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INSULIN RESISTANCE – WHAT IS IT AND HOW DO WE MEASURE IT?

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Introduction

Insulin resistance is defined as the diminished ability of cells to respond to the action of insulin in transporting glucose (sugar) from the bloodstream into muscle and other tissues. In humans, insulin resistance typically develops with obesity and heralds the onset of type 2 (non-insulin-dependent) diabetes (Shepherd and Kahn, 1999). In horses, insulin resistance is commonly associated with equine metabolic syndrome (Johnson, 2002; Powell et al., 2002; Hoffman et al., 2003b; Vick et al., 2006), Cushing's disease (pituitary pars intermedia dysfunction) (Garcia and Beech, 1986; McGowan et al., 2004), and some forms of laminitis (Kronfeld et al., 2006; Treiber et al., 2006c; Bailey et al., 2007). In addition, insulin resistance is thought to potentially play a role in other diseases such as hyperlipemia (Forhead, 1994), endotoxemia (Tóth et al., 2008), and osteochondritis dissecans (Henson et al., 1997; Ralston, 1996) in horses. An understanding of insulin resistance requires a brief review of the mechanism of glucose transport into the two major insulin-sensitive tissues, muscle and fat (adipose tissue). Much of this information is derived from humans and animal species other than the horse. This is followed by a review of the tests currently available to assess insulin resistance in horses.

Glucose Transport Into Muscle and Adipose Cells

INSULIN-MEDIATED GLUCOSE TRANSPORT

Ingestion of meals containing starches and blood sugar produces a rise in blood glucose which triggers the secretion of insulin by the pancreas. Insulin acts to increase glucose transport, metabolism, and storage in skeletal muscle, the largest glucose sink, followed by adipose tissue (Gould, 1993). Insulin also both inhibits glucagon secretion and lowers serum free-fatty-acid concentrations, contributing to a sharp decline in liver glucose production (Shepherd and Kahn, 1999). Prolonged elevation of blood glucose concentrations has toxic effects on cells (Yki-Jarvinen, 1992). Low blood glucose concentrations result in seizures. Therefore, blood glucose concentrations need to be maintained within narrow limits by finely tuned hormonal regulation.

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Glucose transport is the rate-controlling step in skeletal muscle glucose metabolism. The lipid bilayers in cell membranes are naturally impermeable to glucose and therefore a special transport system is required for glucose to enter cells. Facilitated diffusion down glucose-concentration gradients is mediated by transmembrane proteins, GLUT-1, 2, 3, 4, and 5, that are encoded by distinct genes (Gould, 1993). In skeletal muscle and adipocytes GLUT-1 is constantly present in muscle and fat cell membranes and provides basal levels of glucose uptake not influenced by insulin. In contrast, 90 percent of GLUT-4 is sequestered intracellularly and only translocates to the cell membrane under the influence of either insulin or exercise (Holman and Kasuga, 1997). Translocation of GLUT-4 to the cell membrane occurs via a complex process that is initiated when insulin binds to its receptor in the plasma membrane. This results in phosphorylation of the receptor and insulin-receptor substrates. These substrates form complexes with docking proteins, which eventually results in activation of phosphoinositide-3 kinase, a major pathway involved in the mediation of insulin-stimulated glucose transport and metabolism (Shepherd et al., 1998). The functionally important targets further downstream in the phosphoinositide- 3-kinase signaling cascade have not been identified, but they may be proteins that regulate the docking of GLUT-4-containing vesicles at the plasma membrane and their fusion with it (Rea and James, 1997).

NON–INSULIN-MEDIATED GLUCOSE UPTAKE

Although insulin is the chief acute stimulus for glucose uptake into cells, other stimuli, such as thyroid hormone and leptin, can also activate translocation of GLUT-4 into muscle and fat cells membranes. Exercise stimulates glucose transport by pathways that are independent of phosphoinositide-3 kinase and that may involve 5'-AMP–activated kinase (Shepherd and Kahn, 1999). Thyroid hormone increases both basal and insulin-stimulated glucose uptake into muscle and adipocytes, at least partly as a result of increases in GLUT-4 expression (Abel et al., 1996; Kahn, 1992). In horses, levothyroxine improves insulin sensitivity and decreases blood lipid concentrations (Frank et al., 2005). Leptin is secreted by adipocytes and signals the brain in response to changes in energy stores (Berti et al., 1997). Concentrations have been assessed in obese horses (Waller et al., 2006). Administration of leptin improves glucose uptake indirectly, possibly via leptin-induced increases in fatty acid oxidation (Muoio et al., 1997) or via changes in physical activity and thermogenesis mediated by the brain and sympathetic nervous system (Kamohara et al., 1997).

