Evaluation of the induction of vasoactive mediators from equine digital vein endothelial cells by endotoxin

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**Objective**—To determine the effect of endotoxin (lipopolysaccharide [LPS]) on vasoactive mediator production by cultured equine digital vein endothelial cells (EDVECs).

**Sample Population**—EDVECs obtained from forelimb digital veins of 7 healthy adult horses.

**Procedures**—EDVECs were incubated with or without LPS (1 µg/mL) for 0, 2, 4, 6, 22, and 24 hours. The EDVECs were incubated for 18 hours with LPS (10 pg/mL to 1 µg/mL) with or without ibuprofen, cycloheximide, or L-nitroarginine methyl ester. Medium concentrations of prostacyclin, cyclic guanosine monophosphate, endothelin-1, and thromboxane A2 were determined. Changes in inducible nitric oxide synthase and cyclooxygenase-2 expression were determined.

**Results**—LPS stimulated mean 4.2- and 14.1-fold increases in EDVEC prostacyclin and cyclic guanosine monophosphate production, respectively, after 22 hours. These effects were LPS concentration–dependent (LPS concentrations that induced a response halfway between the maximum response and baseline of 1.50 and 1.22 ng/mL, respectively). The LPS-induced cyclic guanosine monophosphate production was significantly inhibited (to basal concentrations) by L-nitroarginine methyl ester, and prostacyclin production was inhibited by cycloheximide and ibuprofen. Production of thromboxane A2 by EDVECs was not detected. Endothelin-1 accumulated in the medium, but LPS did not enhance its production. Inducible nitric oxide synthase expression in EDVECs was not detected with the available antibodies, whereas LPS stimulated cyclooxygenase-2 expression in a time- and concentration-dependent manner.

**Conclusions and Clinical Relevance**—LPS stimulated vasoactive mediator production by equine endothelial cells, which may play a role in LPS-induced digital hypoperfusion. (Am J Vet Res 2008;69:349–355)

Although the precise mechanisms underlying the pathogenesis of laminitis in horses remain unclear, the prodromal stages appear to be associated with concurrent inflammatory and microvascular perturbations.1 The role of endotoxin (ie, LPS) has long been debated because laminitis is a sequel to certain forms of colic, and endotoxin can be detected in the plasma of horses given carbohydrate overload at the onset of Obel grade-3 lameness.4 Lipopolysaccharide exposure initiates multiple pathophysiologic events, including cellular activation and subsequent systemic release of inflammatory and vasoactive mediators.5 Vascular dysfunction plays a central role in the etiology of both laminitis6,7 and endotoxemia, 8,9 and LPS may damage the vascular endothelium directly10 or indirectly via the effects of endogenous vasoactive or inflammatory mediator production.11

Vascular tone is influenced by circulating endogenous vasoactive substances and by mediators produced locally within the vascular wall (particularly by the endothelium), which cause smooth muscle cells to change shape or express proteins including cytokines and vasoactive mediators.12 Thus, the endothelium plays an important role in the normal physiologic control of vascular tone and in the hemodynamic changes that develop in pathologic states including endotoxemia. Despite this, the role of endothelium-derived mediators in equine digital perfusion regulation and pathologic states associated with endotoxemia and digital hypoperfusion, such as laminitis, remains poorly understood.

**Abbreviations**

- LPS: Lipopolysaccharide
- PGI2: Prostaglandin I2
- NO: Nitric oxide
- COX: Cyclooxygenase
- eNOS: Endothelial nitric oxide synthase
- iNOS: Inducible nitric oxide synthase
- ET-1: Endothelin-1
- TxA2: Thromboxane A2
- EDVEC: Equine digital vein endothelial cell
- DMEM: Dulbecco modified Eagle medium
- cGMP: Cyclic guanosine monophosphate
- L-NAME: L-nitroarginine methyl ester

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Endothelial cells release vasoactive mediators in response to several physical and chemical stimuli. Vasodilator mediators include prostacyclin (i.e., PGI₂) and NO, which are produced by the enzymes COX and NOS, respectively. These enzymes exist in multiple isoforms and endothelial cells express constitutively the isofoms COX-1 and eNOS, and also the inducible isofoms COX-2 and iNOS, which are expressed after cellular stimulation by agents, including LPS, resulting in increased mediator synthesis. Lipopolysaccharide (5 to 100 µg/mL) dose-dependently stimulates increased equine pulmonary vascular endothelial cell PGI₂ production, whereas LPS (20 to 160 µg/mL) increases equine digital artery smooth muscle cell COX-2 expression in a non–dose-dependent manner.

