

New developments in our understanding of what causes different forms of laminitis

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We use the term laminitis to describe the clinical and pathological consequences of disturbances in the attachment between inner hoof wall and distal phalanx that is normally provided by the digital lamellae. Importantly, the lamellae normally act to suspend the bone within the hoof capsule and it is the largely unrecoverable loss of this suspensory function that leads to the lameness, dysfunction and morphological derangements characteristic of chronic laminitis. Pathologic alterations in the lamellae can be mild and insidious, with slow and progressive lengthening of lamellae due to stretch and cellular proliferation (as in many cases of endocrine-associated laminitis) or may be characterized by rapid loss of tissue mechanical integrity due to widespread cellular adhesion and cytoskeletal failure (more common in sepsis-associated and supporting limb laminitis). Once the suspensory function of the lamellae is lost, the pain, morphological derangement and progression of digital pathology are largely consequences of novel mechanical forces now acting between the distal phalanx, sole and ground surface, together with the proliferative and dysplastic response of the lamellar epidermis to injury. These chronic processes are common regardless of the inciting cause. Over the last decade however, researchers have recognized that there are key differences (and some similarities) in the initial events that lead to laminitis depending on the inciting cause. A focus on these early events is leading to a better understanding of why laminitis occurs in different clinical situations and is helping to identify therapeutic targets.

Are there common pathways to lamellar dysfunction?

In all 3 types of laminitis (sepsis-associated, supporting-limb laminitis [SLL] and endocrinopathic laminitis), the lamellar epithelial cell is central to the events that lead to lamellar failure. In each case lamellar failure is caused by a combination of:

1. Loss of the normal cell shape (stretching) of the lamellar epithelial cells and
2. Failure of epithelial cell adhesions (both between cells, and to the dermis via the basement membrane).

Endocrinopathic laminitis is dominated by cell stretch with less evidence of adhesion loss, whereas sepsis-associated laminitis and SLL is dominated by adhesion loss. Cell stretching/loss of shape is caused by disruption of the cytoskeleton, and adhesion loss is due to disruption of hemidesmosome/desmosome dynamics. These events could occur as a result of either aberrant cellular signaling or a lack of cellular energy to maintain the dynamic cytoskeletal components and adhesions. The unique environment of the lamellar epithelium means that the cells are normally subjected to profound mechanical stresses and therefore even minor events affecting regulation of the cytoskeleton and/or adhesion complexes could rapidly escalate and cause loss of mechanical integrity.

Disruption of energy supply as a cause of epithelial cell adhesion loss/stretch could occur as a result of perfusion failure (ischemia) or could be associated with more subtle tissue energy

dysregulation/imbalance. Traditionally ischemia was hypothesized to play a central role in all types of laminitis, however to date there is only evidence for ischemia playing a role in SLL. There is preliminary evidence of subtle oxidative energy failure in sepsis-associated laminitis (see below) and lamellar energy balance has not yet been evaluated during endocrinopathic laminitis development. Since maintenance of epithelial cell cytoskeleton and adhesion complexes involves dynamic processes, aberrant cellular signaling may be involved in disrupting the normal balance and triggering laminitis. There is evidence emerging (from the Belknap laboratory at OSU in particular) that lamellar signaling in models of all 3 types of laminitis converge at the level of the mTOR signaling pathway, which plays a central role in cellular growth and homeostasis. The mTOR signaling pathway regulates epithelial-mesenchymal transition (EMT) which is important in many disease processes and dysfunction of mTOR signaling is implicated in an increasing number of conditions including cancer and diabetes. Since cytoskeletal rearrangement and cell adhesion dissolution are initial events EMT (and laminitis), aberrant mTOR signaling may be responsible for lamellar structural failure by causing disruption of these same cellular processes in lamellar epithelial. Furthermore, the role of mTORC1 as a metabolic sensor and regulator of cellular energy metabolism may be critical in laminitis pathophysiology, underlined by preliminary data demonstrating perturbations of energy metabolism in models of the SRL and SLL forms of laminitis.

