Laminitis, failure of the distal phalanx to maintain its attachment to the lamellae of the inner hoof wall, causes unrelenting pain and a characteristic lameness. During a developmental phase, pathology in organs anatomically remote from the foot generates laminitis trigger factors that circulate to cause separation and disorganization of hoof lamellar anatomy. Laminitis, linked to seasonal variations in grass fructan concentration can be induced experimentally using oligofructose, a closely related compound. There is a strong correlation between the degree of lameness and the severity of histopathology in lamellar samples from horses with laminitis. Matrix metalloproteinase enzymes (MMPs), responsible for normal enzymatic remodeling of the epidermal lamellae, appear to be accidently recruited in the pathogenesis of the laminitis. MMP-2 and MMP-9 have been isolated from normal lamellar tissues and in increased quantities from lamellar tissues affected by laminitis. Although epidermal cells of other species readily increase their production of MMP when exposed to cytokines this is not the case with equine lamellar explants. An enzymatic theory of laminitis etiology based on lamellar MMP activation challenges the alternative view that laminitis develops because of vascular pathology affecting the circulation of the foot. Epidermal cell necrosis, intravascular coagulation, edema and other evidence of ischemia cannot be identified in tissue affected by early carbohydrate-induced laminitis. In fact, laminitis does not occur if the foot is in a state of vasoconstriction-induced laminitis. At acute laminitis onset many of the ultrastructural plaques (hemidesmosomes, HDs) that attach lamellar basal cells to the basement membrane of the connective tissue of the distal phalanx, are absent or disrupted. This is accompanied by BM separation, cytoskeleton damage and rounding of basal cell nuclei. The magnitude of HD loss in lamellar basal cells, affected by laminitis directly correlates to the dose of carbohydrate used to induce it. Sound lamellar architecture depends on the structural integrity of HDs, a fact illustrated when a newborn foal, lacking in plectin (one of the HD intracytoplasmic plaque proteins), developed laminitis as soon as it was ambulatory. The weight-bearing basement membrane of the hoof lamellar dermal epidermal interface is unique to the anatomy of digitigrade equids. However the lamellae are dependent on MMP enzymes for tissue remodeling thus making horses susceptible in situations that accidentally precipitate MMP activation, HD failure, basement membrane disadhesion and ultimately laminitis.

KEYWORDS equine laminitis, matrix metalloproteinase (MMP), basement membrane, Streptococcus bovis, hemidesmosome, fructan
Figure 1  Horse with severe laminitis in both front feet showing typical laminitis gait. The hind feet are placed as far forward as possible before the horse attempts painful shuffling steps in front.

Figure 2  Sagittal section of a foot with severe chronic laminitis and a large lamellar wedge. The attachment between the distal phalanx (DP) and the dorsal hoof wall (DHW) has failed and hoof and bone are now widely separated. The dashed yellow line shows the original position of the distal phalanx. The solid black line shows that the distal phalanx has rotated (in the direction of the curved black arrow) off the normally straight axis of the proximal and middle phalanges. The material now between the inner hoof wall and the bone is abnormal and consists of epidermal tissue proliferating to form a weak, disorganized mass called the lamellar wedge (yellow line). The descent of the unattached distal phalanx into the hoof capsule has distorted the growth of the proximal hoof wall tubules and has caused the sole to become convex instead of concave (dropped sole). Two dark hemorrhagic zones (white arrows) show the sites of greatest pressure and trauma.
Laminitis is the most serious disease of the equine hoof and causes pathological changes in anatomy that lead to devastating loss of function. The simplest definition of laminitis is: failure of the attachment between the distal phalanx and the inner hoof wall. A horse has laminitis when the lamellar architecture of the inner hoof wall, which normally suspends the distal phalanx from the inner surface of the hoof capsule, fails. Without the distal phalanx properly attached to the inside of the hoof, the weight of the horse and the forces of locomotion drive the bone down into the hoof capsule, shearing and damaging arteries and veins, crushing the corium of the sole and coronet, causing unrelenting pain and a characteristic lameness (Fig. 1).

