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Endocrinopathic laminitis in the horse

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Laminitis represents one of the most common and potentially crippling diseases of the adult horse, often resulting in permanent lameness or the need for euthanasia. Over the past three decades, many studies have focused on equine laminitis, the majority of published research centering on laminitis arising due to gastrointestinal disease, dietary indiscretion, and endotoxemia. (1,2) In order to gain further insights into this serious pathological condition, experimental models of laminitis have been devised, employing the administration of large quantities of starch,(3) soluble products of Black Walnut trees,(4) or plant-derived fructans.(2) In utilizing these models, the assumption is made that laminitis is a degradative inflammatory condition of the hoof lamellar interface which arises due to changes induced in the large intestinal bacterial flora, accumulation of toxic bacterial products, increased colonic permeability, absorption of bacterial products, and consequent cardiovascular perturbations. Absorbed bacterial products that have been implicated in the pathogenesis of laminitis include lipopolysaccharide (LPS),(5) *Streptococcus bovis* exotoxins,(6,7) and vasoconstrictive amines.(8,9)

A substantial body of evidence exists to show that inflammation is a pivotal and essential component of acute laminitis and that inflammatory changes occur early in the course of experimental laminitis, prior to the development of lameness. These changes include: activation of hoof lamellar matrix metalloproteinases,(10) activation of platelets and the formation of neutrophil-platelet aggregates,(11,12) expression of interleukin-1 α (13) and increased concentration of endothelin-1 (14,15) in the hoof lamellar interface, increased expression of COX-2 mRNA by vascular smooth muscle cells obtained from digital vessels, (16) absorption of bacterial LPS into the circulation,(5) and the appearance of polymorphonuclear granulocytes in affected lamellae.(17,18)

In some cases, laminitis occurs in the absence of gastrointestinal disturbance, endotoxemia, ingestion of Black Walnut toxins, or other pro-inflammatory conditions (such as primary hoof inflammation).(19) Seemingly unprovoked laminitis arising in horses and ponies on grass pasture (“grass founder”) has traditionally been attributed to perceived (uncorroborated) high levels of starch in certain grasses (“lush spring pasture”) at certain times of the year. Important new information points to a hitherto unrecognized role for nondigestible, but rapidly fermentable plant storage carbohydrates, fructans, in the risk for laminitis in certain pastures.(2) The role of fructans in the pathogenesis of laminitis has been reviewed elsewhere.(2) Risk of laminitis has also been reported as an association with many other conditions including contralateral lameness, ingestion of endophyte-infected Tall Fescue, exertional rhabdomyolysis, obesity, MS, and conditions associated with excess glucocorticoids (GCs).(19-22)

In this paper, we will review the association of laminitis with various disturbances in the endocrinological systems of the horse. Although much has been written regarding the pathophysiology of laminitis arising from inflammatory models, much less information is available regarding laminitis arising from other causative factors. In order to differentiate laminitis occurring in association with pro-inflammatory and intestinal conditions from laminitis developing from putative hormonal influences, the term endocrinopathic laminitis has been adopted.

Endocrinological implications for laminitis

The most widely recognized endocrinopathic laminitis occurs in association with GC administration.(22) Most practitioners recognize that the administration of pharmacological GCs such as dexamethasone and

triamcinolone acetate sometimes results in the development of laminitis, though the occurrence of this undesirable side effect is by no means predictable. Although these pharmaceuticals are recommended for the management of numerous inflammatory conditions, including recurrent airway obstruction, dermatitis, *purpura hemorrhagica*, myeloencephalitis, hepatitis, immune-mediated diseases, cancer, shock, and inflammatory eye diseases, their use must be measured against the well-recognized risk of complicating laminitis.

Laminitis is a common clinical indicator of Cushing's disease, a condition in which increased secretion of pituitary *pars intermedia*-derived pro-opiomelanocortin (POMC) peptides leads to perpetually enhanced adrenal secretion of cortisol (hyperadrenocorticism), the physiological GC in the equine species.(23) It has been further suggested that "stress" might predispose some horses to laminitis; it remains to be determined, however, whether this association is attributable to increased endogenous cortisol secretion. Pain resulting from laminitis may represent *very severe* stress for horses, irrespective of the underlying cause. Therefore, protracted laminitis often results in prolonged elevated cortisol secretion, possibly contributing to its persistence and refractoriness.

It has been reported that the vasoconstrictive responses of equine digital arteries to catecholamines is potentiated by both betamethasone and hydrocortisone.(24) Thus, GCs could be contributing to factors that interfere with hoof lamellar perfusion and there is plenty of evidence that reduced blood flow in the hoof is an important component of established laminitis.(25-28) In another study, protracted administration of triamcinolone acetate to horses led to abnormal hoof growth, but not to laminitis *per se*.(29) We recently reported that the level of the steroid-transforming enzyme, 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), is sometimes elevated in hoof lamellar tissues during laminitis. Increased 11 β -HSD1 activity will likely result in locally higher cortisol concentrations within hoof lamellae, with the potential for deleterious effects at this specific location.(30)

Although there is a strong association between increased GC action and risk of laminitis in horses, a satisfactory explanation for the pathogenesis of laminitis resulting from increased GC action is still lacking.(22) Unlike the situation regarding alimentary-type laminitis, there is certainly a paucity of literature which addresses this problem. In consideration of the fact that, as described above, multiple inflammatory changes attend the development of alimentary-type laminitis, one might anticipate that GCs should actually reduce the risk for developing this condition. Moreover, failed attempts to experimentally induce laminitis using high dosages of dexamethasone or triamcinolone acetate in horses suggest that GCs might not actually be the immediate cause of the condition.(29)

