Recent Research into Laminitis

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INTRODUCTION

Laminitis is a major disease of horses because of the associated pain and debilitating nature that make it a life-threatening condition. A complete understanding of laminitis and its complex pathophysiologic processes remains elusive despite substantial efforts and recent advances by many scientists over the last few decades. For this reason, preventative and therapeutic management strategies remain largely empirical and anecdotal with little information from evidence-based medicine. Laminitis may occur as a consequence of three broad categories of disorders: systemic disease, hormonal disturbances, or trauma. Laminitis research has been confounded by apparently disparate results and theories on pathogenesis. However, recent research has shown that many of these differences may in fact represent different stages of the disease, or different original underlying causes. Recent inflammatory, vascular, and enzymatic research in acute inflammatory or gastrointestinal illness has shown links between these mechanisms, and advances in techniques and early time-course studies have helped elucidate such links. A major trend in recent research on laminitis in the past few years has been the increasing interest in metabolic/endocrine events resulting in laminitis, and interestingly, despite the lack of systemic or gastrointestinal clinical illness in these animals, the laminitis may also involve many of the same mechanisms as those occurring in acute laminitis. This article will review recent research into the pathophysiology of laminitis as well as preventive strategies.

Incidence

The USDA NAHMS report covering 1200 operations and 28,000 horses in 2000 estimated that 13% of operations had encountered at least one case of laminitis in the last 12 months, and the overall incidence was 2.1% (USDA, 2000). The condition is responsible for 15% of all equine lameness and occurs most commonly in horses at pasture. Grazing lush pasture was the most commonly listed cause (45%) followed by complications linked with diet, injury, obesity, and pregnancy. In geriatric horses, the incidence was recorded at 6.4% (Brosnahan and Paradis, 2003) and in the UK a survey of over 100,000 horses found an incidence of 7.1% (Hinckley and Henderson, 1996).

Predisposing Causes

Laminitis is a systemic disease that occurs secondary to the following conditions:

1. Inflammatory/acute gastrointestinal illness
   - grain-based carbohydrate overload
   - pasture-induced carbohydrate overload
   - colic
   - endotoxemia and/or septicemia
   - pleuropneumonia
   - enterocolitis
   - metritis
   - contact with black walnut shavings

2. Metabolic/endocrine (hormonal) disturbances
   - insulin resistance
   - equine Cushing's syndrome
   - obesity
   - glucocorticoid administration

3. Traumatic events
   - excessive weight-bearing on one limb
   - excessive concussion

The pathologic changes that occur in the laminitic hoof are not fully understood, but the following processes are believed to occur at various times during the disease:

- decreased blood flow to the foot and perfusion of the lamellae with possible hypoxia of the lamellae
- injury due to reperfusion after an ischemic episode
- hyperemia
- inflammation
- altered metabolism
- apoptosis
- enzymatic degradation
- edema
- necrosis
- laminar shearing/tearing
- structural failure
- disruption and collapse

Pathology

Key pathologic changes include loss of cellular shape of the secondary epidermal lamellae and loss of attachment of the basement membrane of the lamellae, which is then lysed by matrix metalloproteinase enzymes (MMPs) (Pollitt, 1996). The basement membrane is the key structure bridging the

epidermis of the hoof to the connective tissue of the third phalanx, so it follows that the loss and disorganization of basement membrane leads to the failure of hoof anatomy seen in laminitis. These changes are seen on hoof wall biopsies as early as 12 hours after induction of laminitis using the oligofructan induction model, and well before clinical signs (Croser and Pollitt, 2006). The earliest changes of loss of secondary digital lamellae structure and elongation of secondary epidermal lamellae coincided with the onset of a bounding digital pulse. Another component is loss of the lamellar capillaries, which may explain the resistance to blood flow increases and the bounding digital pulse seen during early laminitis. However, very few thrombi were evident and there were no signs of intercellular edema.

Pathophysiologic Theories

The pathogenesis of laminitis is uncertain (Bailey, 2004; Moore et al., 2004). There are at least five hypotheses of how laminitis initially damages the hoof's lamellae, ultimately resulting in changes to the foot's structure and microanatomy, weakening of the laminar bond, rotation/sinking of the pedal bone, and clinical signs of laminitis. However, recent research is now elucidating links between the different theories, and these may actually represent different events in the complex time course of laminitis rather than opposing theories. They include the enzymatic, inflammatory, vascular, metabolic/endocrine, and mechanical/traumatic theories.