The Phases of Laminitis

A developmental phase, during which lamellar separation is triggered, precedes the appearance of the foot pain (the acute phase) of laminitis. This may be as short as 8 to 12 hours in the case of laminitis caused by exposure to the water-soluble toxins of black walnut (Juglans nigra) heartwood shavings or 30 to 40 hours in the case of excessive ingestion of high starch grain. During the developmental phase and before the clinical appearance of foot pain the horse or pony usually experiences a problem with one or more of the following organ systems: gastrointestinal, respiratory, reproductive, renal, endocrine, musculoskeletal, integumentary and immune. Multi-systemic aberrations in organs anatomically remote from the foot result in the lamellar tissues of the feet being exposed to factors which lead to separation and disorganization of lamellar anatomy. The exact nature of the laminitis trigger factors, apparently reaching the lamellar tissues via the circulation, has yet to be elucidated. Sometimes no developmental phase can be recognized: the horse or pony is discovered in the acute phase with no apparent ill-health or inciting problem occurring beforehand. Obesity and related endocrinopathic problems have recently been incriminated in the pathogenesis of this insidious form of laminitis.

Grass founder can also appear without warning and this has now been linked to seasonal variations in the concentration of the soluble sugar fructan by temperate pasture species. Fructan can suddenly reach very high concentrations in the stems of grass and trigger a laminitis-inducing gastrointestinal disturbance when consumed by horses and ponies. That laminitis can be induced by such sugars has been verified experimentally using oligofructose, a closely related compound. The parenteral injection of potent long acting corticosteroid preparations for the treatment of skin disease may precipitate iatrogenic acute laminitis.

The developmental phase merges into the acute phase of laminitis which lasts from the onset of clinical foot pain and lameness at the trot, to the time when there is clinical (usually radiological) evidence of displacement of the distal phalanx within the hoof capsule (Figs. 2 and 3). After the acute phase, if the horse does not die from the disease process inciting the development of laminitis, it can make an apparent complete recovery.
recovery or develop palmar/plantar displacement of the distal phalanx, the hallmark of chronic laminitis. The chronic phase can last indefinitely with clinical signs ranging from persistent, mild lameness, continued severe foot pain, further degeneration of lamellar attachments, recumbency, hoof wall deformation and sloughing of the hooves. It is important to realize that the process initiating the destruction of the lamellar attachment apparatus begins to operate during the developmental phase before the first clinical sign of laminitis, foot pain, is apparent. During the developmental phase the specific problems of the horse often have to be attended to urgently (eg, acute abdomen, grain overload acidosis, electrolyte imbalance, rhabdomyolysis, retained placenta) and unfortunately the feet are often left out of therapeutic equation until the first signs of foot pain (shifting weight from one foot to the other) appear. By the time foot pain is apparent lamellar pathology is underway. In other words foot pain is the clinical sign that lamellar disintegration is occurring. To wait and see if foot pain is the sequel to a metabolic crisis is to miss the opportunity to prevent or at least ameliorate lamellar pathology. There is a good correlation between the severity of laminitis histopathology, as seen with the microscope, and the degree of lameness [using the Obel grading system] shown by the horse. When a horse first starts to show laminitic pain, the anatomy of the hoof wall lamellae is being destroyed. The higher the lameness grade, the more severe the microscopic damage. Any activity that places stress on an already weakened lamellar attachment apparatus (such as forced exercise) causes further damage and is contraindicated. The use of nerve blocks to eliminate pain will also encourage locomotion and precipitate more damage.

The Laminitis Histological Grading System

As laminitis develops a sequence of histopathological changes occurs. Three grades of histological laminitis have been identified based on the degree of severity of the changes. Making the lamellar basement clearly visible is important and requires staining lamellar tissues with either periodic acid Schiff (PAS), or periodic acid silver methanamine (PASM) stains, or with immunohistochemical methods using basement membrane (BM) specific antibodies.