Obesity has been shown to be a significant risk-factor for the development of laminitis. Obesity-associated laminitis may indeed be similar to the metabolic syndrome (MS) in obese humans.(21) Accordingly, the terms MS, equine syndrome X, and peripheral Cushing's syndrome have been used to describe obese horses that tend to develop laminitis. This same MS has previously been *inappropriately* referred to as hypothyroidism (see below). Metabolic syndrome is characterized by the development of obesity, insulin resistance (IR), hypertension, and an abnormal plasma lipid profile.(31) As it develops in genetically susceptible individuals, MS is broadly attributable to the combined effects of inappropriate dietary intake (quantity and quality) and insufficient physical activity over a long period of time (months to years). In the same sense that development of MS is one of the most important risk factors for numerous cardiovascular diseases in humans, including stroke and atherosclerosis, it has been suggested that a similar risk for laminitis might attend the development of MS in horses.(21) However, the association between aspects of MS in horses and risk for laminitis is certainly deserving of further attention and will be discussed below.

Horses affected with MS have traditionally been diagnosed with hypothyroidism. Such diagnoses are based on affected horses typically being obese, coupled with difficulty in weight reduction even caloric intakes well below those that should maintain a normal body weight and condition score (affected horses are commonly referred to as being "easy keepers"). In this regard, the obesity seen in horses has been likened to that seen in other domestic animal species affected with *bona fide* hypothyroidism. Furthermore, some horses with MS have low levels of circulating thyroid hormones.(32)

The existence of true primary hypothyroidism in horses is rare.(32) It can be categorically ruled out following administration of a thyroid stimulation test, the results of which are normal in horses affected with MS. Therefore, the cause of low levels of thyroid hormones in these horses is more likely attributable to pituitary-dependent or secondary hypothyroidism resulting from insufficient production of thyroid-stimulating hormone or blunted thyrotropin-releasing hormone–induced thyroid-stimulating hormone release. Neither laminitis nor obesity develops in horses in which *bona fide* hypothyroidism has been experimentally induced by surgical removal of the thyroid gland.(33,34)

An increased risk for developing laminitis has been reported in horses that graze Tall Fescue (*Festuca arundinaceae*) that is infected by endophytic fungus (*Neotyphodium coenophialum*).(20) Endophyte-infected fescue grass is widespread throughout the eastern United States, Montana, Wyoming, and the Pacific coastal states, where it is a common source of forage for horses. Loline and ergot alkaloids found in endophytic fescue have been associated with endocrinopathic perturbations in the thyroid gland of newborn foals and the hypothalamic-pituitary axis of pregnant mares.(35) However, in another study, orally administered infected fescue seed failed to cause changes in thyroid functions in mature horses.(36) Risk of laminitis has been primarily attributed to the documented vasoconstrictive effects of these endophytic alkaloids.(37)

Laminitis associated with endocrinological perturbations

The two most likely contributing endocrinological disturbances that might play a role in predisposition to laminitis are those conditions associated with excess GCs(22) and those associated with IR.(21) In as much as GCs cause IR and chronic IR might eventually be shown to predispose to pituitary Cushing's disease in horses, the extent to which these 2 broad categories may be related will be discussed below. For the purposes of this discussion, the relationship between GCs and laminitis will be reviewed first and then the relationship between IR and laminitis will be discussed.

Glucocorticoids and the risk for laminitis

Cortisol, a steroid hormone produced and secreted by the adrenal cortices, is the physiological GC in horses. Production of cortisol is increased in the face of stress, such as trauma, infection, intense heat or cold, surgery, restraint and debilitating diseases, and represents an essential physiological adaptation that promotes survival. For example, stress-induced cortisol secretion ensures that adequate nutrients are supplied to the brain, and other areas of the body that might be compromised by a stressful event or injury. Elevated cortisol secretion causes hyperglycemia and promotes fat mobilization and protein catabolism (amino acid mobilization), in support of CNS energy requirements and an elevated demand for protein biosynthesis at compromised locations.

Cortisol-induced protein catabolism is not indiscriminate; proteins with relatively less important critical functions are degraded into amino acids for mobilization into the circulation sooner than proteins with essential functions, such as brain neurotransmitters and muscle contractile proteins. Cortisol also reverses and suppresses the inflammatory responses which accompany stress.

Syndromes of glucocorticoid excess

Harvey W. Cushing originally described the human syndrome resulting from long-term GC exposure in 1932.(38) The most common cause of Cushing's syndrome in horses is pituitary *pars intermedia* dysfunction (PPID), in which excessive quantities of pro-opiomelanocortin (POMC) peptides, including adrenocorticotropin (ACTH), CLIP, β -endorphin and α -MSH, are released from the pituitary gland in an unregulated manner.(23) Chronic POMC peptide-stimulated elevation of cortisol secretion by the adrenal cortices (hyperadrenocorticism, hypercortisolism) represents the driving force by which the horse is subjected to excess GCs over time.

Cushing's syndrome also arises when exogenously administered synthetic GCs, such as dexamethasone and triamcinalone acetonide are administered to horses. Rarely, sporadic cases of Cushing's syndrome occurring as a consequence of primary adrenal neoplasia in horses have been reported.(39,40)

Consequences of glucocorticoid excess

Most of the pathological consequences of excess GCs may be explained as a simple extension of the physiological effects of cortisol.(23) Glucocorticoid excess leads to protein catabolism in skin, connective tissues, bone and skeletal muscle, resulting in skin atrophy, impaired wound healing, muscle atrophy and weakness, and eventually, bone resorption (osteoporosis). Anti-inflammatory and immunosuppressive effects of elevated GCs contribute to a state of relative immune-compromise resulting from the inhibition of diverse inflammatory mechanisms.