Enzymatic theory

It has been established that activation of lamellar MMPs is important in the separation of the lamellar cells from their basement membrane and a break in the bond which holds the dermal and epidermal lamellae together (Pollitt and Daradka, 1998; Pollitt et al., 1998; Johnson et al., 1998). This hypothesis, originally developed by the Australian Equine Laminitis Research Unit, postulates that increased digital blood flow delivers circulating cytokines or some other laminitis trigger factors to the digit, where they evoke the production and activation of MMPs.

Inflammatory theory

Research at several American institutions using the black walnut extract model and the starch models has shown a marked upregulation of inflammatory cytokines. Increased neutrophil and platelet levels found in laminitic horses can have inflammatory roles and are thought to play a role in the development and progression of laminitis. Inflammatory mediators including cytokines, interleukins 1-beta, 6 and 8, cyclooxygenase-2 (COX-2), and endothelial and intracellular adhesion molecules are involved at an early stage (Blikslager et al., 2006; Loftus et al., 2007a). MMP upregulation has been found at a later stage in these models (Loftus et al., 2007a), indicating that the enzymatic and inflammatory theories may be simply two steps in the pathophysiological process of laminitis.

Vascular theory

The clinical signs of acute laminitis, including a bounding digital pulse and increased hoof temperature, suggest a vascular component. It is postulated that there are blood flow abnormalities including increased capillary pressure, flow in arteriovenous anastomoses, and venoconstriction so lamellae are

deprived of blood flow. It has also been suggested that fluid leaks from the capillaries into the restricted soft tissue space, which leads to edema and ischemia by compressing the small blood vessels. With carbohydrate-induced laminitis models, research has been conflicting with both increased and decreased hoof temperature before the onset of lameness, suggesting increased or decreased digital blood flow. However, increased hoof temperature with the onset of lameness suggests subsequent increased blood flow. Despite these discrepancies, there is little doubt that part of the inflammatory process within the lamellae during laminitis involves endothelial activation (Loftus et al., 2007a) and dysfunction (Eades et al., 2007).

Vasoactive amines have been proposed by the Royal Veterinary Group to play a role in pasture-associated laminitis, with their vasoconstrictive effects shown in vitro (Elliot et al., 2003) and increases in their concentrations in the feces of horses fed fructan-rich diets (Crawford et al., 2007).

Metabolic/endocrine theory

Insulin is a major regulatory hormone in glucose and fat metabolism, vascular function, inflammation, tissue remodeling, and growth. Insulin resistance alters insulin signaling by decreasing insulin action in certain resistant pathways while increasing insulin signaling in other unaffected pathways via compensatory hyperinsulinemia. In humans, altered insulin signaling is implicated in reduced glucose availability to insulin-sensitive cells, vasoconstriction and endothelial damage, and inflammatory response. Insulin resistance was first implicated in the pathogenesis of laminitis in the 1980s using tolerance tests, but recent research in ponies has established a direct link with insulin (Asplin et al., 2007b).

Mechanical/traumatic theory

Laminitis has also been known to result from mechanical overload situations, such as excessive concussion on very hard surfaces or one leg bearing excessive weight when the contralateral limb has a severe, non-weight-bearing injury such as a fracture. In the former case, the mechanism is thought to be damage to the lamellae from direct mechanical trauma, while in the latter, the excessive weight-bearing is thought to either directly damage the lamellae or alter blood supply.

Recent Research

Inflammatory and vascular events

Recent research in several institutions has started to unravel the mystery surrounding the complex inflammatory pathways involved in laminitis and, importantly, their time course in different models of acute disease.

While the type of inflammatory cytokine response during inflammation within the lamellae differs from other organs due to an absence of resident tissue macrophages, especially in respect to the lack of a TNF alpha response from lamellae (Rodgerson et al., 2001; Loftus et al., 2007a), there are a number of inflammatory events occurring in models of equine laminitis including inflammatory signaling, endothelial activation, leucocyte extravasation, activation of degradative enzymes (MMPs), and the presence of oxidative stress that indicate a similarity to remote organ dysfunction occurring in human sepsis (Belknap et al., 2007; Loftus et al., 2007a). This is supported by a recent study on risk factors for

laminitis where endotoxemia, diagnosed on the basis of clinical signs and supportive laboratory findings, was the only factor in the multivariate model associated with a significantly increased risk of laminitis (Parsons et al., 2007).

