

Insulin Resistance in Equids: Possible Role in Laminitis¹⁻³

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ABSTRACT Insulin is a major regulatory hormone in glucose and fat metabolism, vascular function, inflammation, tissue remodeling, and the somatotrophic axis of growth. Insulin resistance alters insulin signaling by decreasing insulin action in certain resistant pathways while increasing insulin signaling in other unaffected pathways via compensatory hyperinsulinemia. In humans, altered insulin signaling is implicated in reduced glucose availability to insulin-sensitive cells, vasoconstriction and endothelial damage, and inflammatory response. Although no direct evidence exists for insulin's role in these mechanisms in the laminitic horse, changes in the glucose availability, vasculature, and inflammation were all demonstrated in hoof separation. Insulin resistance was first implicated in the pathogenesis of laminitis in the 1980s using tolerance tests. Our present findings provide the first specific evidence of insulin resistance as a major predisposing condition for laminitis. Specific quantitative characterization of insulin resistance is essential toward identifying the following: 1) ponies in need of special management to avoid laminitis, and 2) potential management strategies to avoid laminitis by increasing insulin sensitivity, including reducing obesity, increasing exercise, and moderating dietary carbohydrates, particularly starch. *J. Nutr.* 136: 2094S–2098S, 2006.

KEY WORDS: • *insulin signaling* • *laminitis* • *horses*

Insulin resistance was first implicated in the pathogenesis of laminitis in the 1980s using oral glucose and i.v. insulin tolerance tests (1,2). These tests indicated relative glucose intolerance and resistance to exogenous insulin in ponies that had previously experienced laminitis, compared with ponies with no history of laminitis. Field and Jeffcott (3) proposed a mechanism of insulin resistance-mediated vasoconstriction in laminitis. Since then, our understanding of insulin resistance in laminitis has improved, due largely to technological advances in measurement. Advancements include the use of specific and quantitative techniques to characterize the dynamic glucose and insulin system and more convenient proxies that statistically predict specific quantitative parameters (4,5). Using such techniques can better define the roles of insulin signaling and insulin resistance in the development of equine laminitis. Here we provide the background of insulin resistance and its assessment, present existing data connecting insulin resistance to equine laminitis, and discuss possible mechanisms. Our goal

is to promote a better understanding of the role of insulin resistance in the development of equine laminitis, ultimately to improve management to avoid the disease.

Definition

Insulin resistance is a general term for the inability of a normal concentration of insulin to produce a normal response from target tissues (6). Insulin resistance is a characteristic of type 2 diabetes and is different from a reduction in insulin action due to reduced circulating insulin as occurs in type 1 diabetes. Insulin signaling refers to the stimulation of a response by insulin.

Insulin resistance could pertain to a breakdown in insulin-signaling mediators prior to the insulin receptor, which reduce circulating insulin or downregulate insulin-receptors. More commonly observed, however, are alterations in the postbinding signal transduction associated with decreased insulin receptor-autophosphorylation and decreased tyrosine kinase activity, resulting in decreased insulin-receptor substrate-1 phosphorylation and reduced activation of phosphatidylinositol 3-kinase (7–9). In addition, disruption of intracellular glucose metabolism regulated by enzymes such as hexokinase and glycogen synthase could reduce insulin-mediated glucose uptake and storage (7–10). To distinguish receptor-level and intracellular inhibition of insulin action we adopted 2 terms: insulin sensitivity, which describes reduced insulin-mediated glucose transport into the cell, and insulin ineffectiveness, which describes a failure of insulin-facilitated intracellular glucose metabolism (11).

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Assessment of insulin sensitivity

Various types of evidence have been used to document insulin insensitivity in horses. Analogy to human metabolic syndrome provides a tempting cornucopia of possible disease associations, mechanisms, and interventions, but care should be taken to avoid the danger of unsupported assumptions. Similarly, association with risk factors such as obesity or equine Cushing's disease that suggest insulin resistance is an insufficient basis for establishing genuine insulin resistance.