Grade 1 Histological Laminitis

During the developmental phase lamellar basal and parabasal cells lose their normal shape, become elongated and appear to slide over one another and, as a consequence, the secondary epidermal lamellae become attenuated with tapering, instead of club-shaped, tips. While this is going on, the BM of the SEL loses its attachment to the basal cells. This is first noticeable at the tips of the SELs where small teat-shaped bubbles of loose BM form. To render this detectable by light microscopy the tissues should be stained with periodic acid Schiff (PAS), or periodic acid silver methanamine (PASM) stains (Fig. 4).
Grade 2 Histological Laminitis

With disadhesion occurring between the lamellar BM and the SEL basal cells, the BM is drawn further away with each cycle of foot loading by the horse. The lamellar basement membrane is now absent between the bases of adjacent SELs. The BM retracts from between the SELs and takes with it secondary dermal lamellar (SDL) connective tissue and SDL capillaries (Fig. 5). The loss of these capillaries may explain why resistance to blood flow was increased 3.5 times (the bounding digital pulse) in horses during early laminitis and why blood appears to bypass the lamellar capillary bed through dilated arteriovenous anastomoses in horses with acute laminitis. Both of these phenomena occur after, and are thus a consequence of, the triggering of MMP production. The epidermal basal cells that have lost their BM attachment do not appear to undergo necrosis, at least initially, and clump together to form amorphous, BM-free masses, on either side of the primary lamellar axis.

Grade 3 Histological Laminitis

In laminitis, the worse case scenario is a rapid and total BM separation from all the epidermal lamellae. Sheets of BM peel away to form aggregations of loose isolated BM in the connective tissue adjoining the lamellae. The epidermal lamellar cells are left as isolated columns with no connection whatsoever with the dermal connective tissue. The lamellar tips slide away from the BM connective tissue attachments, at first microscopically, but as the degree of separation increases the distance between hoof and distal phalanx becomes measurable in millimeters (Figs. 6, 7, and 8). This is manifest clinically as the “sinker.” Since the BM is the key structure bridging the epidermis of the hoof to the connective tissue of the distal phalanx, it follows that the wholesale loss and disorganization of the lamellar BM inexorably leads to the failure of hoof anatomy so characteristic of the chronic stage of laminitis.

The Pathophysiology of Laminitis

The spectacular disintegration of the lamellar attachment apparatus, initiated during the development phase of laminitis, compromises a normally robust and trouble-free hoof, distal phalanx attachment apparatus in a surprisingly short period of time. Logic suggests that it is a normally tightly controlled metabolic process that is thrown into disarray to cause the lamellar-specific lesion of laminitis during its developmental phase. The enzymatic remodeling of the epidermal lamellae, assumed to be mandatory if the continually proliferating stratum medium of the hoof wall is to move past the stationary distal phalanx, could be accidentally recruited in the pathogenesis of the laminitis disease process. Enzymes capable of destroying key components of the lamellar attachment apparatus have been isolated from normal lamellar tissues and in increased quantities from lamellar tissues affected by lamini-
The enzymes are metalloproteinase-2 and metalloproteinase-9 (MMP-2 and MMP-9) also found in a wide range of human and animal remodeling tissues such as bone, joints and endometrium as well as in metastasizing malignant tumors. It is assumed that lamellar MMP activity is constantly responding to the stresses and strains of normal equine life as well as to constant growth. When called for, sufficient MMP is manufactured locally, to release epidermal cell-to-cell, and cell-to-basement membrane attachments, as required, to maintain the correct shape and orientation of the hoof lamellae. From time to time injury to the basement membrane would require its lysis and reconstruction. The controlled release of specific MMP inhibitors keeps this remodeling process in equilibrium and the hoof lamellae and the hoof itself slowly migrate past the stationary basal cells firmly attached to their underlying basement membrane that is in turn attached to the connective tissue of the distal phalanx.