The inflammatory events in the lamellae occur at an early time point in laminitis induction models including black walnut extract and oligofructose models (Belknap et al., 2007; Loftus et al., 2007a). Recent research has shown that the lamellae have a decreased antioxidant activity (Loftus et al., 2007b), with absent superoxide dismutase indicating its susceptibility to oxidative stress produced during tissue inflammatory events and myeloperoxidase activity from emigrant neutrophils (Riggs et al., 2007).

Many studies have confirmed the in vitro effects of vasoconstrictors (including 5 hydroxytryptamine, PGF2alpha and endothelin-1) of digital vessels, especially digital veins (Peroni et al., 2006). Coincident with pro-inflammatory and pro-oxidative effects during the induction of laminitis is upregulation of endothelial and intracellular adhesion molecules (Loftus et al., 2007a). However, in a recent study using the carbohydrate-overload model, changes in endothelin occurred much later than the inflammatory response and endothelial dysfunction (11 hours compared with 1.5 hours) (Eades et al., 2007), and further in vivo work on the time course of vascular events is warranted.

Enzymatic Theory

Enzymes capable of destroying key components of the hoof lamellar attachment apparatus (Pollitt and Daradka, 1998) have been isolated from normal lamellar tissues and in increased quantities from lamellar tissues affected by laminitis (Pollitt et al., 1998). The enzymes are metalloproteinase-2 and metalloproteinase-9 (MMP-2 and MMP-9). Lamellar tissues affected by laminitis increase transcription of MMP (Kyaw-Tanner and Pollitt, 2004), and increased amounts of active MMP are found in laminitis-affected tissues (Pollitt et al., 1998). More recent evidence suggests MMP-9 is associated with neutrophil migration into the lamellae rather than local production (Loftus et al., 2006) and that the activation of these MMP enzymes may be a later event in laminitis, the end result of the inflammatory cascade, rather than an initiating factor (Loftus et al., 2007a,b).

Microbiology

Carbohydrate-induced laminitis is characterized by marked changes in the composition of the hindgut microflora, with gram positive bacteria dominating an environment that is usually predominantly gram negative. An oligofructose induction model was used to study the changes in the bacterial population of the cecum and feces (Millonovich et al., 2007).

Prior to the onset of histological signs (begins at 12 hours) and clinical signs (begins at 24 hours) of laminitis, there was a proliferation of *Streptococcus bovis/equinus* complex. This began two hours after induction and remained high until the 24-hour point in cecal fluid when numbers began to decline. *Lactobacillus* spp. numbers did not increase until 16 hours after induction and numbers were higher in feces than cecal fluid, indicating a secondary proliferation in the ventral colon rather than the cecum. *Enterobacteria* numbers were low and significant increases were not seen until 16 hours after induction, so the proliferation or disappearance of *Enterobacteria* is unlikely to have a key role in laminitis pathophysiology. One unidentified large gram negative rod proliferated consistently in cecal fluid and feces

prior to the onset of laminitis. Other bacteria did not establish significant populations in the hindgut before the onset of laminitis and are thought not to have a role in pathogenesis. While these studies confirmed the key role played by *Streptococcus bovis* in the cecum of horses during laminitis induction, it is not known if laminitis trigger factors are produced by proliferation or death of the bacteria or by a host inflammatory response to these changes.

Metabolic/Endocrine Events

A major trend in recent research on laminitis in the past few years has been the increasing interest in metabolic/endocrine events resulting in laminitis. Conditions associated with insulin resistance in horses include equine Cushing's syndrome (ECS), also called pituitary pars intermedia dysfunction (PPID); equine metabolic syndrome (EMS) (or pasture-associated laminitis); and iatrogenic corticosteroid administration.

