Equine Laminitis — Another Hypothesis for Pathogenesis

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Abstract — Laminitis is an important condition in horses and ponies, not just because of the seriousness of the clinical signs and systemic changes involved, but because of the potentially poor prognosis and likelihood of recurrence. Laminitis is particularly prevalent in ponies and involves a multiplicity of aetiological factors.

Fat ponies and those having previously suffered laminitis were found to be far more intolerant to oral glucose loading (1 g/kg bwt) than normal ponies or Standardbred horses. These ponies also exhibited a far greater response in plasma insulin levels after glucose loading. Insulin response tests (0.4 iu/kg bwt insulin intravenously) showed only a minimal and very protracted response in both the fat and laminitic ponies establishing the existence of an apparently innate insulin insensitivity in these animals.

These findings are important in regulation of carbohydrate and lipid metabolism and play a role in the pathogenesis of laminitis. The reduction of insulin effectiveness leads to elevation in thromboxane A₂ activity, predisposing the animal to peripheral vasoconstriction, compromisation of blood flow to the foot and the development of laminitis.

Introduction

Laminitis is a multisystemic syndrome seen particularly in ponies, but, also occurring in larger horses with apparently more severe clinical signs (8). Much of the experimental investigation has centred round the development of a consistent and reproducible model. Garner and others (16, 17, 25) have been comparatively successful in establishing a reproducible laminitis model of alimentary origin. This has allowed investigation of the myriad of features that develop secondary to carbohydrate overload (16, 25), including quantitation of changes in caecal bacterial populations, secondary to the development of a markedly acidotic environment. They propose a subsequent loss of caecal mucosal integrity, allows sequestration of lactic acid and endotoxin into the systemic circulation or peritoneal cavity (17, 29).
The vascular response to laminitis has come under scrutiny in view of the proposed coagulopathy hypothesis proposed by Hood et al (23, 24). Using the carbohydrate model to induce laminitis, this group has shown there may be variation in platelet numbers, the presence of fibrin split products and activated partial thromboplastin times compatible with episodes of clotting dysfunction. These changes, although not always consistent, precede lameness episodes by approximately one hour. Other workers (40, 41) have looked at changes of vascular permeability by recording digital blood flow, vessel pressures and vascular resistance. Using a number of vasoactive compounds they could not stimulate either the systemic or digital vascular effects apparent in acute laminitis. Both Purohit (34) and Eyre (12, 13, 14) have investigated vasoactive compounds from a therapeutic view with the former investigating alpha- and beta-blockade and the latter, the effects of corticosteroids on the isolated, perfused hoof.

Another important factor in the pathogenesis involves alterations to the vascular morphology of the digital arcade, terminal arch and proximal vessels. Arteriovenous anastomoses in dermal laminae (42) and sole papillae develop as the syndrome progresses. It is hypothesized that blood shunting proximal to the terminal vessels may predispose the laminar tissues to ischaemia and necrosis (22, 25).

It is clear that the laminitis syndrome evokes response from many different body systems, particularly the cardiovascular and endocrine systems, and the coagulation pathways (15, 16, 18, 24). It can cause severe dysfunction with marked and progressive clinical effects if left untreated. The impact of laminitis on the horse industry is of major economic importance. The lack of precise information on the pathogenic mechanisms initiating the syndrome make it a fascinating scientific enigma.

This paper puts forward an hypothesis that there may be an important endocrinological basis for the susceptibility of ponies contracting the laminitis syndrome. The experimental evidence is taken from a study carried out while investigating another condition affecting ponies, namely hyperlipaemia (27).

Materials and methods

Animals
Nine ponies were used of mixed sex and age with bodyweight (bwt) ranging from 90 to 320 kg (Table 1). Each was brought into the Veterinary Clinical Centre for a period of one week during which time they were kept outside in yards and fed a ration of lucerne hay and concentrate twice daily with ad libitum access to water. At 17.00 h the day before experimentation all food was withdrawn. The testing procedures began at 09.00 h which effectively gave a fasting period of 16 h. Three normal Standardbred horses were used for comparative purposes and were handled in the same manner as the ponies.

Protocol
Following the overnight fast, two horses were placed in retaining stocks where they remained for the duration of the procedure (6 h). An indwelling jugular catheter (Dwellcath; Tuta Laboratories, Victoria, Australia) was inserted for collection of serial blood samples. No tranquilisation or other restraint was necessary and no behavioural problems were encountered.

