Effect of heparin administration on urine protein excretion during the developmental stage of experimentally induced laminitis in horses

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Objective—To investigate the effects of heparin administration on urine protein excretion during the developmental stages of experimentally induced laminitis in horses.

Animals—13 horses.

Procedures—Horses received unfractionated heparin (80 U/kg, SC, q 8 h; n = 7) or no treatment (control group; 6) beginning 3 days prior to induction of laminitis. All horses were given 3 oligofructose loading doses (1 g/kg each) at 24-hour intervals and a laminitis induction dose (10 g of oligofructose/kg) 24 hours following the final loading dose (designated as 0 hours) via nasogastric tube. Serum glucose and insulin concentrations were measured before administration of the first loading dose (baseline) and at 0 and 24 hours; urine protein-to-creatinine (UP:C) ratio was determined at 0 hours and every 4 hours thereafter. Lameness was evaluated every 6 hours, and horses were euthanized when Obel grade 2 lameness was observed.

Results—Mean ± SD time until euthanasia did not differ significantly between the heparin-treated (28.9 ± 6.5 hours) and control (29.0 ± 6.9 hours) horses. The UP:C ratio was significantly increased from baseline at 20 to 28 hours after induction of laminitis (ie, 4 ± 4 hours before lameness was evident) in control horses but did not change significantly from baseline in heparin-treated horses. Serum glucose or insulin concentration did not change significantly from baseline in either group.

Conclusions and Clinical Relevance—Urine protein excretion increased during the developmental stages of carbohydrate-induced laminitis in horses; administration of heparin prevented that increase, but did not delay onset or decrease severity of lameness. (*Am J Vet Res* 2010;71:1462–1467)

Laminitis is a major cause of morbidity and death in horses. Although still incompletely understood, it is likely that the pathogenesis of laminitis is multifactorial, with a combination of local and systemic events leading to altered hoof blood flow and thromboembolism of capillary beds. Altered hemodynamics and inflammation-induced damage eventually lead to laminar separation, necrosis, and irreparable damage to the hoof laminae.[1,2] Acute laminitis has been associated with a variety of clinical diseases, including carbohydrate overload, retained fetal membranes in mares, proximal duodenitis-jejunitis, and colitis, in which systemic inflammatory events precede local damage in the hoof. Diagnosis of laminitis is based on detection of clinical signs (eg, lameness and reluctance to move) and physical examination abnormalities (eg, increased hoof temperature and prominent digital artery pulses); in some cases, radiographic views of affected limbs may reveal divergence of the hoof wall and cranial aspect of the third phalanx. However, because these findings usually indicate disease after anatomic alteration has occurred, a reliable earlier indicator of impending laminitis is needed; such an indicator could alert clinicians that aggressive treatment should be instituted before functional or life-threatening damage occurs.

Several environmental, toxic, and metabolic factors have been implicated as causes of laminitis in horses.[3] Intake of excess dietary carbohydrate is a known risk factor for naturally occurring laminitis, and colonic overloading with nonstructural carbohydrate (oligofructose) is an established experimental method of

Abbreviations

G:I	Glucose-to-insulin
UC	Urine creatinine
UP	Urine protein
UP:C	Urine protein-to-creatinine
UPE	Urine protein excretion

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inducing laminitis in horses.[4] The precise mechanism whereby dietary oligofructose contributes to damage of hoof laminae is unknown.[4] However, increases in laminar vascular permeability that are indirectly linked to carbohydrate administration may alter hoof microcirculation, leading to laminar cell damage.[1,5]

Insulin has also been implicated in the development of laminitis in equids. Plasma insulin concentration increases 12 to 48 hours after experimental induction of laminitis with oligofructose in horses, and hyperinsulinemia induces laminitis within 48 hours in clinically normal ponies.[4,6] Additionally, insulin-resistant horses (ie, horses with pituitary pars intermedia dysfunction or metabolic syndrome) are at increased risk of developing laminitis.[7] However, insulin dynamics during the prodromal stages of experimentally induced laminitis have not been thoroughly investigated, to our knowledge, and it is unclear whether hyperinsulinemia consistently precedes development of clinical signs of laminitis.

