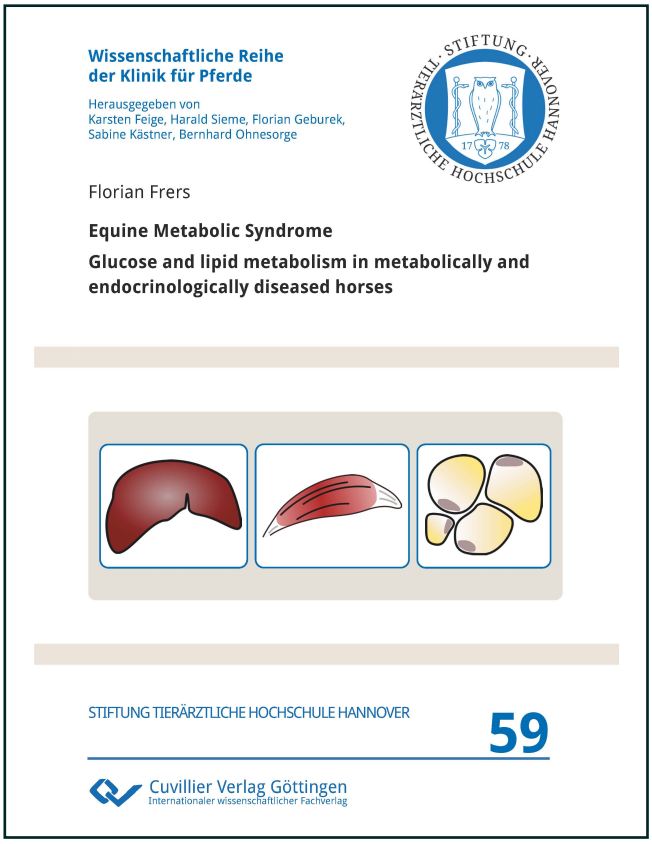
Florian Frers (Autor)

Equine Metabolic Syndrome

Glucose and lipid metabolism in metabolically and endocrinologically diseased horses



https://cuvillier.de/de/shop/publications/8831

1 INTRODUCTION

Over the past two decades, research on endocrine and metabolic disorders in equids has increased tremendously. The two main equine endocrinopathies are the equine metabolic syndrome (EMS) and the pituitary *pars intermedia* dysfunction (PPID). Central to the importance of these two endocrinopathies is the fact, that laminitis can evolve from both pathologic conditions. A link between disturbances of the insulin and glucose homeostasis and laminitis was already documented in the 1980's [1,2]. However, it took about 30 years until the key feature of EMS was recognized as insulin dysregulation (ID) [3]. Insulin dysregulation is a collective term for different imbalances of the insulin and glucose homeostasis. The result of ID is hyperinsulinemia (HI), which is strongly associated with the development of endocrinopathic laminitis [4–6]. Within the horse population, laminitis affects about 3% of all horses [7] and an underlying endocrinopathy is assumed to be the main cause in about 89% of cases [8]. As laminitis is a severe, painful and potentially life-threatening condition, veterinarians as well as horse owners are seeking guidance in diagnosing and treating affected horses. To elucidate the pathophysiological mechanisms contributing to the development of ID and laminitis, current research is based on investigating physiological and pathological modes of action of equine insulin. This research project intends to further contribute to clarify the pathogenesis and consequences of ID in horses suffering from EMS.

1.1 Insulin dysregulation and insulin resistance

Insulin is the main anabolic hormone of the body and regulates the carbohydrate, fat and protein metabolisms. Pancreatic β cells are sensitive to plasma glucose levels and secrete insulin into the blood in response to elevated glucose levels. Circulating insulin is essential to promoting glucose uptake into insulin-sensitive tissues, mainly skeletal muscle (MT), adipose (AT) and, to a lesser extent, liver tissue (LT)