Obesity and EMS

Insulin resistance in horses manifests differently from that in man, although obesity is also a major risk factor in horses with insulin resistance. Obesity has long been associated as a major risk factor for laminitis and this is quantified in the section on risks below. It is difficult for many horses and ponies to lose weight and the syndrome associated with insulin resistance, obesity, and laminitis has recently been termed EMS. EMS was first reported in "Laminitis, hypothyroidism and obesity: A peripheral cushingoid syndrome in horses" (Johnson, 1999). This referred to a syndrome of obese, cresty horses with laminitis. These horses are not hypothyroid as determined by testing for thyroid function (Johnson et al., 2004), but long-term thyroid hormone supplementation can induce weight loss and improve insulin sensitivity (Frank, 2006). The pathogenesis was related back to omental Cushing's in man, which is a syndrome of obesity and abnormal cortisol activity due to activation by the adipose tissue, especially omental adipose tissue as the name suggests. It is thought that fat acts like a gland to produce bioactive substances (adipokines) that reduce insulin sensitivity, cause vascular injury, lead to vasoconstriction, and establish a pro-inflammatory state. There are more than 100 adipokines including leptin, resistin, interleukins, and TNF-α (Johnson et al., 2006). Visceral fat is more biologically active than subcutaneous fat in humans, but of course is harder to assess in the horse or pony. However, it is speculated that fat accumulations in the crest may play the same role as visceral fat in man.

There is a complex relationship between cortisol and adipose tissue production. Situations such as stress or Cushing's disease lead to high cortisol levels that stimulate expansion of adipose tissue and active adipocytes. Active adipose tissue, especially visceral fat, contains the enzyme 11βHSD-1 that can activate cortisol. This leads to further adipogenesis and contributes to insulin resistance by accumulation of fat within skeletal muscle (Davis, 2005; Johnson et al., 2006).

However, irrespective of the name of the condition, the EMS seen clinically is defined by insulin resistance, recurrent painful laminitis despite good management and veterinary care, and often obesity (historically or currently). Despite sometimes appearing similar to horses with ECS, horses with insulin resistance test negative for ECS on specific endocrine tests (i.e., low-dose dexamethasone suppression or basal [ACTH]), are not hirsute and are younger than horses with ECS (McGowan and Riley, 2004). Of

potentially greater interest is that these severe clinical cases probably represent the tip of the iceberg, and there are a number of horses with subclinical disease waiting for the right pasture conditions to develop overt pasture-associated laminitis. These horses have been described by Treiber et al. (2005, 2006) as having "prelaminitic metabolic syndrome," and among other signs, ponies with this syndrome had twice the normal blood concentration of insulin when grazing on winter pasture. In spring, when lush pasture was available, these ponies developed laminitis and had even greater insulin concentrations on basal testing (more than five times the normal values) (Treiber et al., 2006). In more recent studies, it appears that the prevalence of some degree of insulin resistance in horses is 10% (Geor et al., 2007), and even higher (28%) in predisposed breeds such as ponies (McGowan et al., 2008).

Role of insulin in the pathophysiology of metabolic/endocrine laminitis

Insulin has been shown to be the final triggering event in causing endocrinopathic laminitis in ponies (Asplin et al., 2007b). In a study using nine ponies, laminitis could be induced in 100% of ponies exposed to high concentrations of insulin (mean 1036 $\pm$ 55 µIU/mL) while maintaining normal blood glucose concentrations (5.2 $\pm$ 0.1 mmol/L) using a modified euglycemic-hyperinsulinemic clamp technique (Asplin et al., 2007b). All ponies were healthy, young (mean 6.5 years), and nonobese, with no history of laminitis and no evidence of endocrine or other abnormalities on blood tests. Laminitis occurred slowly and in all four limbs, with ponies developing mild signs without actual lameness (Obel grade 1) by 32.6 $\pm$ 5.4 hours and the onset of lameness associated with laminitis (Obel grade 2) by 55 $\pm$ 6 hours. As soon as lameness indicative of laminitis was detected, the infusions were stopped and the ponies were treated with analgesics, resulting in reduction of laminitis by one Obel grade in all cases. Laminitis was confirmed histopathologically in treated ponies. There was no evidence of gastrointestinal involvement and ponies showed no signs of systemic illness throughout the trial (Asplin et al., 2007b).

The common link in endocrinopathic laminitis is insulin resistance, which is manifested as hyperinsulinemia in horses, most commonly with euglycemia (McGowan and Riley, 2004). This new model of laminitis has shown the crucial role insulin plays in laminitis, and shows insulin to be essential in triggering endocrinopathic laminitis. Of importance is that the induction of laminitis occurred independently of glucose or (direct) dietary factors, and also without any evidence of gastrointestinal disturbance. The ponies used were young and had no prior or current obesity. Horses in the study had routine blood tests performed both before and at the onset of laminitis and no changes were found, nor were there any clinical signs indicative of systemic illness or inflammation (Asplin et al., 2007b).