A well-recognized feature of human Cushing's patients is the accumulation of body fat, distributed in an unusual but characteristic manner.(38) Typically, there is an increase in intra-abdominal (omental) adiposity at the same time as fat tissue accumulates in the abdominal wall, face, and upper aspect of the back, whereas the extremities are thin due to muscle wasting. The pathological consequences of excessive intra-abdominal adiposity have received extensive interest during the last decade.(41) The endocrinological consequences of excessive intra-abdominal adiposity will be discussed below. It should be noted that conditions of GC excess lead to the accretion of intra-abdominal adiposity.

Glucocorticoids cause IR by inhibiting the action of insulin. Alongside GC-stimulated hepatic gluconeogenesis, IR is believed to promote the availability of glucose for cells in the CNS and other cells that do not depend on insulin for glucose uptake. Proposed mechanisms for GC-induced IR include a reduction in the number of insulin receptors, a change in receptor affinity for insulin, and defective intracellular signaling (or a combination of these factors). Additionally in some species, several hormones derived from intra-abdominal fat cells contribute to the development of IR.(42) It remains to be determined whether adipose derived hormones contribute to IR in equine Cushing's syndrome.

Risk of laminitis during the treatment of horses using glucocorticoids

Risk of laminitis represents one of the most important potential complications when veterinarians treat horses using synthetic GCs. The likelihood of laminitis appears to be greater with the more potent agents such as triamcinalone acetonide and dexamethasone, and reduced when the less potent GCs such as prednisone and prednisolone are used. It should be noted that the bioavailability of prednisone has recently been shown to be quite low following oral administration to horses, which might contribute to the fact that it is rarely reported to cause laminitis.(43)

In spite of multiple attempts to experimentally induce laminitis, it appears that the development of laminitis following administration of pharmaceutical GCs is unpredictable. The risk of laminitis during short-term treatment with either dexamethasone or triamcinalone acetonide appears to be very small for otherwise healthy horses. It should be considered that laminitis might arise in some horses following GC treatment due to the presence of a pre-existing condition in the hoof lamellar interface that could be exacerbated by GCs.

We have proposed that the action of excessive GCs, over the course of many months, leads to structural changes that weaken the hoof-lamellar attachment thus predisposing to laminitis for any other traditional reason.(22) In horses that have already sustained lamellar weakening, treatment with GCs may precipitate laminitis in a relatively short space of time, creating the appearance that laminitis arose due to the recent treatment.

As noted above, substantial new and experimentally-derived data are pointing to the fact that inflammation plays a pivotal role in the pathogenesis of laminitis. Paradoxically, GCs, which are potent

anti-inflammatory agents, should theoretically *not* cause laminitis and might even be useful for the treatment and prevention of this condition.

Glucocorticoids exert numerous actions that could potentially and theoretically contribute to the pathogenesis of laminitis, including their effects on blood vessels, the integument, the gastrointestinal tract, the action of insulin, and on body fat composition. Possible explanations for the pathogenesis of GC-associated laminitis will be reviewed.

Glucocorticoid effects on blood vessels

Glucocorticoids affect tissue perfusion by virtue of direct actions on vascular smooth muscle and indirectly by causing IR (see below). Impaired perfusion of the hoof lamellar interface is a well-documented pathological aspect of laminitis. Both betamethasone and hydrocortisone potentiate the vasoconstrictive actions of the catecholamines epinephrine, norepinephrine and serotonin on large digital vessels; whether this effect is sufficient to explain the development of laminitis in the face of excessive GCs is currently unknown.(24) Ideally, *ex vitro* studies on the contractile and relaxing functions of blood vessels pertaining to the equine hoof should be performed on arterioles and venules obtained from the lamellar interface (resistance vessels), rather than the large digital vessels (conduit vessels). However, isolation of suitable vessels from this intra-capsular location is problematic, and their relatively small size also presents practical difficulties.

Blood flow through critical tissues is primarily governed by the contractility of vascular smooth muscle cells in strategically located resistance vessels. Results from our laboratory suggest that dexamethasone and triamcinalone treatment affects vascular smooth muscle cells in a manner leading to increased contractility, potentially contributing to a reduced blood flow situation.(44)

Glucocorticoid effects on the integument

The hoof-lamellar interface is a highly specialized part of the integument. Laminitis is characterized by separation of epidermis from the underlying dermis at the level of the basal keratinocyte and its attachment to underlying lamellar basement membrane (LBM).(45) In the same manner that GCs cause skin atrophy, laminitis may result from GC-induced lamellar weakening due to increased protein catabolism. The hoof-lamellar attachment is a highly dynamic interface that is being perpetually re-modeled to meet the needs of tissue “wear and tear”. This attachment normally serves to offset tensile forces derived from the deep digital flexor tendon and the considerable forces applied by the weight of the horse, the rider, saddle, and exercise. Normal physiological repair mechanisms, including fibroblast growth and the biosynthesis of collagen, are inhibited by GCs, and this could further predispose to laminitis (mechanical failure at the attachment interface) over time.(46,47)

We contend that visibly evident changes in the appearance of the hoof can be attributable, in some instances, to the effects of excess GC over time. Asymmetrical palmar/plantar widening of growth lines, widening of the white line zone, and “dropping” of the sole could result from chronic GC action. These structural modifications would appear similar to the appearance of a laminitis-affected hoof but would not be associated with painful laminitis. Thus, GC-induced changes in the hoof may *predispose* to laminitis.(22)

As noted, an essential step in the pathogenesis of acute laminitis is failure of attachment of basal keratinocytes to the underlying LBM. Therefore, factors that might act to weaken the strength of this dermo-epidermal attachment interface might potentially lead to laminitis. For example, keratinocyte attachment failure is attended by matrix metalloproteinase (MMP) induced degradation of the LBM during alimentary-type laminitis.(10) Keratinocytes are richly endowed with GC receptors,(48) and dexamethasone has been shown to decrease specific anchoring proteins that connect basal keratinocytes to the LBM.(49) Moreover, cortisol has been shown to actually inhibit keratinization of the bovine hoof.(50)