Oral glucose tolerance test (OGTT)
A glucose load of 1 g/kg bwt (20 per cent solution) was given by stomach tube. A pre-load blood sample was taken and samples were then collected at 15, 30, 45 and 60 min and thereafter at 30 min intervals for a further 5 h. At the end of the tests the animals were given limited access to feed and ad libitum water.

Insulin response test
Two days after the OGTT and following a 16 h
fast, 0.4 iu/kg bwt of regular insulin (Commonwealth Serum Laboratories, Melbourne, Australia) was given intravenously and blood glucose levels monitored for 6 h. Blood samples were taken at 0 and 5 min and then as for the OGTT protocol.

**Analytical techniques**

Glucose analysis was carried out by the method of Werner, Rie and Wielinger (44) using a kit from Boehringer Mannheim, Mannheim, West Germany.

Insulin assays were carried out on the serum samples taken during the OGTT and covered the 6 h span of this procedure. Serum insulin reactivity was estimated by radioimmunoassay, in a heterologous system, using dextra-charcoal separation of bound and free fractions (1), as previously described (30). Crystalline equine insulin (Sigma Lot 79c-00113) was used for standards and for iodination (19). Tracer was purified by Sephadex G50 filtration. The guinea-pig antiserum (Wright, Indianapolis) cross-reacted equally with human, porcine and canine insulins. As frequently observed in heterologous radioimmunoassays, high concentrations of serum did not dilute in parallel with the standard curve (37, 45). All sera were assayed therefore at 1/40 and 1/100 final protein dilution, where parallelism was uniformly found. Assay sensitivity was 4 mU/litre, of 1.6 per cent. The interassay coefficient of variation at 19.5 mU/litre was 8.6 per cent and at 9.3 mU/litre was 11.6 per cent.

Plasma triglyceride levels were determined according to the method of Eggstein and Kuhlmann (11) using prepacked reagents (Boehringer Mannheim) on plasma samples collected at the beginning of the experiment.

![Graph showing blood glucose levels](image)

**Fig. 1** Mean (± sd) blood glucose values after oral glucose loading (1 g/kg bwt) in 9 ponies and 3 Standardbreds. [Reprinted from Equine vet J (1986), 18: 97–101, with permission].
Results

The animals were divided into four groups. Group 1 consisted of three apparently normal ponies, in Group 2 the ponies were noticeably overweight while in Group 3 they were also overweight and had a history of chronic laminitis. Group 4 were three Standardbred mares used for comparative purposes.

Oral glucose tolerance test

None of the animals showed any adverse clinical effects in response to glucose loading. The mean blood glucose results of the four groups are shown in Figure 1. Impaired glucose tolerance in the ponies of Group 2 and 3 was evidenced by the delay in peak values and failure to return to pre-test levels. Group 1 and 4 showed normal OGTT pattern of response for horses (39) although the normal ponies showed mild glucose intolerance compared to the Standardbreds.

Plasma insulin levels, recorded during the OGTT, showed a considerable difference between all the groups. When compared to the Standardbreds, all the pony groups showed markedly elevated levels of circulating insulin, particularly Groups 2 and 3 (Fig. 2).

Insulin response test

At the dose rate of 0.4 iu/kg bwt iv, no clinical signs of hypoglycaemia were noticed except for one mare in Group 4 which appeared somewhat depressed and unsteady for a short while (less than 30 mins) soon after insulin administration. Blood glucose levels were seen to fall more rapidly from high fasting levels in the Standardbred mares (Group 4) reaching a nadir at 45 mins post loading, compared to 90 mins in the normal ponies of Group 1 (Fig. 3). Ponies in Groups 2 and 3 exhibited only a very small and protracted response.

Triglycerides

Triglyceride levels in the Standardbreds (Group

![Graph of plasma insulin levels](image-url)

Fig. 2 Mean (± sd) plasma insulin levels in 9 ponies and 3 Standardbreds after oral glucose loading (1 g/kg bwt). [Reprinted from Equine vet J (1986). 18: 97-101, with permission].
Table 2  Plasma Triglycerides in 12 Ponies and Standardbreds After a 16 h Fast and Before OGTT and Insulin Response Testing

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Plasma observations</th>
<th>Triglyceride (mg/100 ml)</th>
<th>mean ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ponies</td>
<td>6</td>
<td>26.4 ± 17</td>
<td></td>
</tr>
<tr>
<td>Fat ponies</td>
<td>6</td>
<td>87.1 ± 111</td>
<td></td>
</tr>
<tr>
<td>Fat/laminitic ponies</td>
<td>6</td>
<td>59.0 ± 74</td>
<td></td>
</tr>
<tr>
<td>Normal Standardbreds</td>
<td>6</td>
<td>24.2 ± 21</td>
<td></td>
</tr>
</tbody>
</table>

4) showed little response to 16 h fasting (less than 60 mg/100 ml) (Table 2). In some of the ponies, values of up to 350 mg/100 ml were recorded.