In equids, ID is a key feature of EMS [3,9,10]. The term refers collectively to different aspects of disturbances in the insulin and glucose homeostasis including basal/fasted hyperinsulinemia (HI), a pathological high postprandial HI and/or peripheral insulin resistance (IR) [3]. Peripheral IR exists, whenever normal insulin concentrations

produce a less than normal biological response. One has to distinguish between a decreased sensitivity (e.g. shift in the dose-response curve to the right), a decrease in the maximum response to the hormone, and combinations of decreased sensitivity and decreased responsiveness [11]. In humans, obesity is associated with IR and in consequence with the development of type II diabetes mellitus [12]. About 400 to 500 million people live with diabetes worldwide, of which type II diabetes mellitus accounts for more than 90% of cases [13]. In horses, however, diabetes mellitus type II is rarely reported and only a few case reports exist [14,15]. Peripheral IR was long considered an important factor contributing to endocrinopathic laminitis [16–18]. But, in contrast to humans or carnivores, pancreatic secretion of insulin rarely fails during the lifetime of a horse [19]. Therefore, horses most commonly have compensated IR, a state in which the pancreas responds to IR by producing more insulin [16]. Furthermore, HI in horses may result from a decreased clearance in horses suffering from ID. In healthy horses about 70% of secreted insulin is cleared from the portal blood by the liver. In consequence, HI may result from both, increased insulin secretion and decreased hepatic insulin clearance [20]. Regardless, the consequence of any aspect of ID is HI. Since HI can induce laminitis [4,5,21], research of the past decade focused on the pathogenesis of HI and indicated a contributing role of the gastrointestinal tract (GIT).

Glucose homeostasis is maintained by a complex interaction of different hormones and organs. After carbohydrate intake, plasma glucose concentrations increase and in consequence, insulin secretion by pancreatic β cells is stimulated. Furthermore, different gastrointestinal released incretin hormones stimulate insulin secretion. Incretins are secreted by endocrine cells in the intestinal mucosa [22]. To date, three of them are described in horses, namely glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptides 1 and 2 (GLP-1, GLP-2). They indicate the existence of a functional enteroinsular axis in equids [23–26]. The fact that oral glucose administration stimulates insulin secretion to a greater extent than IV carbohydrate challenges [23,24], further indicates a contribution of incretins in the pathogenesis of ID. Insulin resistant horses have higher GIP and GLP-1 concentrations following an oral carbohydrate challenge in comparison to healthy horses [23,24]. Furthermore, basal plasma GLP-2 concentrations are higher in ID horses compared to healthy

horses [26]. However, results are inconsistent since the incretin response to grazing or a high-grain diet did not differ between healthy and ID horses [27,28].

Besides the complex interactions of glucose, insulin and other hormones described before, the gut microbiome has gained increasing attention in the development of metabolic disorders. In human medicine, the gut microbiota shows alterations regarding its composition and a shift towards increased energy harvest in obese phenotypes [29]. Alterations of the equine gastrointestinal microbiome are also present in horses. Horses suffering from EMS show a decrease in fecal microbial diversity and changes in community structure, an overall measure of the components of the microbiota, and their relative abundances [30]. In a cohort of 35 Welsh-Mountain pony mares, associations between the host phenotype (body fat percentage, markers of IS, age) and the fecal microbiome were investigated. Aged horses (> 19 years) showed increased abundances of *Proteobacteria*, while *Bacteroidetes, Firmicutes,* and *Actinobacteria* were increased in obese animals [31]. Another study investigated the effect of adding pasture to a hay diet on the microbiome in healthy and ID horses. In this study, dominant phyla in all horses were *Firmicutes* and *Bacteroidetes.* Insulin dysregulation and grazing led to a decreased evenness of bacterial populations in this study. Thus, healthy horses had greater microbial stability when challenged with a subtle dietary change [32].

Insulin dysregulation may also occur in association with systemic diseases, sepsis, pregnancy, and stress. However, this will not be described in detail in this research.

1.2 Equine Metabolic Syndrome

The term EMS has gained increasing attention since it was introduced into equine medicine in 2002. Observations, including late-onset obesity, insulin insensitivity, glucose intolerance, dyslipidemia, and insidious-onset laminitis, were combined into the term EMS in relation to the human metabolic syndrome [33]. In 2010, the term EMS was defined in more detail by the American College of Veterinary Internal Medicine [10]. The objective of this consensus statement was to define the clinical features of the EMS phenotype, possible diagnostic approaches, management options, and recommendations. Due to constant new research findings, the EMS was redefined in

a subsequent consensus statement by the European College of Equine Internal Medicine in 2019. The objective of this consensus statement was to summarize and appraise more recent scientific evidence for diagnosing and managing EMS in horses [9].