Equine hoof lamellar keratinocytes appear to have an exceptionally high glucose requirement.(51) It has been suggested that maintenance of the structural integrity of the keratinocyte-to-LBM attachment interface relies on glucose delivery to and uptake by these keratinocytes. Circumstances that might deprive keratinocytes of glucose could theoretically cause laminitis. It remains to be determined whether GC-associated IR, by virtue of inhibited cellular glucose uptake, could compromise the health of keratinocytes and reduce the strength of attachment of these cells to underlying LBM. That being the case, an insulin dependent mechanism for glucose uptake by keratinocytes would be physiologically novel; insulin mediated glucose uptake is not a feature of the integument as a whole.(52)

Glucocorticoid effects on the gastrointestinal tract

Both exogenously administered dexamethasone and increased release of endogenous GCs in times of stress increase the permeability of the mucosal lining of the entire gastrointestinal tract of laboratory animals.(53-56) Stress-associated increases in the permeability of the mucosal lining of the alimentary tract in humans have been shown to facilitate detrimental absorption of antigens, toxins, and other pro-inflammatory molecules from the gut lumen.(56)

Laminitis often arises in the face of intestinal disease in horses, suggesting that toxic factors of intestinal origin play an important role in its pathogenesis.(57) Administering either starch or fructans for the experimental induction of laminitis leads to both increased intestinal permeability and intestinal floral changes. Therefore conditions associated with excess GCs might also contribute to the risk of developing laminitis in horses by virtue of increased intestinal permeability and the absorption of toxic factors from the intestinal lumen.

Glucocorticoids and the action of insulin

High levels of GCs interfere with the action of insulin, leading to IR, a common clinical component of horses affected with GC excess.(23,58) Chronic IR, characterized by hyperglycemia and hyperinsulinemia, subjects those cells that are not dependent on insulin for glucose uptake to relatively high glucose levels over time.(59) This “toxic” glucose effect is especially important for endothelial cells (“glucotoxic endotheliopathy”) and will be discussed later. Glucotoxic endotheliopathy is characterized by increased production of endothelin-1 and reduced release of NO by endothelial cells.(60) Therefore, production of a preponderance of constricting factors for the underlying vascular smooth muscle is another potential causative or predisposing factor for impaired perfusion and risk of laminitis.

Glucocorticoids and obesity

Glucocorticoids stimulate the differentiation of pre-adipocytes (adipose stromal cells) into mature adipocytes. Cushing’s syndromes are characterized by the accretion of both omental (intra-abdominal) obesity and subcutaneous fat (development of a “buffalo hump” and “moon face”).(23) Elevated populations of intra-abdominal adipocytes produce hormones (adipocytokines) such as resistin and leptin, at a greater rate than adipocytes at subcutaneous locations, further contributing to IR.(42) Therefore, GCs act both directly, and indirectly, through increased accumulation of omental adipocytes, to promote IR.

Regulation of cortisol in peripheral tissues

Circulating cortisol, synthesized by and released from the adrenal cortices, is bound to corticosteroid binding globulin, which provides a reservoir to lessen the rapid fluctuations that would arise due to episodic ACTH secretion. Cortisol is subsequently inactivated, primarily in the proximal convoluted tubule and *pars recta* of the kidney, to its 11-keto derivative, cortisone.(61)

The enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which is widely expressed throughout the body, converts inactive cortisone to the active GC, cortisol. There is increasing evidence to

support the notion that 11 β -HSD1 found in intra-abdominal adipocytes significantly elevates the local tissue-specific concentration of cortisol (more so than in adipocytes at other locations).(62) Locally-amplified cortisol production contributes to the perpetuation of these adipocytes through paracrine and autocrine mechanisms.(63)

We recently reported that 11 β -HSD1 activity in both skin and hoof lamellar tissue may be increased in laminitic horses,(30) though the extent to which this elevation in enzyme activity is important for the pathogenesis or clinical progression of laminitis is presently unknown. The role of GC in human omental obesity has recently been described as “tissue-specific Cushing’s syndrome”.(64) Assuming that GCs do cause or predispose a horse to laminitis, increased levels of 11 β -HSD1 may amplify such a predisposition. As new drugs are developed for the specific inhibition of 11 β -HSD1, newer treatment options for GC-associated laminitis in horses may become available.

Obesity and metabolic syndrome

Contemporary equine husbandry practices tend to promote the development of obesity in domesticated horses. Coupled with the provision of grain-rich diets, forage sources (grass and hay) that were artificially selected for the need of *food* animal production are also commonly fed to horses.(65) These forages are considerably higher in soluble carbohydrates (nonfiber/nonstructural carbohydrates) than wild/native grass strains. Obesity is a consequence of the provision of rations that are broadly excessive with respect to metabolic requirements for their level of physical activity and/or that provide calories in a concentrated form that is far removed from their natural diet.(21) Moreover, horses are commonly fed grain-rich rations during long periods of physical inactivity.

Particular importance regarding the development of obesity is placed on the *glycemic index* of the equine ration.(65,66) The accumulation of adipose is strongly attributable to the extent to which ingested food elevates the blood glucose concentration. Rations that contain grain (starch) and forages with high nonstructural carbohydrate content are especially important in this regard.(65) The practice of feeding horses using rations characterized by a high glycemic index leads to 2 specific undesirable outcomes: acquisition of obesity and protracted periods of hyperglycemia.

Horses have inherited “thrifty genes”(67) that permit highly efficient use of their dietary intake, leading to heightened ability to endure periods of environmental harshness. However, when food is abundant, susceptible horses can quickly develop obesity, especially when excessive rich food is coupled with restricted physical activity. In the wild, horses, as herbivores, evolved to produce foals at a time when the primary dietary energy resource – grass – was plentiful and the energy demands of lactation could be met. Herbivores tend to acquire adipose tissue in readiness for winter when grass becomes relatively scarce.