Mechanisms of Insulin Resistance

In horses, a number of factors such as age, breed, state of fasting, diet, access to pasture, exercise, and training all affect the degree of response of the pancreas to blood glucose concentrations and/or the rate of clearance of glucose from the bloodstream into muscle cells (Jacobs and Bolton, 1982; Garcia and Beech et al., 1986; Jeffcott and Field, 1986; June

et al., 1992; Murphy et al., 1997; Williams et al., 2001; Powell et al., 2002; Hoffman et al., 2003a; Hoffman et al., 2003b; de Graaf-Roelfsema, 2006; Pratt et al., 2006; Stewart-Hunt et al., 2006; Treiber et al., 2006b; Bailey et al., 2007). These factors are often adaptive and reversibly affect insulin sensitivity. However, at some point, many horses become resistant to the effects of insulin, resulting in chronic disease. Resistance to the stimulatory effect of insulin on glucose utilization is a key pathogenic feature of obesity, metabolic syndrome, and most human forms of type 2 diabetes (Shepherd and Kahn, 1999). It is also known to be a factor in equine metabolic syndrome (Johnson, 2002; Hoffman et al., 2003b), and Cushing's disease in horses (Garcia and Beech, 1986) as well as a potential cause of some forms of laminitis (Treiber et al., 2006a; Treiber et al., 2006c; Bailey et al., 2007). The precise mechanisms that cause insulin resistance are not known in either humans or horses. Several mechanisms detailed below may possibly be involved.

1. *Changes in GLUT-4 expression or production*. Although mutations in GLUT-4 are theoretically possible causes of insulin resistance, they have not been linked to type 2 diabetes in humans. Further, reduced expression of GLUT-4 has not been found in skeletal muscle of type 2 diabetics (Abel et al., 1996; Kahn, 1992). Thus, in humans at least, a decrease in the production of GLUT-4 does not explain the impairment of whole-body insulin sensitivity.
2. *Defects in the intracellular translocation of GLUT-4 and signaling pathways*. The reduction in insulin-stimulated glucose uptake in skeletal muscle in obese humans is associated with impairment in insulin-stimulated movement of GLUT-4 from intracellular vesicles to the plasma membrane and in reduced activation of phosphoinositide-3 kinase by insulin in muscle (Zierath et al., 1996). To date the precise part of the complex pathway regulating glucose uptake that is impaired with most cases of type 2 diabetes and obesity in humans as well as obese horses is not known.
3. *Impairment of insulin-stimulated glucose transport by circulating or paracrine factors*. The cytokine tumor necrosis factor alpha (TNF-α) has potent inhibitory effects on insulin signaling in isolated muscle and adipose tissue (Hotamisligil and Spiegelman, 1994). TNF-α is released in response to endotoxin, and in horses IV administration of endotoxin has been shown to impair insulin sensitivity for up to 24 hours (Tóth et al., 2008). Chronic elevation of serum free-fatty-acid concentrations, such as that which occurs in many humans with obesity or diabetes and horses with Cushing's or metabolic syndrome, may also contribute to the decreased uptake of glucose into peripheral tissues. In humans this has been shown to be mediated by a loss of the ability of insulin to stimulate phosphoinositide-3 kinase activity (Boden, 1997). Increased circulating cortisol (which occurs in Cushing's disease) also has a direct effect in impairing insulin sensitivity (Firshman et al., 2005; Tiley et al., 2008). Corticosteroids impair phosphorylation of insulin receptors (Coderre, 1992) and cause a large reduction in insulin-stimulated translocation of GLUT-4 (Dimitriadis, 1997).

4. *Hexosamine pathway*. Hyperglycemia itself has detrimental effects on insulin secretion and on the action of insulin in peripheral tissues (McClain and Crook, 1996). The mechanism of glucose toxicity in muscle may involve the hexosamine pathway, in which the enzyme glutamine:fructose-6-phosphate amidotransferase diverts glucose from the glycolytic pathway at the level of fructose-6-phosphate, resulting in the production of glucosamine-6-phosphate and, subsequently, other hexosamine products. Exposure of muscle to glucosamine at very high concentrations reduces stimulation by insulin of glucose transport and GLUT-4 translocation (Baron et al., 1995).