Endothelium-derived vasoconstrictors include ET-1 and TxA₂. Endothelin-1 is the most potent endogenous vasoconstrictor yet to be identified and interacts with endothelial-derived relaxing factors such as NO to maintain normal local vascular flow. Plasma ET-1 concentrations are increased in certain diseases associated with vascular injury, including endotoxia in horses. Thromboxane A₂ is a potent vasoconstrictor produced by some endothelial cells. High LPS concentrations increase equine pulmonary vascular and bovine aortic endothelial cell TxA₂ production, whereas human vascular endothelial cell TxA₂ production has been attributed to contaminating adherent platelets.

Determination of LPS-induced vasoactive mediator expression in equine digital vasculature may aid understanding of the pathophysiologic events that occur in conditions associated with altered vascular tone and endotoxia, including laminis. Although important research has been undertaken to evaluate digital vascular physiologic and pharmacologic phenomena by use of isolated rings of either the digital vessels or, more recently, the lumen microvessels, little research has been done on vasoactive mediator production by equine digital endothelial or vascular smooth muscle cells. The only 2 previous studies addressing these issues have used single, high LPS concentrations, relative to the circulating concentrations in endotoxia in horses or carbohydrate-induced laminitis, and those studies did not evaluate digital endothelial cells in isolation. Because digital venoconstriction has been documented in experimentally induced laminitis and following LPS infusion, equine digital veins appear to be more sensitive than other peripheral veins and digital arteries to the effects of vasoconstrictor agonists, and there is some evidence to suggest that venous endothelial cells are more sensitive to the effects of LPS than arterial endothelial cells, the purpose of the study reported here was to evaluate the effect of clinically relevant concentrations of LPS on the production of vasoactive mediators known to be important in LPS-related diseases by equine digital venous cells, as opposed to arterial endothelial cells.

Materials and Methods

Cell culture—The EDVECs were cultured as described from the digits of horses euthanized at an abattoir by use of the free bullet method. Briefly, as soon as possible postmortem, the digits were flushed to remove the blood with sterile PBS solution (150 mL) by cannulating the medial and lateral digital veins 3 to 4 cm proximal to the coronary band. The medial and lateral digital arteries were ligated, the digit was infused from the venous side with type II collagenase (20 mL [1 mg/mL], prewarmed at 37°C), and the limb was incubated in a water bath for 30 minutes at 37°C. The endothelial cells were flushed out and collected by use of sterile PBS solution before being centrifuged (300 x g for 10 minutes). The supernatant was removed and the cells resuspended in culture medium (DMEM containing 10% fetal calf serum, 10% newborn calf serum, penicillin [100 U/mL], streptomycin [100 mg/mL]) and transferred to a 75-cm² flask for incubation at 37°C in 5% CO₂ and 95% air. After 24 hours, erythrocyte contamination was removed with warm sterile PBS solution before being centrifuged (1 and 0.25 mg/mL, respectively), resuspended in culture medium, transferred evenly to 24-well plates, and incubated at 37°C for 48 hours to allow the cells to adhere and become confluent.

Effect of LPS on mediator production by EDVECs—Confluent EDVEC monolayers were made quiescent in serum-free medium for 3 hours and preincubated with the phosphodiesterase inhibitor, 3-isobutyl-1-methyl-2,6(1H,3H)-putinedione-methyl-3-isobutylxanthine (IBMX [1mM]) for 30 minutes at 37°C to inhibit breakdown of cGMP. The EDVECs were incubated with DMEM containing 1% bovine serum with or without Escherichia coli LPS O55:B5 (1 µg/mL). The culture medium was sampled after 0, 2, 4, 6, 22, and 24 hours of incubation. The EDVECs were also incubated for 18 hours with LPS ranging in concentration from 10 pg/mL to 1 µg/mL with or without either ibuprofen (10µM), cycloheximide (100µM), or L-NAME (100µM).

