Best foot forward

By Sandy Weltan

An investigative approach to laminitis

Laminitis – inflammation of the laminae – is a common, painful and often recurrent problem in equids. It is most often associated with equine metabolic syndrome (EMS) caused by insulin dysregulation (ID). Non-endocrine causes include systemic inflammation and excessive weight bearing. Pregnancy-associated laminitis is due to temporary ID. There is a genetic predisposition: for example, Andalusian horses have decreased insulin sensitivity (Bamford et al., 2014). The interaction with glucocorticoid administration and pituitary pars intermedia dysfunction (PPID) will be discussed. In most cases, laminitis is the clinical consequence of EMS.

Pathophysiology

An understanding of ID requires a brief revisit to the hormonal control of blood glucose concentrations. The main purpose of the hormonal control of metabolism is to provide glucose to cells that are totally dependent on glucose for metabolism, particularly erythrocytes and neural tissue. The key organs involved in energy metabolism are muscle, liver and adipose tissue. In summary, metabolism during the fed (digestive phase) is almost entirely controlled by insulin. In normal animals, insulin drops to low levels during fasting or exercise. Glucagon and catecholamines are the main counterregulatory hormones during the fasting phase. Growth hormone and cortisol also contribute to metabolism during the fasting phase.

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An increase in blood glucose is the primary stimulus for insulin secretion, but fatty acids and arginine also increase insulin secretion. Insulin promotes energy storage in liver, muscle and adipose tissue. Catecholamine directly inhibits insulin secretion and promotes glucagon secretion. Glucagon secretion is stimulated by a decrease in glucose concentration, promotes gluconeogenesis from stored glycogen and amino acids and promotes free fatty acid oxidation. Insulin inhibits glucagon secretion. Stress induces increases in cortisol, decreased insulin and increased glucagon which, with increased catecholamines, promotes glucose mobilisation from liver and lipolysis. Chronic increases in cortisol result in increased insulin and decreases in catecholamines, resulting in increased hepatic glycogen storage and accumulation of triglyceride in adipose tissue.

The term ID is used to indicate disturbances of the balanced interrelationship among plasma concentrations of insulin, glucose and lipids. ID can manifest in several ways, which may overlap. These include increased basal insulin; an excessive or prolonged hyperinsulinaemic response to oral or IV carbohydrate challenge, with or without an excessive or prolonged hyperglycaemia; and tissue insulin resistance (IR). Hypertriglyceridaemia can also be a consequence of IR (Durham et al., 2019). Hyperinsulinaemia induces laminitis in horses and ponies (Asplin et al., 2007; de Laat et al., 2010; de Laat et al., 2012) confirming the results of studies in the 1980s (Durham et al., 2019).

Although the dominant stimulus for the secretion of insulin from β-cells in the pancreatic islets is the presence of glucose in the blood, there are several factors influencing blood glucose concentration. The absorption of glucose from the small intestine into the circulation via sodium-glucose co-transporters (SGLT-1; uptake from gut lumen) and glucose transporter 2 (GLUT-2; transport of glucose from gut epithelial cell to circulation) is an important driver of insulin secretion. However, the rate and extent of glucose absorption vary considerably between individuals, thereby affecting the rate and extent of insulin secretion.

Further, the presence of glucose in the small intestine acts on luminal sensors that signal to SGLT-1, as well as enteroendocrine cells that release incretin peptides, including glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide (GIP), to further augment insulin release. This multi-faceted system is termed the enteroinsular axis and has been extensively studied in humans.

Oral glucose administration stimulates greater insulin secretion than IV administration in ponies, highlighting the effect of incretins in facilitating insulin secretion, although the actual mechanism of action in equines requires further research (de Laat and Fizgerald, 2023). Treatment is beyond the scope of this article, but promising results have been obtained with SGLT-2 inhibitors, which are found in the kidney and reduce the reabsorption of glucose in the kidney. They are available for the treatment of type 2 diabetes mellitus in humans.

Insulin resistance means that tissues that are normally sensitive to insulin, mainly skeletal muscle, adipose tissue and liver, do not respond. The result is increased liver glucose synthesis via gluconeogenesis; impaired tissue glucose uptake due to dysfunction of GLUT-4 receptors in muscle and adipose tissue; and increased lipolysis, resulting in increased free fatty acid concentrations. Fat accumulation in the liver would reduce liver function and reduce insulin clearance, further promoting hyperinsulinaemia. Chronic hyperinsulinaemia results in down regulation of the insulin receptor and downstream signalling. Measuring insulin sensitivity is complex and mostly limited to research environments.

A regional deposition of fat is often associated with EMS, but adiposity is not inextricably linked with EMS. Some cases of EMS occur in lean horses and ponies, and not all cases with regional deposition of adipose tissue have EMS and ID. However, there is an increased incidence of EMS in inactive individuals. Insulin concentrations are higher with increasing age. Hyperinsulinaemia has been shown to alter lipid metabolism and promote obesity.

A possible consequence of increased fat mass is a dysregulation of adipokines – hormones released by adipocytes – which include leptin and adiponectin. Leptin concentrations increase with insulin resistance and adiposity because of increased fat mass and leptin resistance. Resistance results in the loss of the normal effect of leptin in promoting satiety and increased physical activity. Measurement of leptin has been suggested as a diagnostic test for IR (Frank and Tadros, 2014).