An increase in UP concentration is an early consequence of renal vascular damage, either as a result of primary glomerular diseases or systemic inflammatory diseases that cause secondary glomerular vascular damage.[8] Inflammation at any body site may result in intravascular antigen-antibody complex formation, followed by deposition of complexes within the glomeruli, local induction of inflammation and endothelial cell damage, and passage of excess protein into the glomerular ultrafiltrate. Measurement of UP concentration can therefore be used as an early but nonspecific method for detection of disease in dogs, cats, and people.[9] We recently evaluated daily UPE in healthy horses and confirmed that UP:C ratio (measured in a randomly collected urine sample) could be used as a surrogate for 24-hour UP measurement in equids.[10] However, whether UPE increases in horses with naturally occurring or experimentally induced systemic inflammatory diseases such as laminitis has not been determined.

Unfractionated heparin has been used for prevention and treatment of laminitis, particularly in horses deemed at risk, despite conflicting evidence of its efficacy.[11,12] Heparin exerts anticoagulant activity via potentiation of anti-thrombin III and may limit thrombus formation in small vascular beds.[12] In addition, heparin has in vitro anti-inflammatory and indirect vasodilatory effects on vascular endothelium; such effects may prevent the stagnation of blood flow that ultimately results in thrombus formation and may slow or modify the local immune processes that occur in response to and contribute to progression of laminar cell necrosis.[13,14] Interestingly, low–molecular-weight heparin has also been shown to reduce the magnitude of proteinuria in rats with experimentally induced glomerulopathy.[15]

The purpose of the study reported here was to investigate the effects of unfractionated heparin administration on UPE during the developmental stages of experimentally induced laminitis in horses. The hypothesis was that UPE and serum insulin concentration would increase in horses with oligofructose-induced laminitis prior to onset of lameness. In addition, we hypothesized that treatment with heparin prior to and after experimental induction of laminitis would decrease the magnitudes of change in UPE and hyperinsulinemia in affected horses.

Materials and Methods

Experimental animals—The study protocol was reviewed and approved by the Purdue University Animal Care and Use Committee. Thirteen horses that were donated to the Purdue University Veterinary Teaching Hospital for reasons other than lameness of the distal portion of a limb were used in the study. All horses were considered systemically healthy on the basis of results of physical examination, CBC, serum biochemical analysis, urinalysis, and UP:C ratio evaluation. In addition, all horses > 15 years old were also evaluated by use of a domperidone stimulation test to exclude horses with pituitary pars intermedia dysfunction.[16] None of the horses had any clinical signs associated with laminitis, and findings of routine forelimb radiography of all horses were unremarkable. After acquisition, horses were acclimated to individual box stalls for 2 weeks and received fresh water and grass hay ad libitum throughout the study period.

Induction of laminitis and heparin treatment—Horses were randomly assigned to heparin treatment or control groups. Laminitis was induced in all horses by use of a previously described protocol.[4] In brief, each horse received a loading dose of oligofructose[a] (1 g/kg administered via nasogastric tube, q 24 h) on 3 occasions, followed by a single laminitis induction dose of oligofructose (10 g/kg administered via nasogastric tube) that was given 24 hours after the third loading dose. The time at which the induction dose was given was designated as 0 hours. Horses in the heparin treatment group received unfractionated heparin[b] (80 U/kg heparin, SC [in the neck region], q 8 h) beginning at the same time as administration of the first loading dose of oligofructose; heparin treatment was continued until the end of the study period. Control horses received no SC injections.

Assessments—Serum glucose and insulin concentrations were determined immediately prior to administration of the first loading dose of oligofructose (baseline), at 0 hours (the time of administration of the induction dose), and at 24 hours after administration of the induction dose. Urine was collected from each horse before the first loading dose of oligofructose, at 0 hours, and then every 4 hours thereafter by means of indwelling urinary catheters[c] and closed collection bags in females and preputial urine collection bags in males.[10] A lameness evaluation (ie, Obel lameness scoring[17]) was performed every 6 hours after administration of the induction dose by a single investigator (SBA) who was unaware of each horse's group assignment. Horses were euthanized by use of IV injection of pentobarbital sodium when observed lameness was scored as Obel grade 2 (ie, lameness at a walk, without signs of resistance to having a foot lifted) or higher.[17]

Laboratory evaluations—All sample processing was performed at the Purdue University Veterinary Teaching Hospital Clinical Pathology Laboratory. Urine samples were centrifuged for 8 minutes at 3,500

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× *g* prior to analysis, and UP and UC concentrations were then measured by use of standard methods.[d,e] The UP:C ratio of each sample was calculated by dividing UP concentration by UC concentration to generate a unitless ratio. Serum insulin concentration was determined by use of a radioimmunoassay,[f] and serum glucose concentration was determined via dry-slide technology colorimetry.[g] The G:I ratio was calculated by dividing glucose concentration by insulin concentration to generate a unitless ratio.