In parallel with the accumulation of adipose tissue in readiness for a biologically anticipated period of seasonal environmental harshness, homeostatic mechanisms that serve to promote glucose delivery to the CNS are engaged. Insulin resistance, brought about by adipose-derived endocrine signals, represents a key component of this survival mechanism. Simultaneously, adipose-derived pro-inflammatory cytokines act to prime the body’s defense mechanisms, setting into motion a state of enhanced immune surveillance that is believed to further contribute to the individual’s ability to survive environmental harshness.(68)

In the natural seasonal cycle accumulated adipose is progressively depleted throughout the winter and it does not exist in perpetuity. However, horses that are managed using contemporary approaches tend to develop obesity and the excessive adipose tissue persists for years. This process commences when horses are young (< 10 years of age) and continues throughout their teenage years. In fact, excessive feeding of grain to weanling- and yearling-aged horses has been shown to cause IR and increase the risk of developmental orthopaedic disease.(69)

Endocrinopathic consequences of obesity

Fat tissue is not, as previously believed, simply a benign repository of stored energy. Adipocytes represent an important source of numerous diverse hormones (adipokines) that play a role in regulating body mass and body composition.(42) Furthermore, it is clear that heterogeneous populations of adipocytes produce differing levels of the various adipokines. For example, in humans, omental adipocytes are endocrinologically more active than adipocytes at subcutaneous locations. Hence, omental (intraabdominal) adiposity is associated with greater risk for cardiovascular disease than subcutaneous adiposity.(70)

There are differences between different equine breeds with respect to the ease with which an obese state can be attained and maintained. In this regard, the traditional partitioning of breeds between “hot-blooded” and “cold-blooded” may have some relevance. For example, compared to horse breeds, pony breeds are both more insulin resistant and relatively prone to laminitis.(71,72)

The accretion of adiposity is attended by the production of excessive quantities of endocrine signals, including leptin, resistin, adiponectin, mineralocorticoid releasing factors, and certain pro-inflammatory cytokines (i.e., tumor necrosis factor- α , interleukin-6).(42,73,74) Heightened release of free fatty acids from omental adipose also contributes to the development of IR.(75) Omental adipocytes express higher levels of 11 β -HSD-1, which subsequently elevates the local cortisol pool.(76) Thus intraabdominal fat may be likened to a gland that produces a battery of endocrine signals, the net result of which promotes IR and a chronically enhanced inflammatory state.

Insulin

Insulin, a hormone that is associated with dietary abundance, is released from beta cells in pancreatic islets in response to hyperglycemia. Insulin acts through insulin receptor mechanisms to stimulate glucose uptake by skeletal muscle, adipose tissue, and the liver.(77) Stimulated glucose entry into these cells is accomplished by the movement of pre-formed, vesicle-bound GLUT-4 transporter proteins from the cytoplasm into the cell membrane.(78) Insulin normally controls blood glucose concentration within a strict reference range and in the healthy state, effectively protects the body from the adverse effects of post-prandial hyperglycemia (glucotoxicity). Insulin also stimulates lipogenesis and inhibits hepatic gluconeogenesis.(77)

Like the adipokine leptin, insulin is an important regulator of food intake and energy balance for the body as a whole. Both insulin and leptin, which enter the brain from the plasma, act as adiposity signals for the brain and circulating concentrations of these hormones are positively correlated with body weight and especially adipose proportion.(79) Receptors for both insulin and leptin are present in the brain, at locations important for control of food intake and energy balance. When administered to these areas of the brain, both insulin and leptin act additively to inhibit food intake in experimental animals; when administered to the same areas, insulin antibodies cause an increase in food intake and body weight.(79)

Insulin acts as a vasorelaxer for some blood vessels and chronic hyperinsulinemia has been shown to stimulate proliferation of vascular smooth muscle cells.(80,81) In skeletal muscles, insulin normally acts to increase blood flow and to promote capillary recruitment.(82)

The concentration of insulin (and glucose) in the plasma may be affected by many diverse factors including time since feeding, type of ration, diurnal variations in cortisol, excitement and stress, reproductive status, illness, genetics, obesity, and endocrinopathic conditions (PPID, MS, PSSM).(66)

Insulin resistance

Factors that interfere with the effectiveness of insulin at its cellular targets include GCs, free fatty acids, and adipose-derived adipokines.(83) Compromised insulin effectiveness is known as insulin resistance (or impaired insulin sensitivity). If the ability of insulin to facilitate glucose removal from the

circulation is impaired, the plasma glucose concentration tends to remain elevated causing continual stimulation of pancreatic islet β -cells and hyperinsulinemia.(83) Insulin resistance is commonly recognized in horses by demonstrating inappropriate hyperinsulinemia (serum insulin concentration > 300 pmol/l) in the face of a mild-to-moderately elevated plasma glucose concentration.(21) Clearly, it is necessary to rule out other potentially pertinent causes of hyperinsulinemia, such as a recent grain meal. Insulin resistance may be further characterized by undertaking either a glucose tolerance test or, when available in a suitably equipped laboratory, euglycemic-hyperinsulinemic clamping.(84,85)

Clinical effects of IR can be attributable to both inhibition of insulin effect at insulin targets (insufficient glucose delivery) and the direct consequence of exposure of some cells to elevated plasma insulin concentrations over time.(83) For example, hyperinsulinemia per se has been shown to stimulate proliferation of vascular smooth muscle cells.(81) Availability of glucose to skeletal muscle might be reduced as a result of IR making the development of certain types of myopathy more likely. Of further note, IR directly increases the sensitivity of tissues to the effects of GCs.(86)

Most of the clinically important consequences of IR appear to result from exposure of vascular endothelial cells to higher-than-normal glucose levels over time.(59) Endothelial cells do not require insulin for glucose uptake and the intracellular concentration of glucose parallels that of the plasma.