Adiponectin is an insulin-sensitising hormone. Lower concentrations of adiponectin have been found in obese horses, which would result in decreased fatty acid oxidation and decreased glucose tolerance. Adipokines are important to the discussion of EMS and laminitis, because both have direct effects on the endothelium. Adiponectin is also anti-inflammatory, so it would counteract the effect of cytokines such as TNFα on the endothelium.

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When adipocytes reach their maximum storage capacity, hypoxia develops due to limited capillary supply, resulting in energy failure and the release of inflammatory cytokines from stressed and necrotic adipocytes. Adipocytes laden with lipid also lose their ability to buffer lipid fluxes within the body, resulting in deposition of lipid in liver, muscle, pancreas and other tissues, which in turn has a negative effect on insulin metabolism throughout the body. Insulin normally induces nitric oxide-dependent vasodilation in capillary beds, the loss of which results in ectopic deposition of adipose tissue.

Interaction between EMS and PPID

PPID and IR often occur together, but only 32% of horses with PPID are hyperinsulinaemic. Administration of dexamethasone in horses resulted in increased insulin concentrations and lower insulin sensitivity (Tiley et al., 2007). Corticotropin-like intermediate peptide is a derivative of pro-opiomelanocortin and adrenocorticotropic hormone and acts as an insulin secretagogue under experimental conditions. Alpha melanocyte stimulating hormone (αMSH) concentrations are also increased with PPID and, with leptin, plays an important role in energy metabolism. Under normal circumstances, αMSH stimulates neural receptors, opposes leptin and reduces adiposity. A downregulation of this system due to overstimulation of αMSH secretion would have the opposite effect.

Stress hormones catecholamines and corticosteroids are gluconeogenic so that concentrations of carbohydrates, proteins and lipid are adequate to respond to infection. The result is hepatic and peripheral insulin resistance. Gluconeogenic hormones induce inappropriate serine phosphorylation of insulin receptor substrates and receptors. Catecholamines inhibit insulin secretion, which may result in hypertriglyceridemia.

Pathogenesis of lesion formation of laminitis

Basal glucose concentrations are often towards the high end of the reference ranges in horses and ponies with EMS. Glucotoxicity is a consequence of persistent hyperglycaemia in humans with diabetes mellitus, resulting in glycosylation of amino acids. This does not result in laminitis in horses. The mechanism in which high insulin levels cause laminitis has been the subject of many studies in recent years.

Histopathological examination is reportedly difficult, but it demonstrates separation of the basement membrane from secondary epidermal lamellae and disintegration of the basement membrane.

Matrix metalloproteinases were investigated because of their action of breaking down extracellular matrix. No effect has been demonstrated so far, but there are limited studies.

Insulin-like growth factor (IGF-1) is a promising candidate. The growth effect of insulin on lamellar epidermal basal cells through the IGF-1 receptor has been demonstrated in vitro, and the infusion of a monoclonal antibody to selectively block the IGF-1 receptor partially prevented acute laminitis in the euglycaemic hyperinsulinaemic clamp (EHC) model. However, in an in vivo study, IGF-1 gene expression was decreased in insulin-treated horses. There was also no increase in circulating IGF-1 during an EHC. The authors proposed that the downregulation resulted from overstimulation of the receptors during the development of the disease.

Diagnosis of EMS

Because EMS is characterised by ID, diagnosis is by demonstrating hyperinsulinaemia. The 'gold standard' test is the EHC, which is a test of insulin sensitivity, but it is not practical for use in practice. There is also the combined glucose and insulin test.

Oral tests are preferred because insulin concentrations are higher with oral consumption of carbohydrates. Basal insulin may be increased in pasture-fed ponies. They should not

be fasted prior to testing. Dynamic testing provides more consistent results. Several testing protocols have been investigated. The oral glucose test and oral sugar test are used most often.

The European College of Internal Medicine's consensus statement for the oral glucose test (Durham et al., 2019) recommends fasting the horse or pony overnight, then giving them a non-glycaemic feed containing 0.5 or 1g/kg bodyweight glucose powder mixed with a little water to aid mixing and ingestion. Peak plasma insulin occurs between 60 and 120 minutes after the feed, so the sample for insulin measurement is taken two hours after the feed. There have been some problems with palatability, with slow ingestion, possibly delayed gastric emptying and decreased intestinal absorption, so a slightly lower dose of 0.75g/kg bodyweight has been suggested (de Laat and Sillence, 2017). Insulin concentrations suggesting ID at 120 minutes are >68 IU/ml for 0.5 g/kg and >80 IU/ml for 1 g/kg.

The oral sugar test gives similar results. 0.15 mL/kg of Karo Light corn syrup is administered orally via a dose syringe. At this dosage level, the test lacks sensitivity. A dose of 0.45 mL/kg has been suggested, but the study was limited by a small sample number (Jocelyn et al., 2018).

In an effort to provide a more palatable carbohydrate source, a newly developed glycaemic pellet has been tested against the oral sugar test at the 0.15mL/kg dose rate (Thane et al., 2024). Higher blood glucose concentrations were obtained with the pellets, probably because of higher voluntary intake. This is a promising new development as a challenge test for ID but it has yet to be made commercially available.

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The pathophysiology of laminitis, diagnostic tests and treatment protocols continue to advance. More clarity on the pathogenesis would provide new options for treatment and new research is investigating optimal methods for the detection of hyperinsulinaemia. VS

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