Statistical methods—All analyses were conducted by means of a statistical software program.[h] Assessment of data normality was conducted by use of a Shapiro-Wilk test and subjective visual assessment of the raw data. Parametric tests, which were not unduly sensitive to moderate departures from normality (eg, ANOVA), were determined appropriate. Differences in G:I ratio and UP:C ratio over time within each group of horses were each evaluated by use of an ANOVA and a Tukey multiple comparison test. Mean G:I ratio and UP:C ratio were calculated for each group from the data for each heparin-treated or control horse at the following time points: before induction of laminitis, at the time of last measurement, and at the time of euthanasia. Mean G:I ratios and UP:C ratios at each of those time points were each compared between groups by use of a Student *t* test. An ANCOVA was used to compare the slopes of UP:C ratio values over time between the 2 groups. Significance was designated at a value of $P < 0.05$ for all analyses.

Results

Horses—Six mares and 7 geldings were included in the study. The horses were 2 to 30 years old (median age, 7 years) and ranged in weight from 375 to 603 kg (median weight, 497 kg). Each horse was randomly allocated to 1 of the 2 experimental groups (heparin treatment group, n = 7; control group, 6); the distribution of horses by weight or age in each group did not differ significantly ($P = 0.629$ and $P = 0.567$, respectively). All horses developed diarrhea within 20 hours after administration of the induction dose of oligofructose.

Development of lameness—The Obel lameness score significantly increased over time in both the heparin-treated and control horses, but there was no difference in magnitude of Obel score (ie, severity of lameness) or median time until Obel 2 score was attained between groups (**Figure 1**). Horses were euthanized when lameness was scored as Obel grade 2; mean ± SD times until euthanasia (ie, from 0 hours) for the heparin treatment group and control group were 28.9 ± 6.5 hours and 29.0 ± 6.9 hours, respectively. There was no significant ($P = 0.866$) difference in the mean time until euthanasia between groups. The interval from administration of the induction dose of oligofructose to euthanasia ranged from 16 to 40 hours for the heparin treatment group and from 20 to 40 hours for the control group.