Exposure of endothelial cells to relatively high levels of glucose over time, as happens in IR due to a combination of both inefficient glucose clearance via insulin regulated pathways and the use of high glycemic index rations, causes glycosylation of cell proteins and the genesis of reactive oxygen species (ROS).(87) Increased production of ROS may exceed the cell's inherent anti-oxidant capacity and cause oxidative damage (oxidative stress). Endothelial dysfunction resulting from oxidative stress represents the pivotal mechanism through which IR causes cardiovascular disease.(88) Moreover, there is substantial evidence that therapeutic anti-oxidant strategies are helpful in conditions associated with IR.(89) The effect of chronic IR on endothelial cells is referred to as *glucotoxic endotheliopathy*.

As a species, IR-affected horses are unusual in that they appear to be able to maintain stimulated pancreatic insulin secretion for many years. Pancreatic β -cell exhaustion, as occurs in humans and cats affected with protracted IR, rarely leads to type-2 diabetes mellitus in horses.

Glucotoxic endotheliopathy and the risk for laminitis

Pathological changes in the endothelial cells resulting from chronic IR might potentially predispose or contribute to the morbidity associated with laminitis, especially in obese patients. Complex reviews of the pathophysiology of glucotoxic endotheliopathy have been published and should be consulted for additional details.(59) The genesis of ROS resulting from inappropriate intracellular glycosylation leads to reduced nitric oxide (NO) production and enhanced production of endothelin-1 by endothelial cells.(88) These perturbations in NO and ET-1, the two most effective endothelially-derived modulators of underlying vascular smooth muscle tone, lead to increased vasospasticity and potentially reduced perfusion. In human patients, these changes contribute to the development of hypertension in the face of IR. Although it remains to be determined with certainty whether it develops in IR-affected horses and ponies, chronic hypertension has been reported in chronically foundered ponies on the basis of Doppler sphygmomanometry and demonstration of left ventricular hypertrophy.(90)

In addition to dysregulation of vascular smooth muscle tone, glucotoxic changes in the hemostatic properties of endothelia, coagulation proteins, and platelets contribute further risk for poor perfusion and possibly laminitis.(91) The luminal surface of endothelial cells, normally an anti-thrombotic surface, is rendered relatively less so by the effects of IR. The state of platelet activation is relatively up-regulated in IR.(91) The role of activated platelets in the pathogenesis of alimentary-type laminitis has already been addressed.(11,12) Specific pro-thrombotic factors that are increased by IR include plasminogen activator inhibitor-1, von Willebrand's factor, fibrinogen, factor VII and the circulating concentration of thrombin-anti-thrombin complexes.(91) Specific anti-thrombotic factors that are decreased by IR include anti-thrombin-III, protein S, and protein C.(91)

Other aspects of IR that might contribute to the risk for laminitis include attenuation of insulin-dependent vasorelaxation, impaired capillary recruitment, promoted oxidative stress-related damage within the hoof-lamellar interface, and increased MMP activity. Although recent studies have demonstrated a pivotal role for MMP in laminitis,(10) little is known about the effects of IR on MMP regulation. Both MMP-2 and MMP-9 are activated by ROS and their expression appears to be regulated by oxidative stress.(92) It has been recently shown that MMP-9 production by vascular endothelial cells is increased by high glucose conditions and that this glucotoxic effect can be reversed with antioxidants (93). This mechanism of redox-sensitive MMP-9 expression resulting from IR potentially explains elevated MMP activity that has been reported in hoof lamellar tissues obtained from horses affected with PPID.

The attachment of hoof epidermis to underlying LBM appears to depend on adequate glucose uptake by basal keratinocytes.(51) Failure of keratinocyte-to-LBM attachment is a critical early step in the pathogenesis of alimentary-type laminitis.(45) It has been suggested that relative glucose deprivation of keratinocytes could contribute to risk for laminitis in IR-affected horses and ponies. However, this hypothesis would require that hoof lamellar basal keratinocytes significantly depend on insulin-mediated glucose uptake. This hypothesis is deserving of further investigation.

Metabolic syndrome

Human patients affected with IR, obesity, hypertension and dyslipidemia (elevated LDL cholesterol and depressed HDL cholesterol) are often classified as being affected with MS.(94) Other components of MS in humans include impairment of the fibrinolytic system, enhancement of pro-thrombotic processes, hyperhomocysteinemia microalbuminuria, reduced parasympathetic (vagal) neural outflow from the CNS, and heightened inflammation (increased circulating levels of pro-inflammatory cytokines and hepatic-derived acute phase proteins).(77) The extent to which obese horses affected with IR are similar to human patients is unclear; there are few reports regarding lipid profiles in horses, but there is some evidence that hypertension might be present in foundered ponies.(90)

Metabolic syndrome represents a very important risk factor for type-2 diabetes, atherosclerosis, stroke, infertility, cancer, and osteoarthritis in humans.(94) It remains to be determined whether the equine MS is a risk factor for laminitis.(21)

Clinical recognition of equine metabolic syndrome

A diagnosis of MS should be considered in obese adult horses with laminitis or in cases of unexplained laminitis. Additional physical characteristics of MS in horses include abnormal body fat distribution (e.g., thickened, “cresty” neck; fatty accretions at the tail head and near the shoulders; fatty thickening in the prepuce).(21) Affected broodmares are commonly reported to be infertile and often exhibit abnormal cycling. Easy weight gain usually occurs on caloric intakes that are well below those that would be predicted to maintain a normal body weight. Polyphagia is common. Polyuria and polydipsia may be seen in horses that tend to be severely hyperglycemic.