Sepsis-associated laminitis

Laminitis occurs as a consequence of a range of conditions in horses where systemic inflammation is a feature; particularly when this inflammation is driven by bacteria or bacterial products liberated into the bloodstream (sepsis). Gram negative sepsis and endotoxaemia accompany metritis, pneumonia and colitis/enteritis in horses and these conditions are most often complicated by the development of acute laminitis. Experimental alimentary carbohydrate overload models traditionally used to study laminitis feature clinical signs that are characteristic of sepsis and studies have confirmed the presence of endotoxin in the blood and a systemic inflammatory response with these models. It is not surprising that endotoxin, usually of gastrointestinal origin, is an important trigger of sepsis in horses considering their exquisite sensitivity to it, but interestingly investigators have not been able to induce laminitis with experimental infusion of endotoxin alone. Nevertheless, the presence of clinical signs of endotoxaemia/sepsis is an established risk factor for the development of laminitis in hospitalised horses.

Sepsis is a malignant systemic inflammatory process triggered by bacteria and their products (including endotoxin) in the blood with a resultant cascade of haemodynamic alterations, coagulopathy and metabolic alterations. In most species including humans, the development of organ failure ("multiple organ dysfunction syndrome" [MODS]), most commonly affecting the lungs, liver and kidneys, has a major effect on survival in cases of sepsis/SIRS. Laminitis appears to be a form of end organ dysfunction/failure that is ultimately most important in terms of recovery for the adult horse with sepsis. The pathophysiology of organ dysfunction in sepsis (and sepsis associated laminitis) is still poorly understood. Efforts to determine the mechanisms that lead to sepsis-associated laminitis have mirrored those in human MODS research, with the investigation of circulatory derangements, local inflammatory processes, apoptosis and non-ischaemic derangement of cellular energy metabolism receiving the most attention. Although there is some evidence of microvascular dysfunction in MODS and laminitis there is no evidence of true ischaemia. The central role of inflammation in the pathogenesis of acute laminitis has been highlighted experimentally, with endothelial activation, cytokine and

chemokine upregulation, and leukocyte emigration into the lamellar tissue occurring early during the development of experimentally induced laminitis. There is little evidence that apoptosis or oxidative damage play a primary role in laminitis development. Since the lamellae have a unique mechanical role, the lamellar basal epithelial cell adhesions and the integrity of the extracellular matrix have received special research attention, however recent evidence suggests that enzymatic degradation of extracellular matrix components by e.g. matrix metalloproteinase (MMP) enzymes appear to play only a secondary role. There is evidence of non-ischaemic energy failure (mitochondrial dysfunction) in models of human sepsis that is thought to play a role in MODS, but there is debate over whether this may represent a downregulation of cellular metabolic pathways that is actually protective in tissues during sepsis. Similar disruption of energy metabolism in sepsis-induced laminitis has not been definitively documented in the early developmental period, however preliminary results from a study in the author's laboratory suggest that there is dysregulation/failure of oxidative energy metabolism in the late developmental/early acute phase of experimentally-induced sepsis-associated laminitis: this may contribute to the failure of lamellar epidermal basal cells to maintain their critical intercellular and cell-matrix adhesions. Although insulin dysregulation may be a feature of sepsis in horses, it is clear from experimental models that laminitis develops in the absence of clinically significant hyperinsulinaemia. There is evidence that mTORC1 growth factor signaling is upregulated in experimental sepsis-associated laminitis – in this case through IL6 activation. These pathways are consistently blocked by hypothermia experimentally, which also ameliorates the laminitis lesion.

Endocrinopathic laminitis

Endocrinopathic laminitis encompasses laminitis associated with obesity, insulin dysregulation, pasture-associated laminitis, equine metabolic syndrome (EMS), pituitary pars intermedia dysfunction (PPID) or glucocorticoid administration. This type of laminitis appears to be the most common worldwide. Recent research has shown that prolonged hyperinsulinaemia can induce laminitis in healthy ponies and horses. In contrast to sepsis-associated laminitis, lamellar inflammation does not appear to be a major feature and evidence of systemic or gastrointestinal inflammation is not apparent in models or natural disease. The histological changes seen in the lamellae are dominated by epithelial cell stretch rather than adhesion failure, and as the pathology progresses there is increased mitotic activity and cellular proliferation. Current evidence suggests that overstimulation of the growth factor receptor IGF-1R by excess insulin is responsible for triggering a proliferative response in lamellar epidermal cells which involves disruption of normal cell adhesions. Interestingly, excessive activity of the same growth factor receptor, IGF1R, is a major cause of epithelial-mesenchymal transition (EMT) in epithelial cancers and this is mediated through mTORC1. It is therefore likely that the cytoskeletal and adhesion dysregulation of endocrinopathic laminitis is due to mTORC1 activation occurring through activation of IGF1R by excessive insulin. The contribution of cellular energy failure through interference with perfusion, glucose uptake or metabolic pathways has not been specifically studied to date but appears unlikely on current evidence.