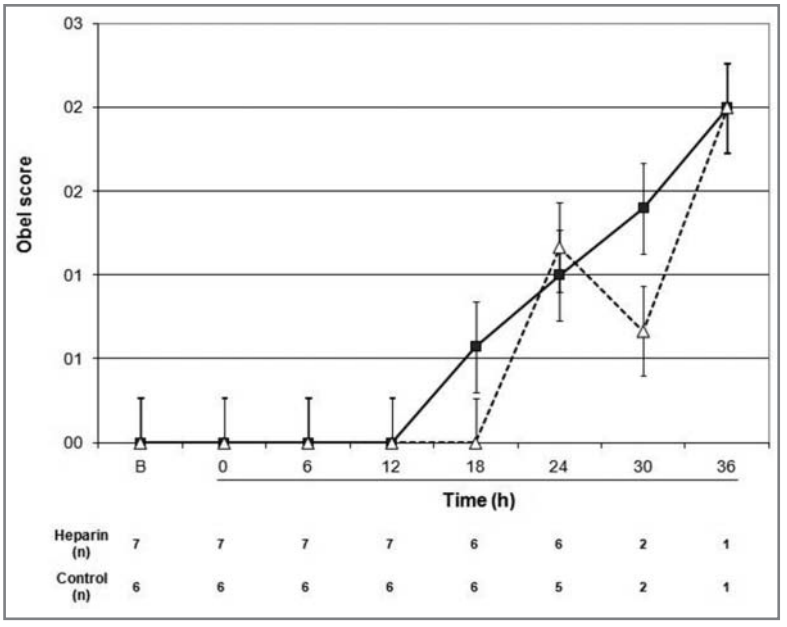

Figure 1—Mean ± SE Obel lameness scores in horses that received unfractionated heparin (80 U/kg, SC, q 8 h; n = 7 [black squares]) or no treatment (control group; 6 [white triangles]) beginning 3 days prior to induction of laminitis via nasogastric administration of oligofructose and continuing throughout the study. To induce laminitis, all horses were given 3 loading doses of oligofructose (1 g/kg each) at 24-hour intervals and a laminitis induction dose (10 g of oligofructose/kg) 24 hours following the final loading dose (designated as 0 hours). Lameness in each horse was evaluated before the first loading dose (baseline [B]) and every 6 hours thereafter. All horses were euthanized when Obel grade 2 lameness was observed; as a result, the number of horses in each group decreased with time (the number [n] of horses remaining in each group at each time point is indicated below the x-axis).

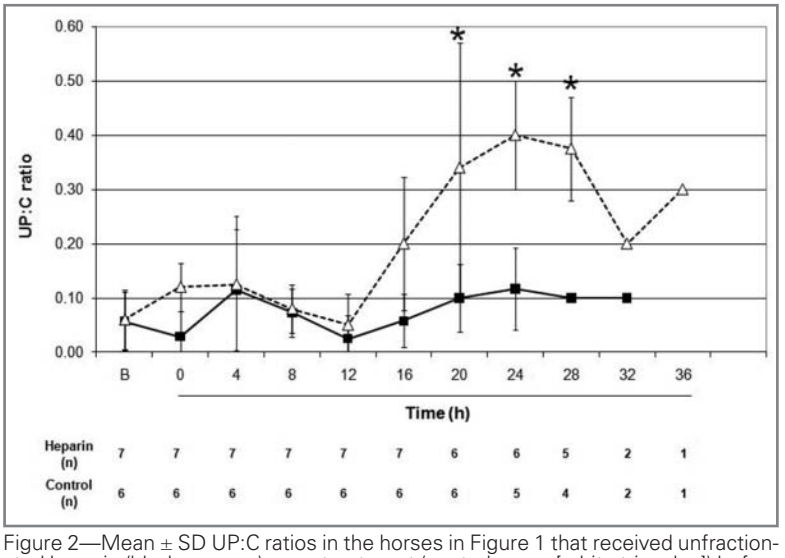

Figure 2—Mean ± SD UP:C ratios in the horses in Figure 1 that received unfractionated heparin (black squares) or no treatment (control group [white triangles]) before induction and during development of carbohydrate-induced laminitis. *At this time point, UP:C ratio in the control group differs significantly ($P < 0.05$) from the baseline (ie, preinduction) value and from the value for the heparin treatment group. *See* Figure 1 for remainder of key.

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Table 1—Mean ± SD values of serum glucose and insulin concentrations and G:I ratio in horses that received unfractionated heparin (80 U/kg, SC, q 8 h; n = 7) or no treatment (control group; 6) before induction and during development of oligofructose-induced laminitis, determined before the first of 3 loading doses of carbohydrate (baseline), before administration of the laminitis induction dose of carbohydrate (0 hours), and 24 hours after administration of the induction dose.

		Time point		
Variable	**Group**	**Baseline**	**0 hours**	**24 hours after induction**
Serum insulin (μU/mL)	Heparin treatment	6.1 ± 2.6	5.7 ± 2.0	5.8 ± 1.5
	Control	7.9 ± 6.0	7.6 ± 8.4	13.2 ± 17.7
Serum glucose (mg/dL)	Heparin treatment	83 ± 9	88 ± 9	135 ± 14
	Control	94 ± 11	89 ± 9	111 ± 15
G:I ratio	Heparin treatment	15.6 ± 5.9	17.3 ± 6.5	24.3 ± 6.9
	Control	20 ± 18.3	18.5 ± 8.5	19.5 ± 11.1

Heparin treatment commenced 3 days prior to induction of laminitis and continued throughout the study. To induce laminitis, all horses were given 3 loading doses of oligofructose (1 g/kg each) at 24-hour intervals and a laminitis induction dose (10 g of oligofructose/kg) 24 hours following the final loading dose.