It should be noted that not all affected horses are obese, and development of MS is believed to predispose some horses to laminitis when it has not yet occurred. Horses that have been subjected to protein energy malnutrition tend to develop IR (survival mechanism).

Currently, identification of IR represents the most useful clinical approach to diagnosing MS in horses. Although the use of euglycemic hyperinsulinemic clamping represents the gold standard for demonstrating IR, the simple demonstration of hyperinsulinemia and mild to moderate hyperglycemia (110 to 140 mg/dl) in fasted horses is clinically practical and strongly suggests that IR is present.(21) Hyperinsulinemia is a far more consistent finding than hyperglycemia, however, and a presumptive diagnosis can be made based on identification of hyperinsulinemia alone, provided that grain has not been fed for a minimum of 5 hours before testing.(65)

Alternatively, an IV glucose tolerance test in IR-affected horses shows that the plasma glucose concentration fails to return to the reference range within 90 to 120 minutes.(84) Use of appropriate diagnostic tests to rule out other potentially similar endocrinopathic conditions, such as hypothyroidism and pituitary *pars intermedia* dysfunction (PPID; pituitary Cushing's disease), lends further support to the diagnosis of MS.(23,32) In some affected horses, the fasting plasma triglyceride concentration is mildly to moderately elevated.(65)

Treatment of metabolic syndrome

Weight reduction and an increased level of physical activity represent the key therapeutic approach to obese horses.(65) Substantial clinical improvement regarding several comorbidities associated with MS (e.g., decreased IR, improved glycemic control, reduced hypertension, improved lipid values) may be achieved with modest weight reductions (5% to 10%). Significant improvements in IR can be achieved using a combination of controlled food intake and enhanced physical conditioning in pony breeds.(95) Exercise promotes increased glucose uptake and use by skeletal muscle via insulin-independent mechanisms, the effect of which persists for up to 24 hours. However, painful laminitis in equine patients precludes enhanced physical exercise for managing obesity and IR. Furthermore, stress associated with the development of laminitis leads to activation of neuroendocrinologic mechanisms (e.g., increased cortisol secretion) that tend to further promote the persistence of IR.

Dietary recommendations pertaining to the management of horses affected with MS have been published elsewhere.(65) Broadly speaking, the ration provided for affected horses should be characterized by a low glycemic index, low nonstructural carbohydrate content, devoid of grain and pasture grass, and balanced appropriately with respect to minerals and vitamins.(65) In light of the fact that the soluble carbohydrate content of grass (pasture) and hay cannot be estimated by visual inspection, analysis of forage by an appropriately equipped laboratory should be sought. Ideally, hay that has been shown to have low nonstructural carbohydrate content should represent the cornerstone of the diet (the combined sugar and starch content of the hay should not exceed 10%).(65)

If the nonstructural carbohydrate content of a specific batch of hay is too high, it may be rendered less dangerous by being soaked under water for 30-60 minutes prior to feeding.(96) Soaking hay in hot water (as opposed to cold water) is a more effective method of reducing its soluble carbohydrate content in this regard. Although soaking hay may represent a practical solution for feeding small numbers of horses, specific problems that could be encountered include the promotion of mold development in non-consumed hay and the freezing of soaked forage in sub-zero winter temperatures.

Ad libitum access to grass pasture for grazing is dangerous for horses affected with MS. Stressed grass (affected by overgrazing, cold temperatures, and insufficient water) often develops a high nonstructural carbohydrate concentration. Moreover, sugars tend to be concentrated in the portions of grass closest to ground level.(97) Laminitis is reported to commonly occur in MS-affected horses that have had access to a very short, overgrazed, or mowed paddock or pasture. The safest clinical approach is to recommend avoidance of fresh grass entirely. Horses can be turned out with completely or partially taped grazing muzzles. If attempting to allow some consumption, owners must be carefully instructed to monitor the horse daily for weight gain, a change in abnormal fat deposits (e.g., crests becoming more hard/firm), and early signs of laminitis (e.g., less spontaneous movement, increased digital pulses, change in foot temperature, reluctance to turn in a small circle).

Well-designed studies to determine whether exogenous thyroid hormone administration is effective or safe for the management of MS are currently lacking.(65) Although thyroid hormones facilitate insulin-mediated glucose uptake by cells in other species, this potentially beneficial effect should be considered in light of the fact that they also enhance intestinal glucose absorption, promoting the tendency toward hyperglycemia seen in horses with MS. Despite the continued controversy regarding thyroid hormone supplementation of horses with MS, its use remains popular among practitioners because of perceived clinical improvement of the animal's energy level and attitude. Thyroid hormone supplementation should be recommended in conjunction with frequent monitoring to prevent iatrogenic

hyperthyroidism. Patients should be gradually tapered off thyroid supplementation as their clinical status and insulin levels improve, and affected horses should not be specifically diagnosed with hypothyroidism.