Protein and mediator determination—A radiimmunoassay was used to measure the stable metabolite 6-keto-PGF₁α, and so indirectly determine PGI₂ concentrations. Cyclic GMP concentrations were measured as an index of endothelial NO production by use of a commercial enzyme immunoassay system according to the manufacturer’s instructions (protocol 2), as described. The TXA₂ concentrations were determined indirectly by use of a radioimmunoassay, as described. The ET-1 concentrations were measured by use of a sandwich ELISA assay for human ET-1 that had been validated for measurement of equine ET-1 in culture medium. Mediator concentrations were expressed per milligram of protein in the well, measured after cell lysis by use of a protein assay kit.

Effect of LPS on COX-2 and iNOS expression by EDVECs—Confluent EDVEC monolayers, in 6-well tissue-culture plates, were made quiescent in serum-free medium for 3 hours at 37°C. The EDVECs were then incubated with DMEM containing 1% bovine serum with or without E coli LPS O55:B5 (1 µg/mL). The
EDVECs were also incubated for 24 hours with LPS ranging in concentration from 10 pg/mL to 1 µg/mL. The culture medium was removed, and the cell monolayers were lysed in Laemmli sample buffer, boiled (10 minutes at 95°C), and stored at −80°C. Western blotting was used to assess COX-2 and iNOS expression, as described.18,30

Determination of COX-2 and iNOS expression—Equal amounts of protein (50 µg/lane) were separated via SDS-PAGE by use of 7.5% (iNOS) and 10% (COX-2) polyacrylamide gels,3 and proteins were transferred onto a nitrocellulose membrane. Immunodetection of iNOS and COX-2 was performed with primary antibodies against iNOS3 and COX-2 and isotype-matched secondary horseradish peroxidase (HRP-conjugated) antibodies.4 Protein bands were detected by use of an enhanced chemiluminescence kit.4 Blots were then scanned, and band density was determined with computer software.3

Statistical analysis—Statistical analyses were carried out with computer software.4 Values represent mean ± SEM from n = 4 to 7, referring to the number of individual animals from which cells were derived. Values at each time point were compared between the LPS-exposed and control cells by either a 2-way ANOVA followed by Bonferroni post hoc test or a 1-way ANOVA followed by a Dunnett post hoc test. The effects of the inhibitors cycloheximide, ibuprofen, and L-NAME on LPS-induced mediator production were assessed by use of a 2-way ANOVA followed by a Bonferroni post hoc test. The effects of the inhibitors on prostacyclin and cGMP production were fitted by a computerized nonlinear iterative regression procedure to a single process logistic equation, as follows:

\[ E = \left( \frac{E_{\text{max}} C^n}{E_{\text{max}} + C^n} \right) \]

where \( E \) is the response, \( E_{\text{max}} \) is the maximum response, \( E_{\text{max}} \) is the LPS concentrations that induced a response halfway between the maximum response and the baseline, \( n \) is the slope that describes the steepness of the curve, and \( C \) is the LPS concentration. The appropriateness of the monophasic equation was confirmed by comparing the sum of squares of the residuals and the scatter of the points about the line (expressed as the coefficient of variation) with a 2-site equation.

The best fit values for \( E_{\text{max}}, E_{\text{max}}, \) and \( n \) obtained from each experimental replicate were used to calculate the geometric mean and 95% confidence intervals (\( E_{\text{50}} \)) and the arithmetic mean ± SEM (\( E_{\text{max}} \) and \( n \)).

The threshold LPS concentration at which a response was first detected was calculated as the LPS concentration corresponding to the upper 95% confidence interval value for the bottom of the dose response curve. Significance was accepted at \( P \leq 0.05 \).

Results

Endothelial cell mediator production—Lipo poly-saccharide (1 µg/mL) stimulated significant mean ± SEM increases of 4.2 ± 1.4 times and 14.1 ± 5.6 times in EDVEC production of PGI, and cGMP, respectively, compared with basal concentrations, after

![Figure 1](image1.png)  
Figure 1—Time course of production (mean ± SEM values from 6 horses) of PGI, by EDVECs exposed to LPS (1 µg/mL [black squares]) or saline (0.9% NaCl) solution (white squares). *Significant (\( P \leq 0.05 \)) difference between LPS-treated and control cells.