UP:C ratios—Prior to administration of the first loading dose of oligofructose, mean UP:C ratios did not differ significantly ($P = 0.751$) between the heparin treatment and control groups (**Figure 2**). Within the control group, mean UP:C ratio was significantly greater than the baseline UP:C ratio at 20, 24, and 28 hours after administration of the induction dose of oligofructose. The mean UP:C ratios were significantly greater in control horses, compared with findings in heparin-treated horses, at 20 ($P = 0.039$), 24 ($P = 0.002$), and 28 ($P = 0.001$) hours. Despite this significant increase in the mean value of the UP:C ratio for the control group, values remained within the previously suggested reference range for UP:C ratio in horses (0.06 to 0.47).[10] In contrast, mean UP:C ratio in the heparin treatment group did not differ significantly from the baseline value at any time point. There was no significant ($P = 0.646$) difference in the final mean UP:C ratio between the heparin treatment and control groups (time point at which the last assessment was made was 32 and 36 hours, respectively). However, there was a significant ($P = 0.039$) difference in the rate of change (ie, slope) in mean UP:C ratio over time between the 2 groups.

Serum glucose and insulin concentrations and G:I ratio—In either the heparin treatment or control group, mean values of serum glucose concentration, serum insulin concentration, or G:I ratios obtained before induction of laminitis, at 0 hours, or at 24 hours after induction of laminitis did not differ (**Table 1**). Likewise, there were no significant differences in mean serum glucose concentration, serum insulin concentration, or G:I ratio between the 2 groups before ($P = 0.595$) or after ($P = 0.530$) induction of laminitis.

Discussion

Results of the present study indicated that there is an increase in UP concentration during the early stages of oligofructose-induced laminitis in horses. This increase in UPE was evident in the control horses between 20 and 28 hours after administration of the laminitis induction dose of carbohydrate and was sustained until immediately before development of Obel 2 lameness. Interestingly, the increase in UPE was not apparent in horses treated with unfractionated heparin (beginning before induction and continuing during the early stages of laminitis), although heparin administration did not prevent or delay onset of laminitis. Moreover, heparin treatment did not affect serum insulin concentration in horses before or after induction of laminitis.

Administration of oligofructose to induce laminitis in horses has been widely used in recent years, primarily because this experimental method consistently results in severe and predictable lesions within an expected time frame (24 to 36 hours).[4,18] Although the hoof lamellae are the primary target organ in laminitis, carbohydrate overload affects multiple organ systems in horses; therefore, carbohydrate-induced laminitis should more accurately be considered a whole-body disease with localized clinical effects.[19] Hindgut microbial changes in horses following oligofructose overloading have been well described.[18] Bacterial overgrowth potentially leads to elaboration of toxins that can cross the damaged intestinal wall; these toxins subsequently cause hoof lamellae damage by increasing circulating amounts of vasoactive agents and activating host lamellar enzymes (eg, metalloproteinases). Although the clinical signs and histopathologic changes induced via carbohydrate overloading are identical to those that develop in equids with naturally occurring laminitis, it is unknown whether the pathogenesis of laminitis is identical in all animals. Therefore, extrapolation of the study results reported here to clinical patients must be done with caution.

The present study was not designed to determine the source of increased UP concentration in horses with oligofructose-induced laminitis. Nevertheless, acute laminitis in horses has been purported to be a final event of an otherwise systemic disease, and it is known that urinary excretion of lipid peroxidation byproducts, which are produced secondary to free radical damage, increases in ponies with chronic laminitis.[20–22] In a pilot investigation related to the study reported here, postmortem examination of a subset of horses that underwent oligofructose-induced laminitis revealed a concurrent nephropathy, including gross renal enlargement and congestion and glomerular edema, in conjunction with hoof laminar edema, cellular swelling, and basal membrane separation; however, no control horses were studied in parallel. Renal damage and subsequent proteinuria also develop secondary to systemic primary and secondary vasculopathies and inflammatory diseases in several other species.[8,23,24] These

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findings, and the well-documented association between systemic inflammatory diseases and glomerular damage in many other species, support that the increases in UP:C ratio detected in the horses of the present study were attributable to loss of glomerular permselectivity rather than to postglomerular inflammation or damage. In people, primary vascular diseases are commonly associated with rapidly progressive glomerulonephritis, and systemic diseases (including diabetes mellitus and a variety of neoplasms and infectious agents) frequently result in glomerular damage. Although primary vasculopathies are rare in dogs, glomerular disease and proteinuria secondary to systemic diseases (typically diseases associated with bacterial or rickettsial infections, neoplasms, or systemic inflammatory autoimmune processes) are commonly diagnosed.