Insulin levels reached in this study are higher than those typically seen in grazing horses (Treiber et al., 2005, 2006). Yet in horses with chronic insulin resistance and endocrinopathic laminitis, insulin concentrations of over 800 µIU/ml were reported in response to glucose administration by Field and Jeffcott (1989) and baseline values over 1000 µIU/ml have been seen frequently in naturally occurring cases of severe insulin resistance syndrome (McGowan and Riley, 2004). However, whether the effect is the same for longer-term exposure to lower levels of insulin or not remains to be determined with further study.

The mechanism by which insulin has triggered laminitis in the model has not yet been determined. However, there are three main theories of the mechanism of laminitis due to the effects of insulin resistance. These are glucose uptake impairment, vascular effects, and pro-inflammatory effects, although

the latter two may well be linked as the vascular endothelium is typically a target tissue for the proinflammatory/ pro-oxidative effects of insulin resistance.

Glucose uptake impairment

Glucose uptake impairment is the classical sign of insulin resistance, due to the principal effects of insulin in stimulating glucose dispersal into the tissues via GLUT4 transport proteins, particularly in muscle and adipose tissue. The underlying problem of insulin resistance is then potential glucose deprivation of tissues, starving them and causing cell death or damage. In support of this, research has shown that healthy hoof tissue has an absolute requirement for glucose such that when hoof explants are incubated in the absence of glucose, or in the presence of a glucose uptake inhibitor, the layers of tissue separate rapidly, as they do when laminitis occurs (Pass et al., 1998). Additionally, the hoof utilizes glucose at an exceptionally fast rate compared with most other tissues (Wattle and Pollitt, 2004) so, in theory, even a small decrease in the rate of glucose uptake could be extremely damaging. Further to classic glucose uptake impairment due to insulin resistance, there could be a combination of hormonal influences including the activity of catecholamines, which can also reduce glucose uptake synergistically to corticosteroids (Hunt and Ivy, 2002). Since corticosteroids also have the potential to increase adrenergic receptors, this synergism could further impair glucose uptake (Huang et al., 1998).

However, recent research has shown that the hoof lamellae are insulin-independent (Asplin et al., 2007a) based on a number of experiments that indicated that glucose uptake in the hoof is neither dependant on insulin, nor is it influenced by the presence of insulin. Further, when the glucose transport proteins were examined, there was a predominance of GLUT1 in lamellae, which are insulin independent, consistent with the hoof having such a high metabolic demand for glucose (Wattle and Pollitt, 2004; Asplin et al., 2007a). Wattle and Pollitt (personal communication) have also shown that the insulin receptor is not even present on lamellar cells, although it is present in the blood vessel walls. Together, these results provide compelling evidence that laminitis cannot be caused by glucose deprivation resulting from insulin resistance.

Inflammation and endothelial cell dysfunction

The pro-inflammatory changes and effects on the microvasculature are a major problem in human diabetics and sufferers of insulin resistance syndrome. Vascular dysfunction is manifested as both vasoconstriction and pro-coagulant activity, and chronically involves vascular remodeling. Insulin resistance and obesity in man result in a pro-inflammatory state, with increased production of pro-inflammatory cytokines and cytokine-like substances (leptin, resistin), impaired endothelial nitric oxide (NO) production, and endothelial dysfunction (Singer and Granger, 2007). Exacerbating the vascular dysfunction in diabetic patients is the accumulation and glycation of glucose in endothelial cells (glucotoxicity). Glucotoxicity promotes the formation of advanced glycation end products (AGEs), further reducing NO production, and leads to an induction of reactive oxygen species and encourages endothelial expression of inflammatory mediators (Hartge et al., 2007). Insulin also has effects on blood flow in both small and large vessels and insulin resistance (Rattigan et al., 2007). Insulin resistance results in both reduced capillary recruitment and reduced vasodilation in animal models and in humans (Rattigan et al., 2007).

While pro-inflammatory effects and vascular effects of insulin resistance may be difficult to separate, it is clear that either or both occurring in horses with insulin resistance could precipitate laminitis by affecting blood flow or by the induction of a pro-inflammatory state and oxidative stress.

Oxidative Stress

The link between oxidative stress and laminitis has recently been studied by a number of groups at the whole-horse level. Neville at al. (2004) found that 20 ponies with chronic laminitis had significantly higher ($P<0.01$) levels of a marker of lipid peroxidation. These ponies had three times the average level of plasma thiobarbituric acid reactive substances (TBARS) which are an indicator of free radical damage.