Effects of Chronic Hyperglycemia

Glucose in chronic excess causes toxic effects on structure and function of many organs, including the pancreatic islet (Yki-Jarvinen, 1992). One potential central mechanism for glucose toxicity is the formation of excess reactive oxygen species that over time cause chronic oxidative stress, defective insulin gene expression, and impaired insulin secretion (Robertson, 2004). In humans, tissues that are particularly susceptible to glucose toxicity include the pancreas, vascular epithelium, retina, and kidneys (Shepherd and Kahn, 1999). The propensity of horses with Cushing's disease and metabolic syndrome to develop laminitis and the ability to induce laminitis with prolonged supraphysiologic infusion of insulin (Asplin et al., 2007) suggest that the laminae in the hooves may have a particular sensitivity to the damaging effects of chronically elevated blood glucose or insulin concentrations. Another consequence of chronic peripheral insulin resistance in humans is damage or exhaustion of the pancreas leading to reduced secretion of insulin and exacerbation of hyperglycemia. It also has been proposed that pancreatic damage or exhaustion may develop in equine metabolic syndrome (Kronfeld et al., 2006).

Measuring Insulin Resistance in Horses

Because both the amount of insulin secreted by the pancreas in response to glucose and the insulin sensitivity of skeletal muscle and adipose tissue affect whole body insulin resistance, both need to be assessed to accurately measure insulin resistance (Firshman and Valberg, 2007). Several procedures have been developed to accomplish this task. The procedures themselves are not particularly difficult to perform. The biggest barrier to their use is the adequacy of normal ranges for each test that accounts for varying ages, breeds, fitness levels, and dietary regimes of horses.

BASAL GLUCOSE AND INSULIN MEASUREMENT

Single fasting glucose and insulin measurements have been used in the field to identify horses with suspected insulin resistance (Kronfeld, 2005b; Treiber, 2006a).

The accuracy of this simple measurement has been questioned because both insulin and glucose concentrations can vary widely in an individual in a short time period (Treiber, 2005; Treiber, 2006a). In addition, it has been suggested that in the later stages of insulin resistance in horses, pancreatic compensation becomes inadequate and hyperinsulinemia may not be detected (Kronfeld, 2006). Therefore more accurate assessments of measuring insulin sensitivity in the horse have been developed.

PROXIES AND REFERENCE QUINTILES FOR BASAL GLUCOSE AND INSULIN

Two proxies for assessing insulin sensitivity and pancreatic beta-cell response have been developed using minimal model testing of healthy horses (Treiber et al., 2005). Insulin sensitivity (SI) is estimated from the reciprocal of basal insulin concentration:

$$SI = (7.93(1/ (\sqrt{[insulin]})) - 1.03$$

The acute pancreatic ß cell response to glucose (AIR_g) is estimated from a function of basal glucose with the reciprocal of basal insulin concentration:

$$AIR_g = (70.1\ (MIRG)) - 13.8$$
$$[\text{where } MIRG = (800\text{-}0.30[insulin - 50]^2) / (glucose - 30)\ ]$$

The combined use of both proxies is suggested to distinguish normal (normal SI and AIR_g), compensatory insulin secretion in normoglycemic insulin resistance (normal or reduced SI and increased AIR_g), and compensatory failure of insulin secretion in hyperglycemic insulin resistance (reduced SI and normal to reduced AIR_g). These proxies have also been used to document insulin resistance in ponies and predict their likelihood of developing laminitis (Treiber, 2006c). Limitations of such proxies include the fact that the reference quintiles developed are not likely universal and should be established to match the type of population of horses or ponies that will be assessed for insulin resistance.

ORAL GLUCOSE TOLERANCE TEST (OGTT)

The OGTT assesses small intestinal absorption of glucose, hepatic glucose uptake, and to an extent the endocrine function of the pancreas and peripheral insulin resistance (Roberts, 1973; Breukink, 1974; Jacobs et al., 1982; June et al., 1992). The test requires an overnight fast, 1 g/kg bodyweight of glucose is administered via a nasogastric tube, and blood glucose is then measured at 0, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after administration. A peak in blood glucose level occurs 90 to 120 minutes hours after administration of the glucose, and blood glucose concentrations should

return to normal after 4 to 6 hours (Roberts, 1973; Kaneko, 1989; Ralston, 2002). An increased glucose response in comparison to normal might suggest reduced pancreatic function or insulin resistance (Ralston, 2002). However, the test is affected by the length of previous fasting, and the age and diet of horses/ponies prior to testing (Breukink, 1974; Jacobs et al., 1982; June et al., 1992; Murphy et al., 1997). In addition, the test may be affected by the stress of nasogastric intubation and variable rates of glucose administration, gastric emptying, and intestinal absorption (Kronfeld et al., 2005a).

