The intersection of PPID and laminitis

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Introduction

Laminitis is a debilitating clinical syndrome of lameness due to hoof pain that equine practitioners are very familiar with. However, it has only been in the last decade that distinct causes of laminitis have been well defined and the importance of endocrine causes of laminitis, including laminitis associated with pituitary pars intermedia dysfunction (PPID) have been understood.[1] This in depth paper will discuss laminitis, focusing on endocrinopathic laminitis and the intersection of PPID and laminitis.

Causes of laminitis

Research has stratified laminitis as occurring from 3 main causes: *SIRS or sepsis associated* laminitis including enterocolitis, pleuropneumonia, retained fetal membranes and grain overload; *supporting limb* laminitis; and *endocrinopathic* laminitis. [1]

Historically, laminitis research was focused on conditions causing severe systemic inflammation (SIRS) or sepsis, such as carbohydrate overload or metritis. Notably, Obel's 1948 thesis used both a starch overload and a metritis model to study laminitis. [2] Between 1948 and the last decade, laminitis research involved exclusively SIRS models including:

starch overload [3], black walnut extract [4] and oligofructose overload [5]. The severe SIRS or sepsis associated form of laminitis was assumed to be more common, since it was more commonly seen at the university hospitals where most of the laminitis research had been conducted. [6] Systemic endocrine disease was known to be associated with laminitis but received relatively little attention. [7,8]

Yet horse owners and frontline practitioners were telling a different story. In a large epidemiological study in the USA involving over 20,000 horses, [9] it was found that causes of laminitis reported by their owners that were associated with SIRS or sepsis related conditions including grain overload, retained placenta, colic or diarrhea only accounted for 12% of the cases of laminitis, with the majority of cases reported as associated with dietary problems, obesity or "unknown". Studies in Europe [10] and the USA [11] showed convincing evidence that the endocrine disorders account for over 90% of horses presenting with lameness due to laminitis. Finally, researchers showed unequivocally that infusion of insulin induced laminitis in healthy, lean ponies [12] or Standardbred racehorses.[13] They also showed that infusion of glucose causing mild hyperglycemia and endogenous hyperinsulinemia resulted in laminitic hoof lesions (without lameness). [14] This combined with some excellent field research looking at predispositions for laminitis in ponies in the USA [15, 16] all supported the conclusion that laminitis could occur without pro-inflammatory and intestinal conditions; fitting in with the term *endocrinopathic laminitis* that had been coined a few years earlier. [17]

Endocrinopathic laminitis

There are two main conditions associated with endocrinopathic laminitis, pituitary pars intermedia dysfunction (PPID) and equine metabolic syndrome (EMS). Common to both these conditions appears to be the development of insulin dysregulation. Insulin

dysregulation results in hyperinsulinemia (either basally, or in association with the ingestion of carbohydrates in forages and feeds). [18]

PPID is a disease of aged horses characterized by loss of dopaminergic inhibition of the pituitary *pars intermedia* and resultant overproduction of pituitary hormones (adrenocorticotropin [ACTH], alpha melanocyte stimulating hormone [α-MSH], beta endorphin [β-endorphin] and corticotrophin-like intermediate peptide [CLIP]). The resultant clinical syndrome is variably associated with a wide array of clinical signs including hypertrichosis and abnormal hair shedding patterns, laminitis, muscle wastage, abnormal fat distribution, polyuria and polydipsia, increased susceptibility to infections and infertility. [19] EMS has been defined in the 2010 ACVIM consensus statement as a phenotype of obesity (regional or generalised), insulin resistance (hyperinsulinemia or abnormal insulin and glucose regulation; now known as insulin dysregulation[18]) and a predisposition to laminitis that has developed in the absence of recognised causes such as grain overload, colic, colitis, or retained placenta. [20]

Laminitis and PPID

Laminitis has been frequently diagnosed in horses with PPID and laboratory submissions suggest it is a common trigger for horse owners to request veterinary investigation of cases of PPID. [21] On pooled analysis from 13 studies, laminitis was the second most common clinical sign in horses with PPID (49%), second only to hypertrichosis (70%). [22] Horses with PPID have 4.65 times the odds of developing laminitis compared to aged matched controls. [23] Yet laminitis occurs variably in studies from 8-82%. [22] This finding may seem conflicting, but can be explained by a number of factors.