The term EMS describes a collection of risk factors for developing endocrinopathic laminitis. Horses suffering from EMS show a cluster of different clinical and laboratory abnormalities. Amongst them, generalized obesity or regional adiposity occur in many affected horses [34–36], although this feature can be inconsistent [37]. In human medicine, general obesity is defined as a pathological condition accompanied by an excessive fat deposition as compared to expected values for a given stature, sex and age [38]. Some authors even go one step further and define obesity as an independent disease [39]. In horses, general obesity is defined as increased adiposity with a negative impact on the horses' health [40]. Clinically, obesity can be assessed through various methods, mostly based on measurement of the body condition score (BCS). Different scaling systems have been established in equine medicine [41–43] with the Henneke scale being the most commonly used [44]. This score is designed as a nine-point scale. One describes very thin horses with a "poor" BCS, while nine describes heavily overweight horses as "extremely fat". A clinical definition of obesity in horses is a BCS ≥ 7 of 9 by the Henneke scale [44], although an exponential relationship between linearly-ordinal BCS points and body fat content could be demonstrated. Therefore, these findings suggest a loss of sensitivity in subjective BCS systems in overweight subjects [45,46]. Regional fat accumulation occurs particularly in the nuchal ligament region (cresty neck), behind the shoulder, around the tail head, and in the area of the prepuce and mammary gland [47].

There are multiple causes for obesity in horses. Epidemiological data related to obesity within the equine population indicate a different prevalence in various regions of the world. The prevalence of obesity ranges from 8% to 51% in different studies including numerous horse breeds from North America, Australia and Europe [48–56]. In summary, the prevalence of obesity was highest in Northern America and the United Kingdom. Amongst all breeds, Shetland ponies, different British native and Welsh breeds, Rocky Mountain Horses, Quarter Horses, Warmbloods and mix-breed horses

were more likely to be obese compared to breeds including Thoroughbreds. Despite studies on breed predisposition, to date no definite evidence of a hereditary component in the pathogenesis of EMS has been found. However, previous studies indicate that potential risk alleles, environmental risk factors, gene-by-environment, and gene-by-gene interaction may contribute to an individual risk to express the EMS phenotype [36,57,58]. Another factor playing a central role in the development of EMS is a lack of exercise, often accompanied by feeding energy-rich diets [59–61]. While obesity can *per se* lead to a systemic inflammatory response [62], the peri-renal and retroperitoneal AT may contribute to whole body ID in horses. Insulin dysregulated horses showed marked adipocyte hypertrophy and increased expression of adipokines and inflammatory cytokines in both AT depots compared to healthy horses [63].

Additionally, alterations of various metabolites can be associated with EMS. Hypertriglyceridemia, increased concentrations of non-esterified fatty acids (NEFA), low-density (LDL) and high-density lipoprotein (HDL) as well as increased cholesterol concentrations were reported in some horses suffering from EMS [64–66]. Furthermore, EMS and especially obesity are associated with hormonal disturbances including hyperleptinemia [67] and hypoadiponectinemia [68,69]. A previous study could also demonstrate that leptin is proportional while adiponectin is inversely proportional to adiposity in horses [70]. However, not all horses suffering from EMS are overweight [37] and laboratory findings are inconsistent.

1.3 Differential diagnosis: pituitary *pars intermedia* dysfunction

An age-related endocrine disorder in horses is PPID. The mean age of horses suffering from PPID is reported to be between 19 [71] and 27 years [72]. The clinical manifestation of PPID varies among horses. A common clinical sign is hirsutism, defined as an excessive hair coat length with inconsistently observed delayed shedding. Furthermore, PPID affected horses show progressive weight loss, muscle atrophy, redistribution of body fat and a pendulous abdomen [73]. Other clinical signs include polydipsia, polyuria and susceptibility to chronic infections [74]. A serious complication of PPID can be the development of endocrinopathic laminitis seen in about 30% of cases [75].

