin-induced changes of laminar tissue as well as metabolic changes of the distal portions of equine limbs. The main advantage of this method is that it allowed a focused investigation of a single factor, in this case the toxic effect of endotoxin on the distal portions of the limbs. Marked histologic changes were detected after the addition of endotoxin to the perfusate. Therefore continuous high-level endotoxemia should be considered as a causative factor for laminitis and not merely as a risk factor.

a. Heidelberger extension tubing, B. Braun, Melsungen, Germany.
b. Sigma-Aldrich Handels GmbH, Vienna, Austria.
c. Hitachi 911, Roche, Vienna, Austria.
d. Digital–Manometer GDH 200–13, Greisinger Electronic GmbH, Regenstaf, Germany.
e. Material testing machine, Walter & Bai AG, Loehningen, Switzerland.
f. ImageJ, version 1.45s, National Institutes of Health, Bethesda, Md. Available at: rsbweb.nih.gov/ij/index.html. Accessed Mar 1, 2011.
g. PASW statistics, version 17, SPSS Inc, Chicago, Ill.

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