a. Firstly, it appears insulin dysregulation is key to develop of laminitis in PPID-affected horses. This is supported by histological research that showed that laminar lesions only occurred in horses with PPID and hyperinsulinemia, and not in horses with PPID but normal basal insulin concentrations. [24] An epidemiological study demonstrated that over a third of horses with PPID had hyperinsulinemia, and 67% of horses with PPID and laminitis had basal hyperinsulinemia [23]. Horses with PPID were 2.7 times more likely to have hyperinsulinemia than aged matched controls. [23] However, when basal hyperinsulinemia occurred in PPID or non-PPID aged horses, they were equally at risk for developing laminitis. [23]

b. Secondly, even when horses are insulin dysregulated, laminitis may not produce clinical lameness. Histological research in naturally occurring cases of endocrinopathic laminitis showed no relationship between the duration of clinical lameness and the degree of pathology found histologically, but did identify the consistent presence of hoof capsular changes in association with pathology such as divergent hoof rings. [27] This was supported by epidemiological research in aged horses where hoof abnormalities indicative of chronic laminitis (including dropped soles, laminitic rings and separated white lines) were more prevalent than a history of laminitis [23, 28]. This implies that horses can have repeated subclinical bouts of laminitis without lameness, which may not be identified or may not be considered significant by horse owners.

c. Thirdly, the presentation of horses with PPID for veterinary attention requires the recognition of the clinical signs of PPID by their owners. Epidemiological research has shown that many of the clinical signs of PPID are considered normal signs of aging by horse owners, including hypertrichosis and muscle atrophy. [29] Indeed, in one study, only 1.6% of horse owners reported their horses with PPID, yet 21.2% tested positive on seasonally adjusted basal ACTH measurements. [23] This may well explain the large

category of "unknown" laminitis cases back in the 1990's when horse owners were asked to identify the causes of laminitis in their horses. [9] Laminitis, at least where it causes lameness, is more likely to result in horses being presented for veterinary attention, therefore, if research studies or case series used owner presented animals for inclusion in studies, the prevalence of laminitis is likely to be higher. [21,22]

Insulin dysregulation and laminitis

It is now well established that hyperinsulinemia associated with insulin dysregulation causes laminitis in affected horses, especially when challenged by dietary carbohydrates. Laminitis has been linked to insulin resistance and hyperinsulinemia in field studies since the 1980's. [7, 8, 15, 16, 30] Experimental research has shown a direct link between hyperinsulinemia and laminitis. [12,13] Laminitis was induced in 100% of normal ponies [12] or horses [13] exposed to high concentrations of insulin (> 1000 µIU/ml) while maintaining euglycemia of 5 mmol/l using a modified euglycemic-hyperinsulinemic clamp technique. All treated ponies or horses were healthy, young and non-obese, with no history of laminitis and no evidence of endocrine or other abnormalities on blood tests. Laminitis occurred slowly and in all 4 limbs, with the onset of lameness associated with laminitis (Obel grade 2) occurring by approximately 48 hours. There was no evidence of gastrointestinal involvement or systemic illness throughout the experiments. [12,13]

The hyperinsulinemic model of laminitis has allowed us to explore different mechanisms that are possibly involved in the development of endocrinopathic laminitis. Prior research does not support glucose deprivation [31] or glucose excess [32] as underlying mechanisms. Metalloproteinases (MMP2 and MMP9 and ADAMTS4) have been consistently upregulated in SIRS or sepsis models of laminitis. [33] However, this has not been the case in

hyperinsulinemic laminitis where minimal MMP or ADAMTS4 activity during the developmental and acute stages of laminitis occurred and only MMP9 was upregulated at later stages of laminitis (48 hours), correlating with histological evidence of neutrophil infiltration. [34]

Mechanisms of hyperinsulinemia and laminitis

More recently insulin's effect on signaling within cells and its effects on blood flow have provided important mechanistic clues. Insulin, when it is working via its appropriate intracellular pathways, has marked effects on stimulating vasodilation and blood flow in both small and large vessels via nitric oxide mediated pathways. [35] Insulin resistance results in inappropriate intracellular pathway activation leading to the opposite effects, which in the vascular endothelium leads to vasoconstriction. Ex-vivo vascular ring models from equine digital vessels showed that just 30 minutes pre-incubation in insulin (inducing insulin dysfunction) resulted in an inappropriate vasoconstriction response to the addition of insulin. [36] This effect was obliterated by a blocker of the inappropriate intracellular pathway of insulin signaling. [36] Further work in horse vasculature has supported this first study finding the same results using laminar vessels. [37] This was further supported by comparing the vascular responses from naturally occurring endocrinopathic laminitis horses and controls using laminar arteries and veins and facial arteries. [38] Vascular dysfunction was evident in the vessels derived from endocrinopathic animals but not the controls. [38] Together there is now compelling evidence that hyperinsulinemia associated with either the hyperinsulinemia model or naturally occurring insulin dysregulation induces abnormal insulin signaling at a cellular level, at least in the vascular endothelium.