Discussion

The OGTT showed clear evidence of impaired tolerance in the obese and laminitic groups when compared with the normal ponies and Standardbred horses. Plasma insulin levels associated with the OGTT were very elevated in these two groups, supporting the concept of insulin resistance as the cause of the abnormal glucose tolerance. The presence of insulin resistance was confirmed by the failure of exogenous insulin to cause a fall in blood glucose comparable to the responses exhibited by normal ponies and Standardbreds in the insulin response test.

Plasma triglycerides after fasting were not generally elevated, probably because the fasting was only for 16 h. Bauer (3) reported similar results for 16 h of fasting although after 40 h very significant elevations occurred.

Coffman and Colles (6) reported significant differences in blood glucose levels after insulin administration in laminitic ponies when compared with normal controls, although these differences were largely nonsignificant when the tests were repeated seven months later. These responses indicated a degree of insulin insensitivity. The presence of a degree of insulin resistance in ponies may be a result of natural selection to survive under harsh conditions such as rugged terrain, severe climate and poor and unreliable food supply. As a consequence they

![Fig. 3](image-url)
may have developed a highly efficient metabolism in which there is a degree of insulin resistance in the muscle resulting in a greater proportion of ingested carbohydrate being diverted to the liver where the coexistent hyperinsulinaemia and hyperglycaemia promotes triglyceride synthesis (20). This triglyceride is transported by the plasma lipoproteins and taken up by the adipose tissue, ensuring that excess oxidisable substrate is stored as triglyceride. A similar metabolic adaptation has been proposed to account for an increased risk of diabetes mellitus in certain human populations (4, 7, 31).

The presence of this metabolic adaptation in the pony breed, which would have been a survival advantage under natural conditions of unreliable food supply, becomes a disadvantage when ponies are domesticated and have a ready supply of good quality food and low levels of physical activity. The hypothesis (26) is further supported by the observation that ponies fatten much more readily than other breeds of horses. Any condition which further exacerbates the underlying insulin resistance in ponies is a potential trigger for hypertriglyceridaemia, with obesity, systemic illness and pregnancy among many factors. Hypertriglyceridaemia associated with severe insulin resistance has been reported in man (36).

These findings may also be important in the onset of laminitis in equids. It has been difficult to definitively explain the pathogenic pathway of laminitis particularly as so many different body systems are involved (8, 23, 24). Current hypotheses favour the coagulopathy model induced by bacterial endotoxins and a secondary metabolic acidosis. Two mechanisms are thought involved in producing the subsequent digital lesion, one a vasoactive process, the other a coagulopathy. Vascular shunting supposedly develops in the acute phase due to the presence of vasoactive hormones or toxins. This occurs in conjunction with alterations to the intrinsic and extrinsic coagulation pathways (23). The vascular lesion (vasospasm) results in a damaged endothelium which in turn stimulates the coagulation pathway, the final situation is not being dissimilar to disseminated intravascular coagulation. During the acute and early chronic phases there is an increase in total digital blood flow, which bypasses the distal capillary bed via arteriovenous shunting. Congestion of the distal vasculature is followed by epidermal lamellar necrosis and oedema. These changes are concurrent with systemic hypertension, tachycardia and a stress haemogram. Stimulation of a positive feedback pathway is hypothesized to result in progressive necrosis of the digit.

The chronic phase, with resultant loss of laminar suspensory function, sees the weight of the horse, together with leverage on the toe and mechanical interference by the deep digital flexor (DDF) tendon, rotate or displace the pedal bone distally. Thus the weakened interconnecting laminae are disrupted provoking an inflammatory response. Epidermal hyperplasia ensues and, by increasing the mass of tissue between the hoof wall and the pedal bone, rotation persists chronically.

