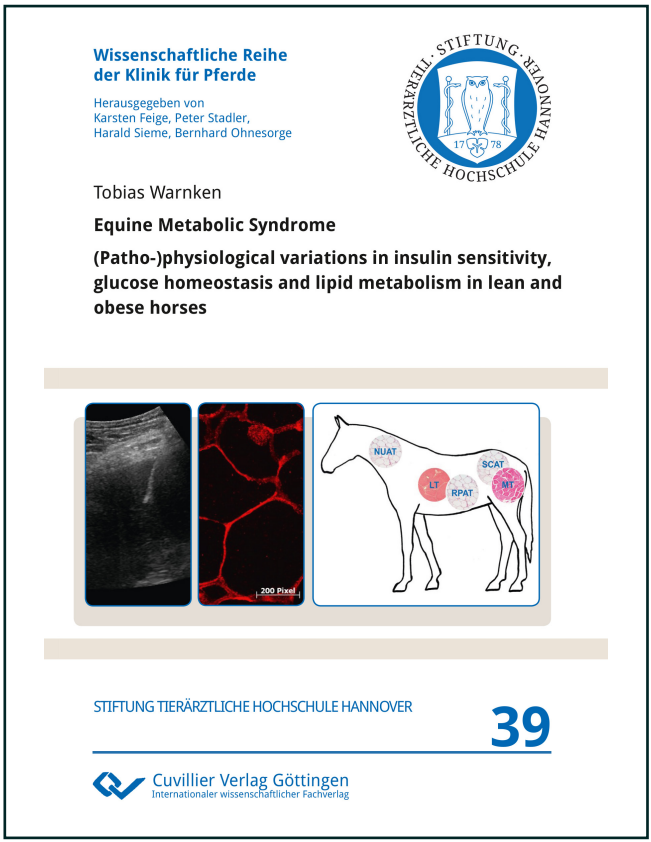
Tobias Warnken (Autor)

# Equine Metabolic Syndrome

(Patho-)physiological variations in insulin sensitivity, glucose homeostasis and lipid metabolism in lean and obese horses

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# 1 INTRODUCTION

## 1.1 The Equine Metabolic Syndrome

The Equine Metabolic Syndrome (EMS) was first introduced into veterinary medicine by Johnson (2002). The term was adopted from human medicine. The human metabolic syndrome describes a disease pattern in which obesity, diabetes type 2 and cardio-vascular diseases are the major symptoms. Similar symptoms in equids compared to the situation in human medicine had been observed (Johnson 2002). The EMS was defined by the American College of Veterinary Internal Medicine consensus statement in 2010 (Frank et al. 2010). According to this statement, affected horses show a cluster of the following signs:

a) generalized obesity or regional adiposity
b) altered insulin regulation characterized by insulin resistance (IR) represented by hyperinsulinemia (HI) or abnormal glycemic and insulinemic responses to oral or intravenous (IV) glucose and/or insulin challenges; and
c) a predisposition towards laminitis or laminitis that has developed in the absence of other recognized causes.

Additional clinical conditions must be considered in association with EMS. Hypertriglyceridemia or dyslipidemia (Frank et al. 2006, Treiber et al. 2006, Carter et al. 2009c), hyperleptinemia (Cartmill et al. 2003), arterial hypertension (Bailey et al. 2008), altered reproductive cycling in mares (Vick et al. 2006) and increased proinflammatory markers (Vick et al. 2007) have been described in horses and ponies suffering from EMS.

Despite concurrent conditions, equine laminitis is the most common result (Karikoski et al. 2011) and is a severe disease which causes an acutely painful condition of the feet, often resulting in acute and/or chronic lameness. Laminitis is defined as failure of the hoof lamellar-distal phalangeal attachment apparatus (Pollitt 2004). Although multiple inducing factors and etiologies have been identified, the exact pathogenesis is still not known in depth. However, acute and recurrent pasture-associated laminitis

is most frequently encountered and associated with HI or IR (Carter et al. 2009c, Karikoski et al. 2011, Patterson-Kane et al. 2018). In accordance with these findings, Walsh et al. (2009) demonstrated that enhanced insulin concentrations correlate with laminitis severity.