The concentrations of insulin in blood (and therefore tissue) vary widely in naturally occurring endocrinopathies and peak in association with soluble carbohydrate ingestion in particular. Episodes of hypersulinemia in natural cases are also much less profound and more transient compared to those achieved in the protocol of insulin infusion used to induce laminitis experimentally. Transient hyperinsulinemic insults in natural cases are likely to contribute to the insidious and sometimes subclinical disease course of laminitis in many endocrinopathic cases.

More severe and profound hyperinsulinemia upon exposure to carbohydrate-rich pasture for instance can still result in more severe acute laminitis in prone horses through these mechanisms.

Supporting-limb laminitis

Although the severity and duration of lameness are considered risk factors, the development of supporting limb laminitis (SLL) is still unpredictable, both in terms of timing and also with respect to which cases will succumb to it (and what degree of pain in the limb with the primary condition is necessary for SLL development). Despite making significant advances in our ability to treat complicated fractures and other painful limb conditions in horses, SLL remains the primary limiting factor to treatment success in these cases. This form of laminitis tends to be commonly associated with rapid and severe failure of the lamellae, with subsequent distal displacement (“sinking”) of the distal phalanx (DP) within the hoof capsule. The incidence has been estimated at approximately 10-15% of horses that present for painful limb problems (or require limb casts) in North American studies, however the threat of SLL likely leads to a reduced propensity to even attempt treatment in many complicated painful limb conditions, with many of these horses instead being euthanased.

Mechanical and vascular mechanisms have been considered by authors as potential contributors to the pathophysiology of SLL but there is very little published evidence. It is well accepted that in the standing horse, the body mass is divided between the fore and hind limbs in a 60:40 ratio. Peak ground reaction forces increase to be equivalent to 0.25 x bwt at the walk, 0.5 x bwt at the trot, and up to 3 x bwt at the gallop. It therefore seems unlikely, from a mechanical perspective, that compensatory load redistribution in the standing horse bearing weight on a single limb could exceed the mechanical “strength” of the lamellae. The lamellar tissue has a high requirement for glucose, yet there is no means for local glycogen storage, therefore reduced supply of blood glucose may rapidly lead to energy failure. Almost 30 years ago it was first demonstrated that there was a valve-like mechanism in the digital arteries of the loaded limb leading to a cut-off of arterial blood supply during the loading phase. This work has been repeated recently by the author’s group using 3D computed tomography (CT) studies, demonstrating arterial attenuation and occlusion under load in cadaver limbs that affects contrast fill in the arterial vasculature within the hoof capsule. Recent studies from the author’s group utilising microdialysis, (a technique that allows real-time detection of local energy metabolites and blood flow in the lamellar interstitium) showed that lamellar perfusion and energy balance are largely determined by the frequency of limb load cycling (weight shifting frequency), which appears to override other manipulations such as attempts modulate vasomotor tone pharmacologically. These studies showed that the act of walking had a marked positive effect on lamellar perfusion and interstitial glucose concentrations, more so than just repeated unloading of the limb in a static horse, indicating that the weight bearing phase itself (and perhaps break over) may play an important role in lamellar perfusion. Preliminary microdialysis studies using an experimental model of preferential weight bearing show that a combination of increased load and decreased limb load cycling frequency can cause reduced perfusion and negative energy balance consistent with ischaemia in the lamellar dermis. The mechanism of this is not clear from preliminary data but it appears more complicated than simple load dependent arterial occlusion: the results of these studies will be discussed.