Horses with clinical or subclinical Cushing's disease with a previous history of laminitis were found to have significantly lower levels of plasma thiol levels indicative of oxidative stress. However there was no difference in other markers of antioxidant function (glutathione peroxidase) or lipid peroxidation (malondialdehyde) (Keen et al., 2004).

Treiber et al. (2007) studied oxidative stress in a pony herd of 42 laminitis-prone ponies and 34 ponies with no history of laminitis. Ponies with a previous history of laminitis did not show reduced antioxidant function or increased oxidative stress when compared to control ponies. However it was considered that ponies may have lower antioxidant capacity than horse breeds. This study found a significant ($P<0.001$) elevation in levels of tumor necrosis factor-alpha (TNF-α) in the laminitis-prone group. This is a pro-inflammatory adipocytokine, which may limit fat accumulation by inducing insulin resistance and thus have a role in the development of laminitis.

Free radical accumulation is thought to result from a number of factors including ischemia during the initial stages of the disorder, reperfusion injury, neutrophil activity in chronic laminitis, and insulin resistance (Neville et al., 2004). Reactive oxidative species (ROS) produced in situations of oxidative stress activate MMP-2 and MMP-9 and high glucose levels can increase production of ROS and MMP-9 by vascular endothelial cells. This glucotoxic action can be reversed by antioxidants (Johnson et al., 2004). More study is needed to determine the extent to which oxidative stress plays a role in laminitis and how antioxidant function may assist in prevention. Supplementation with 8,000-10,000 IU of vitamin E per day has been recommended in the clinical management of EMS (Davis, 2005).

Forages

Grazing lush pasture is the most common perceived cause of laminitis although recent research would indicate insulin resistance is a key risk factor in the development of the disorder. It was thought that high levels of fructans accumulated in grasses under certain conditions (Longland et al., 1999) and that these were not digested in the small intestine, but were instead fermented in the large intestine to produce acid, which decreases pH and can launch a cascade of events leading to laminitis.

Routine analyses of forages do not include fructan levels. However, recent changes in reporting of forage analyses may provide more information on the starch, simple sugar, and fructan content of feeds. Some recent changes in available assays and terminology offer greater evaluation of the carbohydrate content of forages, but this means that comparison of research results from different testing eras is diffi-

cult. Dairy One Laboratory in New York now measures carbohydrates by NIR as ethanol soluble carbohydrates (ESC), which are simple sugars; water-soluble carbohydrates (WSC), which combines simple sugars and fructans; and nonstructural carbohydrates (NSC) as starch plus WSC. Thus it is possible to crudely estimate the fructan content of a forage as WSC - ESC. Nonfiber carbohydrate (NFC) numbers are calculated by subtraction; however, to complicate matters, this was previously referred to as NSC.

Analysis of grass/legume pastures in Germany found that fructan levels varied from 5.7% in spring to 1.8% in late summer (Vervuert et al., 2005). There was a significant negative relationship between fructan and protein content of the pastures. Fructan concentrations were lower in hay and silage than pasture, and these levels were much lower than those recorded by Longland et al. (1999). In addition, the calculated daily maximum intake of 2.5 g fructan/kg BW based on a pasture intake of 3% BW is much lower than the levels of fructans shown to induce laminitis (Pollitt and Van Eps, 2002).

Treiber et al. (2006) also cast doubt on the "fructan in pasture leads to laminitis" model. In a study with a single herd of 150 ponies on a farm in Virginia, they found there was no change in water-soluble carbohydrate (WSC) levels in samples taken in March and May. There was a dramatic increase in pasture availability and clover content from March to May and clinical cases of laminitis were detected in May. It was assumed that no change in WSC content equated with no change in fructan content, but there was a significant increase in starch content of the pastures sampled, from 4.2% in March to 7.8% in May. On this basis, the authors hypothesized that starch may be more important than sugars and fructans in the pathophysiology of laminitis. However, the starch levels recorded were very low compared to those found in grains.