### 1.1.1 Obesity and assessment of body condition in horses

Obesity is a pathological condition associated with altered adipokine production and IR and is common in the equine population (Johnson et al. 2009). Generalized obesity in horses and ponies, which is simply defined as an expanded mass of adipose tissue (AT) in the body, is observed. Furthermore, regional adiposity occurs, which is characterized by an accumulation of fat in certain locations of the body. The body regions mainly affected are the neck, the shoulder region and the tail head (Carter et al. 2009a, Frank et al. 2010). Assessment of body condition and fat mass can be performed by multiple methods. Henneke et al. (1983) developed a body condition scoring (BCS) system with a nine-point scale, one described as “poor,” representing an emaciated horse, and nine as “extremely fat,” representing a pathologically obese horse. The score can be easily used by visual appraisal and palpation of six specific body locations, including the rib area, shoulder region, the area along the withers, the tail-head region, the neck region and along the back of the horse (Henneke et al. 1983). Despite this scoring system, six-point scores (Webb and Weaver 1979) and multiple new scoring systems or adaptations have been described to meet current demands and take into account breed-specific variations in the exterior (Kienzle and Schramme 2004, Dugdale et al. 2012). Regional adiposity can be evaluated in horses by using a five-point cresty neck scoring system (CNS) (Treiber et al. 2006).

The prevalence of obesity in horses and ponies has been investigated in multiple studies. Exemplarily, 32 out of 319 pleasure riding horses in Scotland were classified as obese with an BCS of 6/6 and 112 out of the 319 horses attracted attention with an BCS of 5/6 and were assessed as overweight (Wyse et al. 2008). Studies performed in the USA provided similar results regarding overweight and obese horses (Thatcher et al. 2008, Stewart-Hunt et al. 2010). Furthermore, Giles et al. (2014) compared the BCS in a cohort of ponies determined at the end of the summer period with the BCS

assessed at the end of winter and found out that only 28 % of the horses were obese at the end of the winter period compared to 36 % at the end of the summer period, indicating physiological variations and seasonal changes in BCS in horses (Giles et al. 2014). In addition to limited storage in all cell types, white AT is the major site for the storage of triglycerides (TRG). The AT is a central organ and key player in whole body energy regulation and is responsible for the release of free fatty acids as an energy supplier for other tissues.

### 1.1.2 Hyperinsulinemia, insulin resistance and insulin dysregulation in horses

Glucose homeostasis is closely regulated by insulin to maintain essential homeostasis of the organism. Following food intake, plasma glucose concentrations rises due to enteral absorption. Insulin is secreted by the pancreas as counter-regulatory response to promote glucose uptake by insulin-sensitive (IS) tissues. Therefore, a postprandial increase in circulating plasma insulin concentrations is essential for glucose homeostasis. However, pathophysiologically enhanced postprandial or even fasting HI can occur if insulin regulation is impaired. The HI occurs in horses in an IR state (Frank et al. 2010). In contrast to humans who develop hyperglycemia under IR conditions, horses usually maintain glucose homeostasis with normoglycemia (Divers 2008).

Insulin resistance is defined as a decreased ability of IS tissues to respond adequately to insulin (Muniyappa et al. 2008). In humans, IR is generally a reflection of mainly skeletal muscle IR, as skeletal muscle is responsible for approximately 85 % of glucose disposal in a euglycemic, hyperinsulinemic state (Defronzo et al. 1981). Increased pancreatic beta cell secretion of insulin compensates for impaired tissue sensitivity, resulting in high circulating concentrations of insulin and HI. In humans, the pancreas loses its ability to compensate as the disease processes and will secrete insufficient amounts of insulin in response to hyperglycemia, resulting in a hyperglycemic, hypoinsulinemic state, which is known as type II diabetes mellitus (Shanik et al. 2008). By contrast, horses are rarely reported to develop type II diabetes (Durham et al. 2009). The IR can be caused by several impairments (Kahn 1980). Under physiological conditions, IR can occur during gestation as gestational diabetes in humans and horses (Fowden et al. 1984, Maresh 2001). Studies in horses have shown that IR can