The epidermal cells of other species have been shown to readily increase their production of MMP when exposed to cytokines. Cultures of human oral mucosal keratinocytes respond to the addition of tumor necrosis factor (TNF), interleukin-1 (IL-1) and transforming growth factor-1 (TGF-1) by increasing production of MMP-9. Lamellar tissues affected by laminitis also increase transcription of MMP but whether in response to circulating cytokines or some other trigger factor is yet to be established. Evidence from in vitro studies, using equine lamellar explants, suggests that lamellar MMPs are not activated by exposure to cytokines.

The enzymatic theory of laminitis etiology based on lamellar MMP activation challenges the alternative view that laminitis develops because of vascular pathology affecting the circulation of the foot. A current theory is that venoconstriction and high hydrostatic interstitial fluid pressure (compartment syndrome) impede the flow of blood in the lamellar microcirculation to cause ischemic necrosis of epidermal lamellae. Epidermal cell necrosis, intravascular coagulation and edema were not identified in sections made from tissue in the early stages of laminitis. The vessels in the primary dermal lamellae, even the smallest, were generally dilated without evidence of microvascular thrombi. Further, no abnormalities in the systemic coagulation and fibrinolytic cascades are found in horses with carbohydrate-induced acute laminitis. The gross anatomical appearance of freshly dissected laminitis tissue is one of dryness. The lamellae peel apart. Tissues affected by a compartment syndrome exude fluid.

How do the trigger factors of laminitis reach the lamellae? There is now strong evidence from three independent experimental sources that the foot circulation during the developmental phase of laminitis is dilated. Laminitis does not
occur if the foot is in a state of vasoconstriction during the developmental phase suggesting that the trigger factors will only cause laminitis if they reach the lamellar tissues via dilated blood vessels at a high enough concentration and over a long enough time period. It follows that therapy aimed at keeping the feet of horses in danger of developing laminitis as cool as possible (and therefore vasoconstricted) is logical. Indeed acute laminitis can be prevented in a single cooled limb while laminitis develops in the three remaining limbs maintained at room temperature.27 Horses, unlike humans, do not regard extremely cold feet as uncomfortable and can tolerate having their feet in iced water for 48 hours with no discomfort or ill effect.28 Scintigraphic studies comparing the circulation of iced feet versus normal showed profound vasoconstriction in the cold feet (80.5% of precooled values).29

What are the laminitis trigger factors? Since the carbohydrate overload model of laminitis is characterized by endotoxin production it would seem a safe presumption that macrophages in the peritoneal cavity and elsewhere in the body would be subject to endotoxin stimulation just as they are during other acute gastrointestinal diseases.30 Mononuclear phagocytes express tumor necrosis factor along with other cytokines such as interleukin within minutes of exposure to endotoxin. The cytokine cascade originating from an acute abdomen is responsible for most of the pathological effects of endotoxemia. However, laminitis has never been triggered by the experimental administration of endotoxin into the bloodstream31 or the peritoneal cavity and the actual trigger factors of laminitis remain unidentified. What appears certain in the light of recent research is that the lamellar disintegration of laminitis is mediated by the uncontrolled release of excess MMP.17