Nonspecific methods. Common methods for determining insulin sensitivity are unstandardized and indefinite. These include the basal values of glycemia and insulinemia, which represent the end point of considerable regulation, and tolerance tests, which provide curves for which trends can be inferred, but no specific characteristics at the level of the tissue derived. Nonspecific tests therefore only suggest, often ambiguously, alterations in the complex interactions among glucose, insulin, and tissue.

Specific methods. Specific quantitative methods of determining insulin sensitivity characterize distinct properties of the dynamic glucose and insulin system. These methods, like the tolerance tests, involve observing the response of the system to controlled perturbations. Unlike tolerance tests, however, specific methods quantify the physiological response for statistical comparison and standardization.

Clamp methods. Clamp methods include the euglycemic-hyperinsulinemic clamp, the hyperglycemic clamp, and the insulin suppression test (12). These techniques infuse glucose and insulin, fixing one at a steady state to observe concomitant infusion requirements to maintain a steady-state of the other. For example, the euglycemic-hyperinsulinemic clamp fixes insulin at a constant hyperinsulinemic level, then measures the rate of glucose infusion required to maintain steady-state glycemia. The glucose infusion rate is assumed to represent the simultaneous rate of glucose clearance from the plasma and is relative to the clamped level of hyperinsulinemia. The euglycemic-hyperinsulinemic clamp has been used extensively to study insulin sensitivity in numerous species, including the horse (13) and has been considered historically as a "gold-standard." However, the contribution of clamp studies in ponies and horses has been overstated (14,15), and questions remain regarding the nonphysiological nature of the clamp.

Minimal model of glucose and insulin dynamics. The minimal model is a unique quantitative method that currently provides the most detailed assessment of glucose and insulin dynamics. The minimal model is a physiological compartmental representation of the glucose and insulin regulatory system (16). Application involves an i.v. glucose tolerance test superimposed after a delay by an i.v. insulin dose. The pattern of glucose clearance is then modeled according to the compartmental design.

The minimal model allows for several determinations (16) as follows: 1) the change in the rate of glucose clearance in response to a known exogenous insulin dose illustrates the insulin sensitivity index (SI)⁵ of the tissue; 2) the difference between the rates of insulin-stimulated glucose clearance and total glucose clearance reveals the insulin-independent component of glucose clearance (Sg); 3) the endogenous insulin response following the glucose dose (AIRg) quantifies the pancreatic β -cell response; and 4) the appropriateness of this response (DI) can be determined by comparing insulin secretion to insulin sensitivity.

Measurements of insulin sensitivity determined by the minimal model were shown to be correlated with results from the euglycemic-clamp (17). However, the minimal model is now widely accepted on the basis of its merits as a more physiological test of the glucose-insulin regulatory system. Unlike the clamp, the minimal model observes insulin activity at physiological levels. Due to its physiological and dynamic nature, the minimal model has proven robust and flexible, allowing application to a number of physiological states. Finally, the minimal model is the only method currently differentiating insulin-dependent and insulin-independent glucose clearance.

Basal proxies for specific methods. An attempt was made to standardize simple proxies derived from basal glucose and insulin concentrations to test for insulin sensitivity and insulin response. Proxies derived in humans include the homeostasis model assessment, the quantitative insulin sensitivity check index, and ratios of basal glucose and insulin (18–20). These proxies provide standardized, quantitative estimates of insulin sensitivity and response that have been correlated with specific tests for insulin sensitivity or response. These proxies are also still based on circulating insulin and glucose values that are easily perturbed and describe the outcome (not the process) of glucose and insulin dynamics. Nevertheless, basal proxies provide an example of the chronic state of the subject; to date, they are the most practical test for insulin resistance and insulin response for large numbers of tests.

Proxies for the horse. We recently determined and statistically standardized basal proxies, i.e., the reciprocal of the square root of insulin (RISQI) and the modified insulin to glucose ratio (MIRg), for insulin sensitivity and insulin response in horses (5). Unlike other proxies, RISQI and MIRg were designed to estimate specific quantitative parameters, i.e., SI and AIRg of the minimal model, respectively, with determined variability. These proxies were also subjected to statistical tests of equivalence and predictive power.