Increases in albuminuria and total UPE are well-established early markers of disease progression and risk of death associated with many proteinuric diseases of humans and other animals.[8,23–25] Although the present study revealed an increase in UPE during the developmental stage of acute laminitis in horses, we cannot infer that horses that are predisposed to development of laminitis and have proteinuria should be treated more aggressively with anti-laminitis protocols. However, measurement of UPE as an early marker for the risk of laminitis should be explored as a potential aid for the clinical management of patients with systemic inflammatory diseases; determination of the sensitivity and specificity of UP protein concentration measurement as a predictor of development of laminitis in those patients is warranted.

Unfractionated heparin is often administered empirically to horses to prevent development of laminitis, but its efficacy in slowing or otherwise altering patient outcome is unknown.[11,12] Proposed mechanisms for heparin's effects initially included its anticoagulant role as a potentiator of anti-thrombin III activity.[12] More recently, heparin has been proposed to mediate several anti-inflammatory and indirect vasodilatory effects on vascular endothelium.[13,14] The mechanism whereby heparin may reduce or prevent UPE in horses with laminitis and presumptive secondary glomerular damage is likewise unknown. The precise mechanisms of the anti-proteinuric effects of heparin and related glycosaminoglycans may include repelling of negatively charged proteins after deposition within the glomerular basement membrane or direct modulation of endothelial cell, podocyte, or inflammatory cell activity.[26,27] Glycosaminoglycan-containing molecules such as heparin sulfate may modulate podocyte activity, and low–molecular-weight heparin decreases proteinuria in rats with experimentally induced glomerular disease and in people with various diseases.[15,27–29]

Low–molecular-weight heparin is smaller in size and has better bioavailability, is associated with more predictable responses, and has a decreased incidence of adverse effects, compared with unfractionated heparin.[12,30,31] However, the anti-inflammatory effects of unfractionated and low–molecular-weight heparin on equine digital endothelium do not differ.[14] Low–molecular-weight heparin is considerably more expensive than unfractionated heparin and is not widely available. Consequently, we chose to use the latter in the present study. Although administration of unfractionated heparin did not delay or prevent the onset of laminitis in the study horses, if the increase in UPE in these horses is truly attributable to glomerular damage resulting from pathophysiologic processes similar to those in other species, then findings of our study suggest that heparin has an effect on the functional integrity of the equine glomerular apparatus.

On the basis of the results of the present study, we cannot determine whether the beneficial effects of heparin on the hoof laminae may have been overcome by the severity of the experimentally induced laminitis, whether the pathogenesis of oligofructose-induced laminitis is not affected by heparin in general, or whether other forms of experimentally induced or naturally occurring laminitis would be modulated by heparin. Further investigation of heparin treatment and its potential benefits in a clinical setting is needed, where onset of laminitis may be slower and attributable to a variety of concurrent systemic diseases and where severity of the disease may be milder.

Naturally occurring endocrine disorders in horses are known causes of laminitis, and experimentally induced hyperinsulinemia results in the development of laminitis in ponies.[3,6,7] In other species, hyperglycemia increases permeability of the endothelium (ie, glucotoxicosis); therefore, because plasma glucose and serum insulin concentrations have been previously reported to increase during the developmental stage of oligofructose-induced laminitis in horses, we elected to measure circulating glucose and insulin concentrations to determine whether heparin administration affected their dynamics.[4,32] In the present study, there were no significant changes in serum insulin concentration or G:I ratio before or after induction of laminitis in heparin-treated or control horses and no significant differences in those variables between groups at any time point. Detailed assessment of insulin sensitivity in horses is ideally done by more invasive methods, such as the euglycemic-hyperinsulinemic clamp technique or the insulin-modified frequently sampled IV glucose tolerance tests.[33] Although measurement of blood insulin concentration and G:I ratios are less sensitive and more subject to variation introduced by moment-to-moment biological variation and differences in sensitivity of insulin assays, they are more practical to perform in the clinic or research setting and are often considered to be sufficient indicators of insulin sensitivity.[34] However, because of the difference in sensitivity of these assays for determining insulin sensitivity, we must acknowledge that the lack of significant differences in these variables between our study groups may be a result of the test chosen rather than lack of a difference between groups. Alternatively, the type and quality of feed may also play a role in insulin dynamics, and substitution of an alternative energy source may have an effect; therefore, extrapolation of the findings of the present study to naturally occurring cases of laminitis should be done with caution. Further studies with larger sample sizes, more precise assay techniques, and more frequent sample collections may be needed to clarify the discrepancy in study results.