![Figure 2](image2.png)  
Figure 2—Time course of production (mean ± SEM values from 6 horses) of cGMP by EDVECs exposed to LPS (1 µg/mL [black squares]) or saline solution (white squares). See Figure 1 for key.

Table 1—Concentration response curve values (n = 6 horses) for PGI, and cGMP production by EDVECs after 18 hours of exposure to LPS (10 pg/mL to 1 µg/mL).

<table>
<thead>
<tr>
<th>Mediator</th>
<th>( \text{EC}_{50} ) (geometric mean ng/mL [95% CI])</th>
<th>( E_{\text{max}} \text{ SEM} ) (mean mg/mg of protein)</th>
<th>( E_{\text{50}} \text{ SEM} ) (mean mg/mg of protein)</th>
<th>Threshold LPS concentration (mean ± SEM ng/mL)</th>
<th>Hillslope (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGI</td>
<td>1.50 (0.75–3.04)</td>
<td>1,682 ± 54</td>
<td>373 ± 44</td>
<td>0.25 ± 0.07</td>
<td>1.19 ± 0.24</td>
</tr>
<tr>
<td>cGMP</td>
<td>1.22 (0.75–1.98)</td>
<td>22.62 ± 0.6</td>
<td>4.23 ± 0.59</td>
<td>0.126 ± 0.06</td>
<td>1.09 ± 0.26</td>
</tr>
</tbody>
</table>

*Significant (\( P \leq 0.001 \)) difference from \( E_{\text{max}} \) values. \( E_{\text{50}} \) is LPS concentrations that induced a response halfway between the maximum response and the baseline, \( E_{\text{max}} \) is Maximum response, \( E_{0} \) is Basal response. CI = Confidence interval.
22 hours (Figures 1 and 2). These stimulations were LPS concentration-dependent (Table 1). The LPS-in-
duced cGMP production was significantly (\(P = 0.01\)) inhibited to basal concentrations by L-NAME but was unaffected by cycloheximide and ibuprofen (Figure 3). The LPS-induced PGI\(_2\) production was partially inhibited by L-NAME, although significance was not reached (\(P = 0.09\)), and was significantly inhibited to less than basal concentrations by cycloheximide and ibuprofen (Figure 4). The TxA\(_2\) concentration, at all time points and irrespective of LPS concentration, was not significantly different from that of the culture media (0.78 ng/mL). Endothelin-1 accumulated in the medium, with the medium concentration increasing by 82 ± 35 times during the 24-hour incubation period, but mediator production was not enhanced by LPS (Figure 5). There was a large amount of variation in ET-1 production after 22 and 24 hours of incubation both in the presence (313 ± 168 pg of protein/mg and 328 ± 123 pg of protein/mg, respectively) and absence (210 ± 87 pg of protein/mg and 299 ± 131 pg of protein/mg, respectively) of LPS.

Endothelial cell COX-2 and iNOS expression—Lipopolysaccharide (1 \(\mu\)g/mL) induced a significant 5-
times and 23-times increase in COX-2 expression after 6 and 24 hours, respectively. This increase was concentration-dependent (Figure 6), with 1 ng/mL of LPS significantly increasing expression to 273 ± 51% of basal expression (n = 7). Inducible NOS expression could not be detected in EDVECs with the commercially available antibodies used in this study.

Discussion

Endotoxin induced time- and concentration-de-
pendent increases in PGI\(_2\) and NO production by
EDVECs through increased COX-2 expression and
increased eNOS activity that was evident over the range
of LPS concentrations reported in clinical equine endo-
toxemia\(^{28}\) and carbohydrate overload-induced lam-
imitis.\(^4\) Although ET-1 accumulated in the medium and

![Figure 3](image3.jpg)

![Figure 4](image4.jpg)

![Figure 5](image5.jpg)

![Figure 6](image6.jpg)
this was unaltered by LPS, there was considerable variation in the results obtained. Endothelial cells did not appear to release TXA₂.