McIntosh et al. (2007a,b) investigated circadian and seasonal patterns in sugar, starch, and fructan and the relationship to glucose and insulin values in grazing Thoroughbred mares. Strong circadian rhythms were seen in pasture NSC content, with lowest levels seen between 4:00 a.m. and 5:00 a.m. and peak levels 12 hours later. Pasture NSC levels were strongly correlated ($P<0.001$) with temperature, humidity, and solar radiation. Samples taken in April contained 20% NSC, but this dropped to 13% in May and less than 10% in August, October, and January. Simple sugar content was much higher than fructan content and starch contents were low in all samples taken. Peak fructan level was 5.5% in April and this dropped to 3.9% in May and was below 3% in other months. Ten mares were assigned to grazing and compared with four stabled controls fed timothy/alfalfa hay. Plasma insulin was highest in grazing horses in April followed by May and was significantly higher than controls in those months. Plasma glucose in grazing horses was higher in April than controls and other sample times. A circadian pattern was seen in plasma insulin levels in April with peak levels seen in the evening. Plasma insulin was significantly correlated with NSC and sugar content in April, May and January. It was thought that in susceptible horses, this change in glucose and insulin dynamics could increase the risk of laminitis.

Soaking hay has been shown to reduce the starch and sugar content and this resulted in a reduced glucose and insulin response to feeding (Cottrell et al., 2005). Soaking two orchard grass hay samples for 30 minutes in cold water reduced the sugar content from 12% to 5.6% and 22% to 13.4%. The starch content was unaffected. To test the effect of soaking on the glycemic index of the hay, it was fed to 12 weanling fillies in a crossover study. This led to a significant ($P<0.01$) reduction in area under curve for plasma glucose and insulin. This practice is an important management aid to reduce the glucose/insulin response to feeding hay in insulin-resistant horses that are prone to laminitis.

Recognition of Horses at Risk of Laminitis

Research at Virginia Tech has examined risk factors for laminitis in ponies to allow the identification of ponies with pre-laminitic metabolic syndrome (PLMS) at high risk of developing the disorder (Treiber at al., 2006; Carter et al., 2007). The initial study identified ponies with three of a possible four criteria at risk of developing laminitis. The criteria were obesity (body condition score (BSC) >6), insulin resistance, hypertriglyceridemia, and increased insulin secretory response.

In the follow-up study, 76 pony mares on one farm were assessed. Thirty-four ponies had no history of laminitis (NL) and 42 had previously had laminitis (PL). In the spring, six of the PL ponies developed laminitis and the criteria picked four of the six as at-risk horses with PLMS. The odds ratio for prediction of laminitis risk was 3.75, so horses chosen as PLMS cases were nearly four times more likely to develop laminitis than those not identified. There was no difference between NL and PL ponies in plasma glucose, cortisol, and leptin, although plasma leptin was positively correlated ($P<0.001$) with BCS on a 1-9 scale and cresty neck score (CNS) on a 1-5 scale.

Assessment of obesity provided a simpler and more accurate means of diagnosing PLMS. Criteria of BCS >6 and CNS >3 predicted the same number of cases but with a higher specificity and predictive power (75% vs. 65%) and odds ratio (6.12 vs. 3.75). To reduce subjectivity in BCS and CNS assessments, morphometric measurements could be substituted. A girth-to-height ratio of >1.28 can replace BCS and crest height >10 cm could replace CNS. The authors concluded that further study in other populations and seasons is warranted.

Genetics

In dairy cattle, the heritability of laminitis is estimated to be between 0.1 and 0.15, although no clear mode of inheritance has been determined (Huang and Shanks, 1995a,b). A recent study in Virginia examined the heritability of grass founder in an inbred herd of Welsh and Dartmoor ponies (Splan et al., 2005). The pedigrees of 257 ponies born from 1933 to 2002 were traced back up to 10 generations. Ninety-five ponies (36.7%) were reported to have suffered laminitis and nearly half the affected ponies had a parent that was recorded as laminitic. Increased severity was noted when a pony's sire and dam had both suffered laminitis. This analysis suggests a major gene with a dominant action. In theory, a dominant gene would mean that 100% of cases needed to have an affected parent, but the researchers postulated that the number was reduced by incomplete recording of cases born earlier in the study population and ponies having a genetic predisposition but environmental thresholds for clinical disease not being crossed.

The development of a test to identify those ponies with a genetic predisposition to laminitis would assist the management of these ponies so that the incidence of the disorder can be reduced.