We have successfully developed an in vitro model18,21 for equine laminitis using small explants of tissue taken from the inner hoof wall of normal, freshly killed, abattoir horses. Each explant consists of stratum medium, the lamellar layer and the sublamellar connective tissue. After incubation for 48 hours in tissue culture medium, plus the laminitis trigger factor under investigation, each explant is subjected to tension. The force required to separate epidermal from dermal lamellae is recorded. When dermal–epidermal lamellar separation occurs readily (as occurs in field cases of laminitis) we consider the tissue to have developed in vitro laminitis. Lamellar explants can be cultured for up to 7 days in normal medium and no lamellar separation occurs. It is virtually impossible to separate normal lamellar explants. One event that readily causes separation of lamellar explants is MMP activation. The addition to the culture medium of the organomercurial compound aminophenylmercuric acetate (APMA), a well known nonphysiological MMP activator, readily induces explant lamellar separation. Treatment of lamellar explants with APMA is the in vitro laminitis control against which naturally occurring laminitis induction factors can be measured. The presence or absence of MMP activation in explant supernatants is detected zymographically using gelatin polyacrylamide electrophoresis and all explant tissues are fixed and examined histologically. Histological sections show a clear zone of complete separation between the base-
ment membrane and the basal cells of the epidermal lamellae. This is a characteristic of in vitro laminitis and resembles the basement membrane lesion of natural in vivo laminitis.

We have used the in vitro laminitis explant model to investigate most of the proposed causes of equine laminitis. The equine lamellae have tested resistant to virtually all known cytokines, tissue factors and prostaglandins. Gram-negative bacterial endotoxin, extract of black walnut \( (Juglans nigra) \) and even anaerobic culture conditions fail to induce lamellar separation or significant MMP activation. There is one notable exception however. A factor present in the supernatant of cultures of \( \text{Streptococcus bovis} \) isolated from the equine cecum activates equine hoof MMP-2 and causes lamellar separation.\(^{21}\) During grain overload \( S. \ bovis \) is the principal microorganism responsible for the rapid fermentation of carbohydrate to lactic acid in the equine hindgut. In the presence of virtually unlimited substrate its population explodes exponentially. We are currently investigating the role of the \( S. \ bovis \) MMP activator in natural cases of equine laminitis.\(^{21}\) If it crosses the mucosal barrier of the hindgut and enters the circulation it may be a “cause” of laminitis (at least in the carbohydrate overload model). In other words it may be an exogenous laminitis trigger factor.

The activity of tissue MMPs has long been shown to correlate strongly with the degree of malignancy and invasiveness of lethal human tumors such as malignant melanoma, breast and colon cancer. We have recently cloned the gene responsible for MMP-2 expression in lamellar hoof.\(^{17}\) Horses with acute laminitis show increased expression of the MMP-2 gene, 48 hours after alimentary carbohydrate overload (Fig. 9). For mean MMP-2 gene expression to have doubled by the time lameness is manifest implies that the factors signaling the increased expression have been present for some time. This places perturbation of MMP equilibrium early in the cascade of events leading to the foot pain of acute, clinical laminitis. Indeed biopsies of lamellar tissue taken at 24, 36, and 48 hours (Wattle and Pollitt, unpublished data) all showed some of the histopathology described in the pub-

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**Figure 8** Diagrams showing normal lamellar histology and three grades of laminitis histopathology in order of increasing severity.
lished laminitis grading systems. At 24 hours lamellae had intact basement membranes but SELs were attenuated with round basal cell nuclei. At 36 hours, SEL attenuation had progressed and SEL basal cells with rounded nuclei were disorganized; SEL tips were pointed instead of rounded. Only at 48 hours was the BM not attached to SEL basal cells suggesting that the disadhesion process commenced somewhere between 36 and 48 hours. However the molecular and biochemical events contributing to BM disattachment, as evidenced by nuclear rounding and SEL attenuation, were in place by 24 hours. The basement membrane lesion of laminitis is insidious in nature and well under way by the time clinicians are aware of laminitis foot pain. Any preventive or treatment strategies must be in place before overt foot pain develops if horses are to survive the development phase of laminitis without significant lamellar damage.

There is a wide range of chemical agents capable of inhibiting MMP activity both in vitro and in vivo. We have shown that one of these (Batimastat or BB-94, British Biotech, Oxford) blocks the activity of the laminitis MMPs in vitro and has the potential to be a useful tool in the prevention and management of acute laminitis. Trials to test whether MMP inhibitors can prevent or ameliorate field cases of laminitis are currently underway in the Australian Equine Laminitis Research Unit at The University of Queensland.