To the authors’ knowledge, the production of vasoactive mediators by equine digital endothelial cells has not been evaluated previously. Lipopolysaccharide (10 µg/mL) increased PGI₂ production by segments of rat aorta after 24 to 48 hours of incubation and by rat cerebral endothelial cells after 6 hours. Incubation of human umbilical and bovine aortic endothelial cells with LPS (0.001 to 1 µg/mL for 24 hours) revealed a time- and concentration-dependent (EC₅₀ not reported) increase in COX metabolite accumulation, enhanced COX activity, and COX-2 protein induction that was inhibited by cycloheximide, dexamethasone, aspirin, and indomethacin pretreatment. Lipopolysaccharide (5 µg/mL for 1 hour) significantly increased PGI₂ production by equine pulmonary arterial endothelial cells and increasing LPS concentration (5 to 100 µg/mL) further increased PGI₂ production. Lipopolysaccharide (20 µg/mL) induced COX-2 upregulation in equine digital artery smooth muscle cells. Lipopolysaccharide (1 µg/mL) also increased cultured equine digital blood vessel PGI₂ production, an effect that was completely inhibited by cycloheximide and ibuprofen and partially inhibited by L-NAME. Human umbilical vein endothelial cells expressed COX-2 and produced prostacyclin in a NS-398–sensitive manner, suggesting that PGI₂ production was derived principally by the COX-2 pathway. Results of the present study support and extend these previous findings and revealed time- and LPS concentration–dependent increases in EDVEC PGI₂ production. The threshold concentration at which LPS-induced PGI₂ production was detectable (250 ± 70 pg/mL) suggests that these findings are relevant to clinical endotoxemia in horses but are at the high end of the LPS concentrations reported in horses with carbohydrate overload–induced laminitis.

Prostacyclin production was inhibited to less than basal concentrations by cycloheximide and ibuprofen and reduced by L-NAME. The inhibitory effects of the nonselective COX inhibitor ibuprofen and the protein synthesis inhibitor cycloheximide suggest that LPS–induced endothelial prostaglandin release probably results from increased inducible COX, that is, COX-2 synthesis and activity. This was confirmed by the LPS-induced time- and concentration-dependent increase in COX-2 expression. Cross talk between the NO and COX pathways, with NO stimulating COX-2, has been detected in murine macrophages. The apparent partial inhibitory effect of the NOS inhibitor L-NAME on PGI₂ production by EDVECs may suggest that this also occurs in equine endothelial cells. A link between NO and regulation of eicosanoid synthesis could represent an important mechanism in controlling vascular and inflammatory responses in pathophysiologic states. Induction of COX-2 by LPS in bovine aortic endothelial cells is mediated by a tyrosine kinase and LPS-stimulated COX-2 gene and protein expression, and PGI₂ release by bovine pulmonary artery endothelial cells is mediated by activation of p38 or p42/44 mitogen-activated protein kinases. However, the signalling pathways involved in equine digital endothelial cell COX-2 activation remain unknown.

In some species, LPS upregulates vascular NO production. High LPS concentrations (10 µg/mL) stimulated NO production by cultured segments of rat aorta that peaked after 48 hours but failed to stimulate NO production by murine aortic endothelial cells. Slightly lower LPS concentrations (1 µg/mL) failed to stimulate nitrite production by either bovine aortic endothelial cells or segments of equine digital vessels. In all of those studies, the Griess reaction was used to quantify NO production, which is not very sensitive. One study found that LPS-induced endothelial cell NO production was detectable by use of the oxyglobin assay but not the Griess reaction. Evidence of iNOS induction was not detected in human and rat aortic endothelial cells exposed to LPS or cytokines by use of the chemiluminescence assay. Nitric oxide production may have been detectable in those studies if cellular cGMP production had been used as a marker of its biological activity, as used in the present study. Lipopolysaccharide, in combination with other cytokines, induced iNOS activity in murine endothelial cells, whereas LPS alone did not increase nitrite production or iNOS mRNA. Thus, it is also possible that LPS requires cytokines, generated in vivo from several cell types directly or indirectly, to induce iNOS. In the present study, L-NAME–sensitive NO production was significantly increased in response to LPS in a time- and LPS concentration–dependent manner in the absence of additional cytokines. Thus, it would appear that EDVECs differ from other endothelial cells in their LPS sensitivity, resulting in increased NO production and lack of requirement for cytokines for NO upregulation. Similar to PGI₂, LPS-induced cGMP production was detectable from 126 ± 60 pg of LPS/mL, suggesting that these responses may be relevant to clinical endotoxemia in horses but occur at the high end of the LPS concentrations reported in horses with carbohydrate overload–induced laminitis.