occur in pregnant mares up to 270 days of gestation, indicated by higher levels of insulin release in response to exogenous and endogenous glucose (Fowden et al. 1984). Multiple mechanisms leading to IR have been proposed such as fewer insulin receptors (InsRs) due to downregulation, the decreased function of receptors themselves or a breakdown of insulin-signaling mediators (Kahn 1980, Kahn and Flier 2000, Shanik et al. 2008). Despite the fact that the mechanisms are not completely known so far, alterations in post-receptor signaling are discussed most.

Generalized obesity in humans is associated with the development of IR (Kahn and Flier 2000). Similarly, equine obesity is negatively correlated with IS (Hoffman et al. 2003, Carter et al. 2009b) and associated with an increased risk of HI (Carter et al. 2009c). Experimental studies in horses and ponies have revealed a significant cross-link between equine HI and the occurrence of laminitis. Prolonged HI for 48 hours induced by hyperinsulinemic clamps resulted in laminitis in previously healthy Standardbred horses and normal ponies (Asplin et al. 2007, De Laat et al. 2010, De Laat et al. 2015). Moreover, horses presented to a first opinion hospital for evaluation of laminitis were hyperinsulinemic in 86 % of cases (Karikoski et al. 2011). A recent study showed the direct link between a pathological high insulin response assessed with a glucose challenge test and the occurrence of experimentally induced laminitis by a dietary challenge high in nonstructural carbohydrates. Ponies with insulin concentrations higher than 65 µIU/mL developed laminitis after consuming 12 g NSC/kg BW/d for a period of up to 18 days (Meier et al. 2017). This is in accordance with the identification of high basal serum insulin concentrations as a risk factor for the development of laminitis (Menzies-Gow et al. 2017).

The IR and alterations in insulin regulation in horses have been extensively studied in the last decade and provided new information on potential pathophysiological mechanisms. Recently altered insulin regulation, including tissue IR and basal or postprandial HI, have been subsumed under the term insulin dysregulation (ID) (Frank and Tadros 2014, The Equine Endocrinology Group 2016, Bertin and De Laat 2017). De Laat et al. (2016) showed that ID can occur independently of tissue IR and that IV and oral tests did not supply similar results regarding the insulinemic state of ponies. Since then, equine HI has been considered a counter-regulatory response to IR.

However, there is growing evidence in recent research to support a gastrointestinal etiology by incretin hormones released from the proximal intestine, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide, which may augment insulin secretion (De Laat et al. 2016).

### 1.1.3 Assessment of disturbances in insulin regulation in horses

Although, several studies postulated a link between BCS or CNS and basal HI or an increased risk of laminitis (Carter et al. 2009a, Carter et al. 2009c), disturbances in insulin regulation may not be identified correctly by phenotype in all cases (Firshman and Valberg 2007, Bertin and De Laat 2017). Clinical experience indicated that high BCS or CNS does not necessarily implicate disturbed insulin regulation, alternatively, low BCS does not preclude ID or IR.

Basal measures of insulin and/or glucose are often used to assess disturbed insulin regulation. Nevertheless, single measurements of both or even further calculation of indices or ratios (Treiber et al. 2005, Carter et al. 2009c) based on these measures may not be sufficient in all patients (Dunbar et al. 2016). In addition to these simple and static diagnostic procedures, dynamic stimulation tests are proposed for the assessment of IR and ID (Frank and Tadros 2014, The Equine Endocrinology Group 2016, Bertin and De Laat 2017). Research in recent years has established several testing protocols for stimulation tests based on either oral challenge tests performed by the application of sugar or glucose formulations or IV testing protocols with injections of glucose or insulin, or even both to assess disturbed insulin regulation.