These proxies require a single basal blood sample collected between 0800 and 1000 from relaxed horses that had grazed overnight on pasture or hay. The equations to calculate the proxies from basal plasma insulin (mIU/L) and glucose (mg/dL) are as follows:

Reciprocal of insulin square-root index (for determining insulin sensitivity, SI) $RISQI = 1/\sqrt{\text{insulin}} = \text{insulin}^{-0.5}$

Modified insulin ratio to glucose (for determining insulin response, AIRg) $MIRg = [800 - 0.30 \cdot (\text{insulin} - 50)^2] / (\text{glucose} - 30)$.

RISQI and MIRg were evaluated, selected, and standardized by correlation, Bland-Altman plot, and concordance. We also considered the sensitivity, specificity, and total predictive power of each proxy based on appropriate differentiation of horses in the lowest quintile of SI or AIRg. The linear relation between each proxy and its minimal model parameter was well-defined and conserved within subpopulations. There was some individual variation attributable to horse and/or sampling, an important consideration when using single-sample surrogates for individual cases.

The need for proxies exists when constraints (in particular large sampling size) preclude the use of time- and labor-intensive tests such as the minimal model or the clamp method. Proxies would be beneficial for repeated monitoring of individual cases or, as shown here, for studies on large populations.

Observations on insulin resistance and laminitis

Tolerance tests. The ability for exogenous insulin to induce hypoglycemia was shown to be reduced in ponies with previous

⁵ Abbreviations used: AIRg, minimal model acute insulin response to glucose; DI, minimal model disposition index; MIRg, modified insulin to glucose ratio; MMP, matrix metalloproteinase; RISQI, reciprocal of the square root of insulin; Sg, minimal model glucose effectiveness; SI, minimal model insulin sensitivity index.

incidence of laminitis compared with normal ponies (1,2). In addition, glucose intolerance in previously laminitic fat ponies was observed after oral glucose loading (1 g/kg body weight) (2). Peak glucose values were higher in these ponies compared with normal ponies, and glucose did not return to baseline. A similar pattern but to a lesser degree was observed in fat ponies that had no history of laminitis compared with normal ponies. Concomitant insulin responses were also higher in ponies with previous laminitis. This result further suggests insulin resistance as well as compensatory insulin response in the predisposition to laminitis.

Basal proxies. The proxies RISQI and MIRG were used to evaluate insulin sensitivity and insulin response in 160 ponies (21,22). Consistent with nonspecific observations, ponies predisposed to laminitis had lower insulin sensitivity (RISQI) and higher insulin response (MIRG), indicating a compensatory exaggeration of pancreatic β -cell insulin secretion (5). In addition, cut-off values for RISQI or MIRG could be defined to differentiate ponies predisposed to laminitis from ponies without any predisposition with an accuracy (specificity, sensitivity and total predictive power each) of at least 70%.

Minimal model. The population survey using basal proxies was followed by an application of the minimal model in 7 previously laminitic and 7 control ponies matched for obesity (23). Results from the minimal model validated the basal proxies in ponies and corroborated conclusions of compensated insulin resistance in previously laminitic ponies. Further, previously laminitic ponies were shown to be at greater risk for failed glycemic control. With specific quantitative characterization, this test consummates the association between altered insulin signaling and the risk of developing laminitis.

Mechanisms of insulin and laminitis

Insulin resistance has been associated with a predisposition to laminitis in ponies (1,2,22,23). It is important to recognize the juxtaposition of insulin resistance and compensatory hyperinsulinemia, such that insulin-resistant pathways of insulin signaling will be suppressed, whereas other pathways will be overstimulated. In humans, altered insulin signaling is implicated in reduced glucose availability to insulin-sensitive cells, vasoconstriction and endothelial damage, and the inflammatory response (24,25). These processes were shown to promote hoof separation. Perhaps insulin signaling mechanisms similar to those in humans exist in the pony and in combination with "trigger factors" associated with digestion of starch/fructan contribute to hoof failure.

Suggested trigger factors in horses include exotoxins, endotoxins, and amines (26). Exotoxins (proteases) are released by disturbed gut microflora and activate collagenases, which break down hoof connective tissue. Matrix metalloproteinases (MMPs)-2 and -9 are particularly implicated in this pathogenesis of laminitis (27,28). In human cells, insulin was shown to suppress MMP-9, whereas glucose stimulates MMP-9 (29,30). Therefore, insulin resistance and associated glucose intolerance could contribute to MMP activity and dissolution of the lamina.