Cryotherapy for Prevention

Continuous cryotherapy by keeping the foot in an iceboot containing water at 1°C for 48-72 hours is well tolerated and has been proven an effective and safe method in horses at risk of laminitis, when

applied *before* lameness develops. One study that cooled one foot on normal and oligofructan laminitis-induced horses for 48 hours found slightly elevated activity of MMP-2 in treated vs. normal feet, but the activity in cooled feet was significantly lower ($P<0.05$) than in untreated feet. The cooled feet showed no lameness and better tissue architecture scores than untreated feet (Van Eps and Pollitt, 2004).

A later study by the same authors used 72 hours of cryotherapy after experimental oligofructan-induced laminitis to significantly reduce ($P<0.05$) the lameness score and total epidermal length in treated horses compared to controls at seven days after induction (Van Eps and Pollitt, 2006a,b). The use of water at 1°C maintained internal hoof temperatures at 1.8°C to 3.6°C for the majority of the period in six normal horses also undergoing cryotherapy. Lamellar histology was normal in these horses.

This is not a new concept for preventing/moderating laminitis and many horses with clinical laminitis have been observed to stand in cold water. Potential benefits include vasoconstriction, resulting in decreased blood flow and decreased delivery of LTFs; decreased cell and tissue metabolism in lamellae; decreased activation of MMPs; and anti-inflammatory activity. Cryotherapy is an effective preventative strategy for horses at risk of developing acute laminitis.

However, the optimum temperature has not been established. The treatment period may be extended in association with some clinical conditions such as metritis and pleuritis. It is important to cool horses to at least midcannon to cool arterial blood. Horses tolerate cryotherapy well with no signs of discomfort.

Intracecal Buffering

The pH of the cecum drops dramatically related to the production of D-lactate in the period before the onset of clinical signs of carbohydrate-overload laminitis. Souza et al. (2006) examined the effect of an intracecal buffer (CB) or saline (CS) administered eight hours after induction of a carbohydrate-induced laminitis model. Both treatments were also administered to control horses (WB and WS). The buffer was 3.5 g aluminum hydroxide and 65 g magnesium hydroxide administered through a surgically inserted cecal cannula.

Horses receiving the buffer had delayed onset of clinical signs, increased cecal pH, decreased growth of *Streptococcus* and *Lactobacillus*, and reduced expression of MMP-2 and MMP-9 in the hoof after 48 hours, but horses were not protected against laminitis in this model. Expression of MMP-2 and MMP-9 was higher in laminitic tissues than control tissues. MMP-2 expression was 2.25-fold higher in the CS group and 1.18-fold higher in the CB group than the WS group. MMP-9 followed a similar pattern, with CS tissues 17.8-fold higher and CB only 5.1-fold higher than WS tissues. Further studies are warranted to assess the potential of intracecal buffering in management of horses with carbohydrate-overload laminitis.

Intracecal administration of sodium bicarbonate has been shown to be effective in raising the pH of the cecum when administered by intracecal cannula after a starch-rich grain meal that caused a significant drop in cecal pH (Willard et al., 1977). However, oral administration of sodium bicarbonate will not protect against the pH drop, as it will be digested and absorbed before it gets to the cecum.

Kentucky Equine Research has recently examined the effect of an orally administered hindgut buffer on fecal pH and lactate levels in horses on normal grain and hay diets. Thoroughbred horses in training were fed 4 or 6 kg of an unfortified sweet feed and 4 kg of hay in a switchback study. Administration of the protected sodium bicarbonate buffer EquiShure (KERx, Versailles, KY) in each of two grain feeds pre-

vented the significant ($P<0.05$) drop in fecal pH seen in control horses after feeding, and fecal D- and L-lactate levels were significantly lower ($P<0.05$) in treated horses at six hours after feeding (Pagan et al., 2007). While cecal measurements were not performed in this study, cecal pH has been correlated with fecal pH. It can reasonably be assumed that the protected sodium bicarbonate attenuated the drop in cecal pH seen after a grain meal, and it may have value given by stomach tube in grain overload cases.

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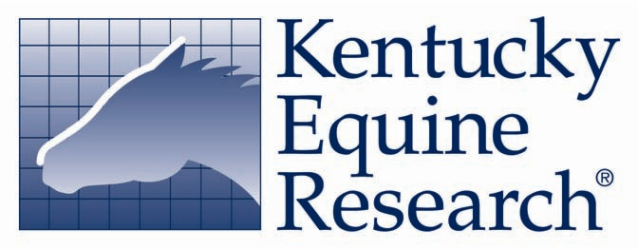

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