The most accurate gold standard test for the assessment of tissue IS is the hyperglycemic or hyperinsulinemic clamp (Defronzo et al. 1979). Patients with normal IS require more glucose to maintain euglycemia than an individual with IR. Put simply, the amount of glucose required to maintain basal concentrations is equal to the amount of glucose taken up by the tissues and, therefore, represents a measure of peripheral tissue sensitivity to insulin. Two types of clamping procedures can be distinguished. The euglycemic hyperinsulinemic clamp (EHC) provides supra-physiological steady-state insulin concentrations, during which the rates of glucose infusion required to maintain euglycemia are used to measure the IS of muscle and AT. By contrast, the

hyperglycemic clamp fixes plasma glucose at an acutely elevated level. Consequently, endogenous hepatic glucose production is suppressed and glucose infusion rates reflect pancreatic insulin secretion, allowing quantification of the sensitivity of the pancreatic beta cells to glucose. The EHC is ideal for assessing IS, as it assesses tissue IS isolated from the impact of pancreatic insulin secretion or enteral glucose absorption on glucose homeostasis (Defronzo et al. 1979). In addition, endogenous glucose production is largely suppressed by the insulin infusion. Several studies performed in horses used EHC procedures as the gold standard to test insulin-dependent tissue sensitivity (Rijnen and Van Der Kolk 2003, Kronfeld et al. 2005, Pratt et al. 2005, Pratt-Phillips et al. 2015). However, these tests are usually reserved for research approaches due to their complex implementation. Furthermore, EHCs do not reflect disturbed insulin regulation in addition to tissue IR or impaired insulin clearance.

Oral glucose challenge tests allow the assessment of postprandial HI under standardized conditions. The oral glucose tolerance test (OGTT) was initially designed for use in horses to evaluate small intestinal malabsorption (Roberts and Hill 1973). Subsequently, the OGTT was used to evaluate glucose tolerance in equids by administration of 1 g/kg body weight (BW) of glucose (Jeffcott et al. 1986). Physiologically, a peak in blood glucose concentrations can be observed 90-120 minutes after the administration of glucose and should decline and return to normal pre-stimulation baseline concentrations within 4-6 hours (Roberts and Hill 1973). More profound or prolonged hyperglycemia is reported to be indicative of impaired pancreatic insulin secretion, decreased tissue IS or enhanced enteral absorption.

The oral testing protocol has undergone profound changes over time and several variations have been described for the indirect assessment of IR or direct assessment of ID. Protocols differ regarding the different application routes and dosages of glucose or other sugar formulations used. Most oral tests protocols are subsumed under the term OGT.

Nowadays, OGT is most often performed as an in-feed OGT, by introducing 0.5, 0.75 or 1.0 g/kg BW glucose or dextrose powder mixed in low-glycemic meal followed by blood sampling after 120 minutes (Smith et al. 2016, De Laat and Sillence 2017). The

analysis of insulin with a commercially available chemiluminescent immunoassay in samples collected allows the classification of patients as ID during standard dose in-feed OGT with 1 g/kg BW glucose when the insulin concentration is > 85 µIU/mL and > 68 µIU/mL in the case of 0.5 g/kg BW glucose used (The Equine Endocrinology Group 2016). However, incomplete ingestion and prolonged consumption times can preclude reliable results for interpretation (Kronfeld et al. 2005, De Laat and Sillence 2017). The oral sugar test (OST), using commercially available corn syrup as a glucose substrate, was established to simplify the application of glucose (Schuver et al. 2010). In order to implement the OST, 0.15 or 0.25 mL/kg BW corn syrup is administered via syringe into the oral cavity of the horse, followed by the measurement of insulin and glucose (Schuver et al. 2014, Jacob et al. 2017). Insulin concentrations of > 45 µIU/mL are generally suggested as being indicative of ID (The Equine Endocrinology Group 2016). The most invasive but most precise oral test approach is the OGT via nasogastric tubing (Ralston 2002). The substantial benefit of this protocol is the exact intragastric administration of a defined glucose dosage in a short time. Standard dose OGT is performed with 1 g/kg BW glucose dissolved in water and administered via nasogastric tubing directly into the stomach of the horse. Application is followed by analyses of glucose and insulin concentrations at specific time points, usually 120 minutes. Although this remains the most precise procedure, it requires nasogastric tubing and often raises debates about the impairment of clinically relevant test results by activation of the hypothalamic-pituitary-adrenal axis.