Relationship between metabolic syndrome and pituitary pars intermedia dysfunction

The relationship between MS and PPID is deserving of further investigation. Traditionally, PPID is characterized by hirsutism (failure to shed out the haircoat), advanced age, and poor bodily condition (muscle wasting and accretion of subcutaneous fat pads).(23) Some affected horses are affected with laminitis and exhibit polyuria and polydipsia (PU:PD).(23)

Although the dexamethasone suppression test (DST) is a reliable test for PPID with very high sensitivity and specificity (supported by extensive studies and autopsy confirmation), some believe that use of dexamethasone in these patients engenders an unacceptable risk of laminitis.(98) Therefore, additional diagnostic tests have been advocated. For example, elevation of plasma e-ACTH concentration may be as useful as the DST for diagnosis of PPID without the attending risk of laminitis.(99,100)

However, increasing use of an elevated plasma e-ACTH concentration as the diagnostic criterion for PPID has led to the observation that PPID may be more prevalent in relatively younger horses than previously recognized. Diagnosis of PPID in teenage (and younger) horses prior to the development of hirsutism and weight loss is being reported more commonly using the e-ACTH criterion.(98,101)

Pituitary dependent Cushing's disease results from clonal expansion of POMC-secreting cells in the *pars intermedia*.(23) This situation is unique to the equine species and has been attributed to loss of dopaminergic inhibition of melanotropes in the *pars intermedia* resulting from primary hypothalamic perturbations.(23) Interestingly, a role for oxidative stress in loss of dopaminergic innervation of the *pars intermedia* from horses affected with PPID has been supported by work published recently.(102)

We have speculated that PPID arises as a consequence of MS and that chronic IR and attending oxidative stress are directly responsible for the loss of dopaminergic innervation that, in the healthy state, normally acts to suppress POMC-peptide secretion from the *pars intermedia*. Therefore, identification of PPID in younger horses (based on results of e-ACTH) that have not developed the well-recognized physical appearance of the old Cushingoid horse should not be surprising. This observation might also partially explain why PPID appears to be more common in pony breeds than horse breeds (as pony breeds tend to be at greater risk for laminitis and tend to be more insulin resistant than horses).

Laminitis is a potential complication of both MS and PPID. The extent to which these two endocrinological conditions are related is yet clearly deserving of further investigation.

Endocrinopathic hoof lamellar remodeling versus laminitis

We believe that conditions associated with GC excess (exogenous or endogenous) and IR lead to structural changes in the connective tissues of the hoof-lamellar junctional zone that might be viewed simplistically as a "weakening" effect on the attachment interface. Over time, these changes result in lengthening and attenuation of the primary and secondary dermal lamellae, not necessarily associated with pain, inflammation, or lameness *per se*. An important result of these lamellar changes is a gradual and progressive "pulling apart" of the underlying dermis (and *os pedis*) from the lamellar interface.(22)

On inspection, the characteristic features of the affected hoof include progressive widening of the growth lines ("laminar" or "stress" lines), palmar divergence of growth lines and widening of the white line zone. Radiographic examination of the affected hoof reveals changes that are similar to those seen in classic laminitis, including pedal bone rotation and pedal osteitis. Osteopenia and remodeling of the *os pedis* (atrophied appearance with a distal "ski tip") arise from the combined effects of GC-induced osteoporosis, the pull of the deep digital flexor tendon, and "weakening" of the lamellar connective tissue matrix between the *os pedis* and the lamellar interface.

Conclusions

Conditions and circumstances associated with either or both GC excess and IR are attended by increased risk for the development of laminitis in adult horses. However, there exists substantial controversy as to whether IR or excess GCs can cause laminitis *de novo*. If IR and elevated GCs are causative factors for laminitis, the pathogenesis of the laminitic condition arising from their effects is probably different from that associated with diseases of the gastrointestinal tract and endotoxemia. However, severe disease states associated with endotoxemia are also characterized by hypercortisolism (stress), neuroendocrine activation, and IR (production of inflammatory cytokines). Numerous possible and plausible theoretical mechanisms have been hypothesized and discussed.

It is interesting to note that, in parallel with the fact that GCs cause IR, a state of IR also increases tissue sensitivity to the action of GCs. The combined effects of IR and, by virtue of dysregulated cortisol metabolism, increased cortisol action, in intraabdominal adipose tissue affect the body as a whole through multiple, diverse mechanisms. The consequences of excessive intraabdominal adiposity for the body as a whole (risk for diabetes and cardiovascular disease) have been widely reported and publicized. In light of the fact that cortisol dysregulation (through increased 11 β -HSD-1) is believed to play a pivotal role in this process, proposed endocrine names for this situation are omental Cushing's disease or "tissue-specific" Cushing's disease. It remains to be seen whether the development of endocrinopathic laminitis represents a species-specific example of "tissue-specific" Cushing's disease in horses.

Veterinarians must exert caution with respect to the use of GCs in adult horses. Extreme caution should be exercised in the administration of systemic GCs to horses that have been previously affected by laminitis, and animals so treated must be carefully monitored for the development of laminitic pain. The risk of laminitis appears to be greater with dexamethasone and triamcinalone acetonide compared with prednisone and prednisolone. Whenever possible, GCs should be administered locally.

Horse owners and veterinarians should recognize that obesity is associated with multiple endocrinological changes that might predispose to cardiovascular disease and other conditions. Obesity in horses most commonly results from the combined effects of insufficient exercise and provision of a ration that is grossly excessive with respect to energy requirements, especially glycemic index. Client education efforts should be directed toward emphasizing the value of a fit, lean physical condition over an obese, halter-show class condition. Improved objective methods for the assessment of obesity between horses and between observers would be useful. Currently, horses are graded using a subjective body score that ranges between 1 (extremely thin) to 9 (obese).(103)

New information is emerging regarding better methods of evaluating equine rations. The recognition of the role of non-structural carbohydrates in forages that lead to obesity and IR and the increasing availability of practical laboratory analyses for the purpose of constructing a healthy ration for horses is particularly exciting. Further work is needed to better understand and recognize the glycemic index of equine nutritional components as well as the overall ration. The availability of laboratories for the analysis of forage-based rations for fructans would be similarly helpful for veterinarians.

We suggest that structural changes in the equine hoof that *resemble* laminitis may arise due to the actions of excess GC or IR. Although these changes are not painful *per se* and are not associated with inflammation, they could predispose affected horses to the development of *bona fide* laminitis for other reasons. Further investigation into the effect of endocrinological perturbations at the hoof lamellar interface is clearly needed.