Upregulation of iNOS expression has been reported to be the mechanism by which endothelial cells increase NO production in response to LPS. The expression of iNOS and subsequent high-output NO production is believed to underlie the systemic hypotension, inadequate tissue perfusion, and organ failure associated with endotoxemia in humans. In 1 study, LPS induced human endothelial cell iNOS expression via the p38 mitogen-activated protein kinase signalling pathway. In another study, LPS activated human umbilical vein endothelial NOS through phosphoinositide 3-kinase and Akt-protein kinase B–dependent enzyme phosphorylation with the resultant NO acting as a co-stimulus for the expression of iNOS. The absence of an effect of the protein synthesis inhibitor, cycloheximide, at a concentration that inhibited LPS-induced mediator production by segments of equine digital vessel on LPS-stimulated EDVEC NO production in the present study suggests that increased activity of a constitutively expressed enzyme (eNOS) is involved rather than synthesis of an inducible isofrom. The means by which the activity of eNOS may be upregulated in these circumstances remains unclear. Unfortunately, the commercially available antibodies for iNOS protein tested in the present study did not recognize the equine form of the
protein, so it was not possible to confirm that increased iNOS expression did not occur in response to LPS. Lipopolysaccharide increased equine pulmonary endothelial cell thromboxane production but only at concentrations greater than 10 µg/mL, which is much higher than the concentrations used in the present study and the concentrations measured in clinical endotoxemia. Lower concentrations of LPS (100 ng/mL for 6 hours) stimulated TxA₂ production by rat cerebral endothelial cells but not bovine aortic endothelial cells (1 ng/mL to 1 µg/mL for 24 hours). In the present study, EDVECs did not appear to produce TxA₂.

Cultured equine pulmonary artery, equine digital artery and vein, and human umbilical vein endothelial cells produced ET-1 continuously over 24 hours, although the rate of release decreased after the first 4 hours of incubation. The pattern of ET-1 production by control EDVECs in the present study was similar. Lipopolysaccharide (0.1 to 10 µg/mL) promoted ET-1 release from cultured bovine aortic endothelial cells and from human umbilical vein endothelial cells in a concentration-dependent manner over a concentration range of 1 to 250 ng/mL, whereas concentrations between 250 and 1,000 ng/mL significantly decreased production. Lipopolysaccharide (100 µg/mL) decreased equine digital arterial and venous endothelial cell ET-1 production. Thus, the LPS concentration used in the present study was potentially inhibitory. Nonetheless, basal ET-1 production was not reduced by LPS, suggesting that EDVEC ET-1 production is unaffected by 1 µg of LPS/mL. Furthermore, these results are in agreement with the results from an equine in vivo experiment, in which LPS (30 ng/kg) infusion leading to peak plasma LPS concentrations of 13 pg/mL failed to increase plasma ET-1 concentrations. However, it must be acknowledged that there was a large amount of variability in EDVEC ET-1 production after 22 and 24 hours of incubation both with and without LPS, which confounded interpretation of these findings.

Endotoxin stimulated EDVEC COX-2 expression and constitutive NOS activity, resulting in increased PGI₂ and cGMP production but not ET-1 or TxA₂ production, which was evident over the range of LPS concentrations reported in clinical endotoxemia and carbohydrate overload–induced laminitis. Although cultured endothelial cells are clearly not exposed to the same conditions as they would be in vivo (in particular, shear stress that is believed to modulate endothelial cell vasoactive mediator release), equine digital vein endothelium appeared to be capable of increased vasoactive mediator production in response to LPS. The prodromal stages of laminitis appear to be associated with selective dysfunction of the laminar veins; there is a physiologic predisposition for venoconstriction in the equine digital microvasculature, and there are reduced contractile responses to vasoconstrictor mediators following experimental induction of laminitis. The increased production of vasoconstrictor mediators induced by LPS may in part explain this reduced response to vascularconstrictor agonists and may play an important role in the alterations in digital perfusion evident in experimental and clinical endotoxemia and laminitis. Further investigations are required to determine the intracellular signalling pathways involved.

References
1. Robertson TP, Peroni JF, Noschka E, et al. Prostanoids and iso-prostanes as inflammatory and vasoactive conduits in the development of laminitis. Recent advances in the physiology of equine laminitis Havemeyer Foundation Workshop 2007:120.