A vicious cycle of events is now proposed for laminitis (Fig. 4) which implicates the inherent insulin insensitivity of ponies. Cortisol-induced insulin resistance in man is due to a decrease in both hepatic and extrahepatic sensitivity to insulin (5, 38, 43). Assuming that insulin binding to monocytes and erythrocytes reflects insulin binding in tissues, the proposed decrease in insulin effectiveness is explained by post-receptor defects (21) and significant decreases in insulin binding to its receptor. This reduction in binding results from reduced receptor affinity (9) and reduced receptor number (10). Catecholamines also induce peripheral insulin resistance thereby compounding the tissue refractiveness to insulin (28). It is proposed that this reduced effectiveness of insulin is instrumental in precipitating

![Fig. 4 Flow diagram of the proposed vicious cycle of pathogenesis for equine laminitis in ponies.](image-url)
platelet aggregation and subsequent vasoconstric-
ion particularly in the peripheral vasculature of
the horse.

It has been further demonstrated that platelets
from diabetic patients (insulin dependant)
manifest increased in vitro platelet aggregation
and increased synthesis of the pro-aggregator
vasoconstrictive thromboxane A2 (TxA2). This
occurrence exacerabes vascular complications
and microangiopathy, such as retinopathy and
nephropathy (35). Vascular prostacyclin (PGI2),
a vasodilatory, anti-aggregatory substance, un-
dergoes decreased synthesis in both diabetic
patients and experimental animals with diabetes
mellitus (32, 33). Treatment with insulin reverses
this decreased synthesis of prostacyclin. In insulin
dependant diabetics, the increased ratio of
TxA2:PGI255dj0, favouring an enhanced throm-
botic state, may play a significant role in the
apparently accelerated vascular disease.

Conclusion

The relevance of all this in the pathogenesis of
equine laminitis revolves around the develop-
ment of tissue refractoriness to insulin. In the
normoinsulinaemic animal, the insulin effect is
primarily reduced through the actions of cortisol,
catecholamines and endotoxin at both receptor
and post-receptor levels. In the obese hyperinsu-
lnaemic animal, tissue refractoriness to insulin
manifests in direct reduction of insulin receptor
population (9). Variation in tissue receptor char-
acteristics (affinity and number) could be used to
explain the variable constriction and dilatation of
different vessels in the distal limb. This implies
that primary control of dermal vessels may be
shared between alpha-adrenergic effects and in-
sulin receptor mediated elevation in arterio-
venous anastomosis flow and reduced capillary
flow.

The treatment of either acute or chronic
laminitis currently involves two broad ap-
proaches. Firstly, the mechanistic approach,
centred around corrective foot-trimming, frog
support, dorsal hoofwall resection and corrective
shoeing. Secondly, the medical approach in-
volving anticoagulant therapy, anti-inflammatory
compounds and vasodilatory agents. Each of
these methods concentrates on a specific feature
of the pathogenic pathway; no one therapeutic
modality to date has been shown completely ef-
ficacious in the treatment of the horse or pony
with either impending or fulminating laminitis.

The vicious cycle of pathogenesis described
above proposes an inhibition in the effectiveness
of insulin, which through its various effects, is the
primary causative factor in the onset of laminitis.
Correspondingly, managerial practices and
modifications would seem much more relevant
than treatment after the fact. Therefore, mini-
mizing adrenal stimulation through stress
reduction (ie control of pain and systemic dis-
ease) with subsequent reduction in the insulin
inhibition displayed by cortisol. Control of en-
dotoxin release and tissue effects can be
performed medically using activated charcoal,
mineral oil and flunixin meglumine. Obviously,
avoidance of either grain overload or retained
placenta through good managerial practice is the
ideal. A regular pattern of exercise and dietary
constraint will relieve the contribution obesity
makes to the onset of laminitis. This is particu-
larly pertinent in the pony breeds, which through
changes in use patterns have made them much
more susceptible to metabolic disease. Heparin
therapy serves two functions: it actively removed
 circulating triglycerides from the systemic circu-
lation and is active in the dissolution of platelet
aggregates which occurs in response to elevated
TXA2 release. Finally, the promotion of the syn-
thesis of the vasodilatory, anti-aggregatory
prostacyclin should be undertaken. Selective
thromboxane synthetase inhibition has also been
investigated with equivocal results to date. The
problem with either reducing thromboxane A2
synthesis or increasing prostacyclin production is
overshoot and an inappropriate response.

In conclusion these facts shed new light on the
predisposition of ponies to laminitis particularly
when none of the other inciting causes are
present. There could be important implications
in management and treatment of the condition in
ponies as a result.

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