INTRAVENOUS GLUCOSE TOLERANCE TEST (IVGTT)

Diet, disease states, and breed of horse, pony or donkey also affect the IVGTT (Argenzio et al., 1970; Garcia and Beech, 1986). However, it avoids the variable absorption of glucose by the intestinal tract inherent with the OGTT (Kronfeld et al., 2005a). Horses must be kept off feed for 12 to 24 hours after which 0.5 g glucose/kg bodyweight is infused via an intravenous catheter over about 10 minutes. Blood glucose and insulin concentrations are determined at 0, 5, 15, 30, 60, and 90 minutes and then hourly for 5 to 6 hours after injection. Measures of half-life of glucose disposal and the fractional turnover rate, which is a measure of glucose utilization and thus peripheral insulin resistance, are calculated (Kaneko, 1989). Normal horses usually show an immediate rise in blood glucose concentration and a return to normal levels within one hour. The insulin response curve should parallel the glucose response curve with a peak at around 30 minutes post injection of glucose (Garcia and Beech, 1986; Ralston, 2002). Insulin resistance would potentially produce a higher peak in blood glucose and a consistent delay in return to baseline of > 2 hours. Insulin concentrations must also be measured in order to determine whether this is due to an impairment of insulin secretion from the pancreas or from impairment of insulin-stimulated glucose disposal. If a horse has impaired pancreatic beta cell function, a delayed glucose curve might be observed and the insulin response may be blunted or delayed. However, the IVGTT is not a sensitive means to measure diminished pancreatic response (Firshman and Valberg, 2007).

INSULIN TOLERANCE TEST (ITT)

The ITT measures the blood glucose response to an injection of insulin. It is a direct measure of the sensitivity of tissues to the test dose of insulin, which is highly variable but may range from 0.2 IU/kg to 0.6 IU/kg and also assesses the response of the animal to the insulin-induced hypoglycemia. Normally, depending upon the dose of insulin used, the blood glucose levels drop to 50% of the original value within 20 to 30 minutes and return to the fasting level within 1.5 to 2 hours (Kaneko, 1989; Ribeiro et al., 2004). Typically when the ITT is performed in an animal that is resistant to insulin, blood glucose levels will not fall as dramatically and will return to normal levels more quickly compared to a normal individual. It should be emphasized, however, that the

response to this test depends on a number of factors such as, but not limited to, age, diet, stress, and others.

FREQUENTLY SAMPLED GLUCOSE INSULIN TOLERANCE TEST (FSGIT)

A combined IV glucose and insulin tolerance test has recently been developed as a means to assess both pancreatic insulin secretion as well as peripheral insulin resistance (Treiber et al., 2005; Pratt et al., 2005). Horses are not fasted, 300 mg/kg of glucose solution is administered rapidly IV, and blood is drawn via a catheter prior to administration of and at 0,1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 minutes afterward. At 20 minutes after glucose administration, a small IV dose of insulin (20 mU/kg) is given and blood is collected at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after glucose administration. Glucose and insulin dynamics are assessed via minimal model analysis. This test is designed to assess both the pancreatic response to elevated blood glucose and peripheral tissue sensitivity to insulin. Normal values for horses of similar breed, age, diet, and fitness are required for its interpretation.

HYPERGLYCEMIC AND HYPERINSULINEMIC CLAMPING

The disadvantage of the previously described tests is that endogenous insulin secretion cannot be controlled and any potential fluctuations during the test alter glucose homeostasis (Firshman and Valberg, 2007). To break the endogenous glucose-insulin negative feedback loop, two types of glucose clamp techniques have been used in horses (DeFronzo et al., 1979; Rijnen and van der Kolk, 2003; Annandale et al., 2004; Firshman et al., 2005). The hyperglycemic clamp fixes plasma glucose at an acutely elevated level for two hours, thus suppressing endogenous hepatic glucose production. The glucose infusion rate becomes a measure of pancreatic insulin secretion and therefore the technique allows quantification of the sensitivity of the pancreatic beta cells to glucose. Hyperinsulinemic euglycemic clamping provides supra-maximal steady state insulin concentrations during which the rate of glucose infusion required to maintain euglycemia during the clamp serves as a measure of the insulin sensitivity of muscle and adipose tissues. Arguments against the clamping technique have centered on the nonphysiological nature of the test and technical difficulties performing the test. However, it remains one of the most accurate means to assess sensitivity of tissues to hyperglycemia and hyperinsulinemia (Firshman and Valberg, 2007).

Conclusion

Insulin resistance is an important component of many equine diseases including Cushing's disease, metabolic syndrome and forms of laminitis. In advanced cases,

clinical signs are often sufficient to establish a diagnosis of these diseases. However, in the prodromal stages of disease, diagnosis of insulin resistance may be an aid to identifying susceptibility to these disorders and instituting early treatment. The tests described may become a useful clinical means to assess the degree of insulin resistance in horses and responses to treatments. However, it is clear that at present there is not one ideal test that is both practical and accurate. To interpret results of insulin sensitivity testing, normal values need to be established for various breeds and ages of horses as well as for varying stages of fitness and diets ranging from pasture to concentrates.

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