The Ultrastructure of Laminitis

Laminitis studied by transmission electron microscopy (TEM) and immunofluorescence microscopy (IFM) has provided new insight into the mechanism of the disease. The hemidesmosome (HD) is the attachment plaque responsible for maintaining contact between the SEL basal cell and its underlying basement membrane. In lamellar SEL samples taken at the onset of acute laminitis many HDs are absent or disrupted (Fig. 10). Loss and disruption of HDs is accompanied by BM separation, cytoskeleton damage and rounding of the basal cell nucleus. Indeed the magnitude of HD loss in SEL basal cells affected by laminitis directly correlates to the dose of carbohydrate used to induce it; data that support a bacterial pathogenesis of laminitis. The hypothesis is that increasing amounts of carbohydrate substrate support greater numbers of microbes that generate higher concentrations of laminitis trigger factors.

Hoof lamellae cultured in vitro, separate under tension and the intracytoplasmic components of their HDs denucleate and fade if not provided with sufficient glucose; a mechanism that may be operating in vivo when toxemia and the various endocrinopathies associated with laminitis limit the supply of lamellar glucose. Activation of constituent lamellar MMPs also causes lamellar separation under tension but without affecting HD ultrastructure. Activated MMPs appear to cleave laminin5 anchoring filaments and set the BM adrift; also a process now shown to occur in vivo.

The dependence of sound lamellar architecture on the structural integrity of HDs is well illustrated when foals are born lacking just one of their HD proteins. A 45-day-old Quarterhorse foal showed the clinical signs and gross pathology of chronic laminitis since birth. Characteristic laminitis histopathology was present only in the front feet. In all feet TEM showed the intracytoplasmic plaques of lamellar hemidesmosomes were small, misshapen and not associated with the cytoskeleton. In all feet IFM showed the hemidesmosomal, intracytoplasmic plaque protein, plectin, was absent. The foal was a rare case of congenital epidermolysis bullosa simplex, an inherited failure to express plectin.
ing plectin, the cytoskeleton and hemidesmosomes of the hoof lamellae were unstable, resulting in laminitis when the front feet first bore weight. The loss of even a single protein causes structural failure and laminitis. The presence of histological lesions only in the front feet illustrated the influence of weight distribution on laminitis severity and supports the validity of preventive strategies that unload the feet.

Ideas on laminitis pathophysiology abound and this review has focused on the MMP, enzymatic theory of laminitis pathogenesis, a hypothesis that depends on the generation of circulating toxins, or proinflammatory mediators (laminitis trigger factors) in the gastrointestinal (carbohydrate overload) or reproductive (septic metritis) tracts. A weakness of this hypothesis is how can laminitis trigger factors pass through the lung, kidney and liver without inducing significant pathology? Perhaps it all comes down to the unique anatomy of digitigrade equids. MMP activation and basement membrane disadhesion may be ubiquitous to the epithelia of many organs but without the influence of weight bearing any resultant pathology is transient. However weight bearing BMs such as those within the lamellar dermal epidermal interface of horse’s feet, separate under tension, a process that may escalate into a cascade of ever increasing severity. The validity of this proposition will be tested when veterinary researchers learn how to unload the feet of horses during the developmental phase of laminitis.

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**References**


**Figure 10** Transmission electron micrographs (TEMs) of lamellar SELs at the onset of acute laminitis. In (A) many hemidesmosomes (arrow) are absent or faded. Anchoring filaments (arrowhead) are present where hemidesmosomes are still relatively normal. Loss and disruption of hemidesmosomes is accompanied by the commencement of BM separation and damage of the cytoskeleton (*). Bar = 500 nm. In (B) the BM has separated from the attenuated, pointed SEL tip and formed a typical, empty BM enclosed bubble. There are few recognizable hemidesmosomes and only fragments of cytoskeleton (*). Bar = 200 nm. D, dermis; E, epidermal basal cell.