Although horses with oligofructose-induced laminitis had a significantly increased UP concentration when

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not concurrently treated with heparin, values were not greater than the upper reference limit reported previously.[10] Nevertheless, detection of an increase in this variable may have a role as a marker of early stages of laminitis. To detect an increase in UP concentration in horses that are at risk for development of laminitis, baseline concentrations would have to be measured when the horses were healthy, if possible, or at the time of initial evaluation. Patterns of changes in UP concentration may be more useful in making management decisions than values determined at single time points. Rather than measurement of total UP concentration, measurement of urine concentration of proteins that may be more specific indicators of glomerular function (eg, albumin, N-acetylglucosaminidase, or cystatin C) could perhaps be more useful for identification of horses at risk of developing laminitis. Further studies in clinical patients are required before any monitoring or intervention recommendations can be made, and we acknowledge that early detection of laminitis on the basis of UP concentration measurements in horses may prove to be challenging despite the preliminary results reported here.

a. BENEO-Orafti P95 oligofructose powder, BENEO-Orafti, Tienen, Belgium.
b. Heparin sodium for injection, 10,000 USP U/mL, Abraxis Pharmaceutical Products, Schaumburg, Ill.
c. Silkolatex Rusch Gold Foley catheter, Teleflex Medical, Bannockburn, Ill.
d. Vitros Crea quantitative color test, VITROS Chemistry, Ortho-Clinical Diagnostics, Rochester, NY.
e. Bio-Rad Microprotein dye-binding test, Bio-Rad Laboratories, Hercules, Calif.
f. RIA Gamma Counter 7000, Organon Teknika, Durham, NC.
g. Vitros Fusion, version 5.1, Ortho-Clinical Diagnostics, Rochester, NY.
h. SAS, version 9.1, SAS Institute Inc, Cary, NC.