Laminitis in horses has long been considered to involve a vascular component (31–33), perhaps similar to the vascular changes observed in diabetes mellitus and cardiovascular disease in humans. Ischemia could reduce nutrient flow to sensitive tissues, whereas subsequent reperfusion would instigate inflammation and possibly overload the weakened tissue with exacerbating factors such as activated MMPs.

Endotoxins (lipopolysaccharide) are released from the upset gut and were shown to have detrimental effects on the vas-

culature and blood supply to the hoof (34,35). Lipopolysaccharide was shown to increase MMP-9 (36) and the inflammatory cytokine tumor necrosis factor- α (37), which induces insulin resistance (38–40). In healthy humans, insulin may counter these inflammatory signals through stimulation of anti-inflammatory IL-6 release from adipose tissue in humans (41–43). Insulin resistance could reduce this protective effect. Thus, acute insulin resistance may be superimposed on chronic insulin resistance and other trigger factors for laminitis.

Amines are also released by bacteria under acidic condition in the cecum (26). They were shown to cause constriction in horse vascular tissue (34,35) and reduce digital blood flow in standing horses (44). Insulin resistance may attenuate the sensitivity of tissue to amines, increasing their vascular effects (24,25).

Insulin itself is a vasoregulatory hormone, invoking vasodilation through pathways similar to those of insulin-mediated glucose metabolism in human cell cultures (45). Thus, insulin insensitivity would occur simultaneously to a reduction in insulin's vasodilatory effect. Similarly, insulin's ability to counteract endothelin-1-associated vasoconstriction might be compromised in insulin resistance (46), whereas compensatory hyperinsulinemia might stimulate increased endothelin-1 production (47,48). Altered insulin signaling could also affect growth factors, neurohormones, and oxidative stress, factors that are also associated with endothelial damage (25,49).

Even when the blood supply reaches laminar tissue, insulin insensitivity (exacerbated by inflammatory factors) could compromise glucose transport into insulin-dependent cells such as the laminar keratinocytes (50). Glucose deprivation was shown in vitro to result in separation of equine hoof-to-bone connective tissue (51).

Countermeasures to insulin resistance

No countermeasures for pasture laminitis have been tested by randomized control trials. However, likely mechanisms were suggested by pathophysiological and nutritional experiments, partially justifying intervention, which is known to improve insulin sensitivity.

Diets that cause large postprandial fluctuations in plasma glucose and insulin are associated with decreased insulin sensitivity in horses (52). Numerous factors influence digestion and absorption of glucose from starch and sugar; therefore, the contents of starch and sugar are not the sole indicator of a feed's glycemic response (53). Fat and fiber feeds were shown to reduce glycemic responses and promote normal glucose metabolism and gut function (54,55). Certain polyunsaturated fats, such as (n-3) or (n-6), may modulate inflammatory signals (56), which could improve insulin sensitivity (37) in the horse, and it was claimed that (n-3) prevented starch-induced laminitis (57).

Exercise was shown to improve insulin sensitivity in healthy horses for as long as 24 h after a single bout (58,59). In addition to glucose metabolism, exercise stimulates fat metabolism, an effect influenced by diet (60–62).

Obesity is associated with diet and exercise, but obesity alone has consequences for insulin sensitivity and vascular function. Obesity in humans resembles an inflammatory state, with elevated circulating acute-phase proteins and inflammatory cytokines associated with insulin resistance (40,63). These factors increase with the degree of obesity in humans (40,63).

Weight management, proper diet, and exercise comprise a synergistic recommendation for improving insulin sensitivity to avoid pasture laminitis. Recently, levothyroxine was shown to induce weight loss and improve insulin sensitivity, suggesting a

possible supplement to reduce the risk of laminitis (64,65). Because laminitis is associated with pain, biomechanical stress, and increased risk of hypermetabolism, management of laminitic horses requires clinical judgments in association with the timing of exercise and weight reduction (66).

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