In addition to these oral stimulation protocols, IV challenge tests have been developed. Protocols range from a simple IV glucose tolerance test (IVGTT) (Garcia and Beech 1986, Giraudet et al. 1994) or insulin response tests (IRT) (Caltabilota et al. 2010, Bertin and Sojka-Kritchevsky 2013) to more complex combined procedures using glucose and insulin stimuli. The combined glucose-insulin test (CGIT) (Eiler et al. 2005) and the frequently sampled IV glucose tolerance test (FSIGTT) (Hoffman et al. 2003, Pratt et al. 2005, Treiber et al. 2005) can be used to assess the capacity of exogenous insulin to shift glucose into the IS tissues.

Regarding the CGIT, glucose solution is administered intravenously to the horses, followed directly by a second injection of insulin. Blood samples are taken following a

specific protocol for at least 150 minutes, including analyses of glucose and insulin. Insulin is measured in the initial sample before the glucose administration and after 45 minutes, whereas glucose is measured in all samples. Typical healthy horses show a biphasic blood glucose curve during the CGIT procedure. The first phase shows a positive hyperglycemia and the second one a negative hypoglycemia, in which glucose concentrations drop below the baseline. In IR horses, the first positive phase is prolonged with a slower return to baseline (Eiler et al. 2005). The 45-minute value is used as a clinical cut-off value to distinguish between IS and IR individuals. Horses should achieve normal glucose concentrations, return to previous baseline levels and have insulin concentration under 100 µIU/mL within 45 minutes (Frank and Tadros 2014). Horses with insulin concentration above 100 µIU/mL are considered to secrete more insulin than normal or are clearing the hormone from the circulation at a slower rate. Therefore, values above these ranges are interpreted as an indication of reduced IS (Eiler et al. 2005).

## 1.2 Glucose homeostasis and insulin action

Glucose is an important energy source for mammalian cells, and glucose homeostasis is essential for survival and metabolic health. The blood glucose in healthy mammals is derived from enteral absorption, gluconeogenesis in the liver and kidneys, and glycogenolysis in cases of hypoglycemia. Postprandial blood glucose concentration is normally regulated primarily by pancreatic insulin secretion and insulin-mediated glucose uptake by IS tissues. Insulin-independent tissues account for a lesser amount of glucose uptake. Plasma glucose concentration in horses is tightly controlled within physiological ranges. Depending on which literature is consulted, physiological ranges have been reported to be between 3.3 and 5.0 mmol/L (Ralston 2002).

Glucose absorption in the equine small intestine occurrs mainly in the proximal to mid small intestine and is directed via two types of insulin-independent glucose transporters: sodium-glucose linked transporter 1, a sodium/glucose cotransporter, on the luminal membrane and glucose transporter 2 on the basolateral membrane (Shirazi-Beechey 2008, Shirazi-Beechey et al. 2011). Both hyperinsulinemia and hyperglycemia, as postprandial consequences, suppress hepatic glucose production

by gluconeogenesis, inhibit the breakdown of glycogen to glucose in the liver, and stimulate the uptake, storage and use of glucose in tissues, such as skeletal muscle and AT, to restore normoglycemia.