However, the vascular endothelium is only part of the story and does not correlate well with the histological lesion which provides even more important mechanistic clues. [1] As early as 6

hours post exposure to hyperinsulinemia, marked elongation of the secondary epidermal laminae (SEL) is observed, developing tapered tips and with SEL angled more acutely to the primary lamellar (PEL) axis. [39] Cellular changes are dominated by cellular elongation and stretching then apoptosis and mitosis, all of which precede the pain and inflammation of Obel grade 2 laminitis. [40] The current theory of endocrinopathic laminitis is that the epithelial cells that make up the laminae are affected by hyperinsulinaemia, just like the vascular endothelial cells were shown to be affected above, resulting in abnormal intracellular signaling in insulin dysregulated horses. This results in activation and dysregulation of certain pathways or 'downstream events' within the cells resulting in cellular damage and disruption of cytoskeletal organisation, allowing these cells to stretch under mechanical forces and leading to the well-defined initial lesion of laminar and cellular stretching. [1, 41]

PPID and insulin dysregulation

Although it is known that over a third of horses with PPID are insulin dysregulated, and that insulin dysregulation causes the laminitis, it is not entirely clear how PPID induces insulin dysregulation. Due to the high prevalence of PPID (over 20%) [23] and also of insulin dysregulation without PPID (EMS; over 25% in susceptible breeds such as ponies) [42], and the fact that in both conditions the prevalence increases with age [23, 42] it is possible that both conditions could simply be occurring in the same animal. It may be that PPID itself does not cause insulin dysregulation at all and laminitis only occurs when PPID occurs in an animal that already had insulin dysregulation. However, this is not supported by the observation that horses with PPID were 2.7 times more likely to have hyperinsulinemia than aged matched controls. [23]

Several mechanisms have been considered as to why PPID may lead to insulin dysregulation including abnormal central or peripheral glucocorticoid activity. [17] Although much of the secreted ACTH in horses with PPID may be biologically inactive or inert [19] and circulating cortisol may not be elevated in horses with PPID [43], there could be local upregulation of corticosteroids in peripheral tissues such as adipose tissue. [17] Evidence for prolonged glucocorticoid administration inducing insulin dysregulation exists. [44] Or it could be that pituitary peptides such as CLIP interfere with insulin regulation. [19] But ultimately, the relationship between PPID and the development of insulin dysregulation is not completely known.

Irrespective of how horses with PPID develop insulin dysregulation, it is increasingly apparent that the combination of PPID and insulin dysregulation leads to a poorer prognosis than either PPID horses without insulin dysregulation [45] or than insulin dysregulated horses without PPID[i].

Conclusions and clinical implications

Laminitis is a clinical syndrome with a cause, and endocrinopathic laminitis accounts for over 90% of laminitis cases seen in the field for lameness. Therefore, for laminitis that presents as lameness, clinical evaluation can often rapidly eliminate SIRS and sepsis associated conditions and supporting limb laminitis as potential causes. If these are ruled out, efforts to appropriately diagnose the underlying endocrinopathy should be made. Specifically, clinical examination and diagnostic testing can be used to determine if the horse has EMS, PPID or PPID with insulin dysregulation. Veterinarians should be aware that horses with PPID may not be presented with clinical laminitis or a history of laminitis, but hooves should be checked for evidence of sub-clinical laminitis in the form of divergent hoof rings, dropped

soles or separation at the white line) due to the possibility of these being missed by their owners.

It is important to assess insulin regulation in horses with PPID, as the presence of insulin dysregulation can guide determination of the likelihood of laminitis as well as the prognosis. Where insulin dysregulation is not well controlled, the prognosis for long term survival is lower. Knowledge of the relationship between insulin dysregulation and laminitis can direct veterinarians to utilize the results of the diagnostic tests for insulin dysregulation (and PPID) to guide longer term management.

Veterinarians should utilize the understanding of the early lesion of stretching due to cellular compromise in laminae to guide clinical management of horses with endocrinopathic laminitis. Management changes that should be considered include providing hoof support , thereby limiting mechanical forces and providing dietary and pharmacologic intervention to reduce circulating hyperinsulinemia as rapidly as possible.

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[i] McGowan CM, unpublished data