References

1. Eades SC, Holm AMS, Moore RM. A review of the pathophysiology and treatment of acute laminitis: pathophysiologic and therapeutic implications of endothelin-1, in *Proceedings*. 48th Annu Conv Am Assoc Equine Pract 2002;353–361.
2. Moore RM, Eades SC, Stokes AM. Evidence for vascular and enzymatic events in the pathophysiology of acute laminitis: which pathway is responsible for initiation of this process in horses? *Equine Vet J* 2004;36:204–209.
3. Johnson PJ, Messer NT, Ganjam VK. Cushing's syndromes, insulin resistance and endocrinopathic laminitis. *Equine Vet J* 2004;36:194–198.
4. van Eps AW, Pollitt CC. Equine laminitis induced with oligofructose. *Equine Vet J* 2006;38:203–208.
5. Bailey SR, Marr CM, Elliott J. Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet J* 2004;167:129–142.
6. Asplin KE, Sillence MN, Pollitt CC, et al. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet J* 2007;174:530–535.
7. Frank N. Endocrinopathic laminitis, obesity-associated laminitis, and pasture-associated laminitis, in *Proceedings*. 54th Annu Conv Am Assoc Equine Pract 2008;54:341–346.
8. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001;286:421–426.
9. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum consensus statement (small animal). *J Vet Intern Med* 2005;19:377–385.
10. Uberti B, Eberle DB, Pressler BM, et al. Determination of and correlation between urine protein excretion and urine protein-to-creatinine ratio values during a 24-hour period in healthy horses and ponies. *Am J Vet Res* 2009;70:1551–1556.
11. Belknap JK, Moore JN. Evaluation of heparin for prophylaxis of equine laminitis: 71 cases (1980–1986). *J Am Vet Med Assoc* 1989;195:505–507.
12. Moore BR, Hinchcliff KW. Heparin: a review of its pharmacology and therapeutic use in horses. *J Vet Intern Med* 1994;8:26–35.
13. Baldus S, Rudolph V, Roiss M, et al. Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase. *Circulation* 2006;113:1871–1878.
14. de la Rebiere G, Franck T, Deby-Dupont G, et al. Effects of unfractionated and fractionated heparins on myeloperoxidase activity and interactions with endothelial cells: possible effects on the pathophysiology of equine laminitis. *Vet J* 2008;178:62–69.
15. Deepa PR, Varalakshmi P. The cytoprotective role of a low-molecular-weight heparin fragment studied in an experimental model of glomerulotoxicity. *Eur J Pharmacol* 2003;478:199–205.
16. Miller MA, Pardo ID, Jackson LP, et al. Correlation of pituitary histomorphometry with adrenocorticotrophic hormone response to domperidone administration in the diagnosis of equine pituitary pars intermedia dysfunction. *Vet Pathol* 2008;45:26–38.
17. Garner HE, Hutcheson DP, Coffman JR, et al. Lactic acidosis: a factor associated with equine laminitis. *J Anim Sci* 1977;45:1037–1041.
18. Milinovich GJ, Trott DJ, Burrell PC, et al. Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environ Microbiol* 2006;8:885–898.
19. Hood DM. Laminitis as a systemic disease. *Vet Clin North Am Equine Pract* 1999;15:481–494.
20. Bailey SR. The pathogenesis of acute laminitis: fitting more pieces into the puzzle. *Equine Vet J* 2004;36:199–203.
21. Nourian AR, Baldwin GI, van Eps AW, et al. Equine laminitis: ultrastructural lesions detected 24–30 hours after induction with oligofructose. *Equine Vet J* 2007;39:360–364.
22. Neville RF, Hollands T, Collins SN, et al. Evaluation of urinary TBARS in normal and chronic laminitic ponies. *Equine Vet J* 2004;36:292–294.
23. de Jong PE, Brenner BM. From secondary to primary prevention of progressive renal disease: the case for screening for albuminuria. *Kidney Int* 2004;66:2109–2118.
24. Whittemore JC, Gill VL, Jensen WA, et al. Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. *J Am Vet Med Assoc* 2006;229:958–963.
25. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998;339:1448–1456.
26. Wijnhoven TJ, Lensen JF, Rops AL, et al. Anti-proteinuric effects of glycosaminoglycan-based drugs. *Curr Opin Mol Ther* 2007;9:364–377.
27. Chen S, Wassenhove-McCarthy DJ, Yamaguchi Y, et al. Loss of heparan sulfate glycosaminoglycan assembly in podocytes does not lead to proteinuria. *Kidney Int* 2008;74:289–299.
28. Guo Y, Wang Z, Dong L, et al. Ability of low-molecular-weight heparin to alleviate proteinuria by inhibiting respiratory syncytial virus infection. *Nephrology (Carlton)* 2008;13:545–553.
29. Benck U, Haeckel S, Clorius JH, et al. Proteinuria-lowering effect of heparin therapy in diabetic nephropathy without affecting the renin-angiotensin-aldosterone system. *Clin J Am Soc Nephrol* 2007;2:58–67.
30. Weitz JI. Low-molecular-weight heparins. *N Engl J Med* 1997;337:688–698.
31. Feige K, Schwarzwald CC, Bombeli T. Comparison of unfractioned and low molecular weight heparin for prophylaxis of coagulopathies in 52 horses with colic: a randomised double-blind clinical trial. *Equine Vet J* 2003;35:506–513.
32. Wardle EN. How does hyperglycaemia predispose to diabetic nephropathy? *QJM* 1996;89:943–951.
33. Pratt SE, Geor RJ, McCutcheon LJ. Repeatability of 2 methods for assessment of insulin sensitivity and glucose dynamics in horses. *J Vet Intern Med* 2005;19:883–888.
34. Treiber KH, Kronfeld DS, Hess TM, et al. Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. *Am J Vet Res* 2005;66:2114–2121.

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