High luminal glucose concentrations in the gut trigger signaling pathways in endocrine cells causing the secretion of gastrointestinal hormones, so-called incretins. Incretins are synthesized in the endocrine cells of the gastrointestinal tract and promote the release of insulin under hyperglycemic conditions (Marks et al. 1991, Shirazi-Beechey et al. 2011). The glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are the incretins most investigated in horses (Duehlmeier et al. 2001, Chameroy et al. 2010, Bamford et al. 2015, Chameroy et al. 2016, De Laat et al. 2016). While increased plasma GIP concentrations occurred in horses and ponies during an OGTT, GIP concentrations remained normal in these animals during an IV glucose tolerance testing procedure (Duehlmeier et al. 2001).

Insulin controls the activities of several metabolic enzymes by phosphorylation and dephosphorylation and regulates the expression of genes involved in gluconeogenesis and glycolysis (Pilkis and Granner 1992).

Insulin is a peptide hormone synthesized by the β cells within the islets of Langerhans of the pancreas in response to hyperglycemia. It is synthesized in the ribosomes of the rough endoplasmic reticulum (RER) as pre-pro insulin, consisting of an A-chain, a B-chain and a connecting peptide (C-peptide) (Wahren et al. 2000). Pre-pro insulin is cleaved to pro-insulin by the removal of a signal peptide. Thus, pro-insulin acquires the characteristic tertiary structure in the RER. Pro-insulin is transported to the Golgi apparatus in secretory vesicles and forms soluble pro-insulin hexamers containing zinc (Dodson and Steiner 1998). The C-peptide is removed by enzymes during secretion of the pro-insulin vesicle from the Golgi, resulting in the conversion of pro-insulin to insulin and C-peptide (Steiner 2004). Consequently, insulin forms insoluble hexamers containing zinc, precipitating as chemically stable crystals at a pH of 5.5 stored in granules.

Insulin and C-peptide are co-secreted by exocytosis of mature granules into circulation in equimolar amounts. Cell membrane depolarization and opening of voltage-

dependent calcium channels force the influx of extracellular calcium, which, in turn, triggers the exocytosis of insulin granules (Prentki and Matschinsky 1987). Insulin secretion in humans is characteristically pulsatile and biphasic. In response to stimulation, for example, by glucose, the initial rapid phase of insulin secretion is followed by a less pronounced but sustained insulin release (Bratanova-Tochkova et al. 2002). However, monophasic and biphasic secretion patterns have been observed and discussed in equids (Hoffman et al. 2003, Bamford et al. 2014, Smith et al. 2016). It has been shown in humans that GIP is suggested to primarily amplify initial insulin secretion by promoting the exocytosis of previously docked insulin granules in humans, while GLP-1 also stimulates second-phase insulin secretion in nondiabetic subjects (Schou et al. 2005).

If secreted into the blood stream, insulin binds to its receptor in IS tissues, allowing the activation of the insulin signaling cascade and glucose uptake by target tissues. Insulin-mediated glucose disposal occurs primarily in the skeletal muscle, AT and liver. Despite insulin-mediated glucose uptake, glucose can also be transported by using insulin-independent glucose transporters (GLUT). This is also essential for glucose homeostasis and the maintenance of essential organ functions.

The first pass through the liver clears approximately 50 % of the insulin in humans. Insulin in liver tissue (LT) is degraded by insulinase (Valera Mora et al. 2003), whereas insulin in systemic circulation is removed mainly by glomerular filtration in the kidney together with C-peptide (Rabkin et al. 1984).

The counter-regulatory hormones to insulin, which increase the concentration of glucose in the blood, include glucagon, epinephrine and, to a lesser extent, growth hormone and cortisol. These hormones promote glycogenolysis in terms of negative energy balance and, therefore, provide glucose as a potent energy supplier.

## 1.3 Lipid metabolism and insulin actions

Adipose tissue metabolism is essential in the regulation of energy balance and lipid utilization. Lipids can be divided, based on their chemical structure, into TRG, phospholipids, glycolipids and steroids. The TRG are the most important energy