The pituitary *pars intermedia* dysfunction is proposed to be a neurodegenerative disorder of dopaminergic neurons of the *hypothalamus* induced by oxidative stress [76]. Degeneration of these neurons results in a loss of negative control of the *pars intermedia* and in an increased production of proopiomelanocortin derived peptides. As a result, circulating concentrations of adrenocorticotropic hormone (ACTH), alpha melanocyte stimulating hormone, beta endorphin and corticotrophin-like intermediate peptide all increase [77,78]. The pathophysiology of PPID is unique to horses and stands in contrast to pituitary-dependent hyperadrenocorticism in other species, since horses have elevated plasma ACTH levels without concurrent elevated plasma cortisol concentrations. Furthermore, recent research findings indicate a dysregulation of the cortisol metabolism. Horses with PPID have physiological plasma cortisol concentrations but show enhanced urinary cortisol metabolite excretion. Furthermore, a dysregulation of tissue-specific steroid-metabolizing enzymes may contribute to an increased cortisol clearance in PPID affected horses. Hence, it can be concluded that elevated ACTH concentrations result from alterations in the peripheral cortisol metabolism and the excessive activation of the hypothalamic–pituitary–adrenal axis is compensatory [79]. Additionally, glucocorticoid administration (dexamethasone) is able to induce IR in healthy horses, when administered IV for 21 days [80]. These findings fit very well with the fact that in horses with PPID HI results to a greater extent from IR in contrast to horses suffering from EMS [75]. However, the pathogenesis of ID in horses suffering from PPID remains to be further elucidated since it may be partially different in comparison to EMS-associated ID.

In summary, although the underlying pathomechanisms of EMS and PPID are different, the potential consequence of endocrinopathic laminitis is the same. Especially in older horses, EMS and PPID can coexist. Therefore, accurate diagnosis is highly important.

1.4 Endocrinopathic laminitis

Laminitis is a severe, painful and in some cases life-threatening disease of the distal limb. In general it is defined as the failure of the attachment between the distal phalanx an the inner hoof wall [81]. Various hypotheses regarding the pathogenesis of

endocrinopathic laminitis have been put forward over the past decades, but to date exact mechanisms are still unknown [82–84]. The overall frequency of laminitis varies greatly within the equine population and ranges from 1.5% to 34% [7]. Amongst horses presented with laminitis an underlying endocrinopathy was present in 89% of cases [8]. The term “endocrinopathic laminitis” refers to observations that certain individual horses appear to be predisposed to recurrent episodes of laminitis [85]. Factors including age, breed, and a reduced IS have already been associated with laminitis around the 1980s [1,2], but it took about 30 years until HI was recognized as the causing agent of endocrinopathic laminitis. Hyperinsulinemia results from ID and induces laminitis in horses [21] and ponies [4]. Furthermore, the presence and severity of laminitis is associated with hyperinsulinemia [86]. It has been shown that feeding a diet high in nonstructural carbohydrates (NSC) leads to a pathologically high postprandial HI, confirming a direct association between endogenous HI and the development of laminitis [87].

Although it is well established that HI causes laminitis, underlying pathomechanisms are still not conclusively clarified. Different theories to explain the negative effect of insulin on components of the distal phalanx exist and include vascular, cellular, lamellar and metabolite-related impairments. Prolonged infusion of insulin increases epidermal cellular proliferation while basement membrane disintegration is not observed in cases of HI induced laminitis [88]. Further lesions within the lamellar tissue in natural occurring laminitis cases with associated HI include apoptotic cell death, lamellar fusion, hyperplasia and partial replacement with aberrant keratin containing nucleated debris [89]. Moreover, in experimentally insulin-induced laminitis, secondary epidermal laminae elongation, narrowing and alterations in orientation relative to primary epidermal laminae were present [90,91]. On the other hand, neither marked pro-inflammatory cytokine expression nor increased leukocyte infiltration are seen in pasture-associated laminitis in a cohort of mixed-breed ponies [92], as typically present with sepsis-associated laminitis.

Besides a direct effect of insulin on lamellar tissue, HI induces vascular dysfunction in facial skin arteries and laminar vessels [93]. Furthermore, potential vascular impairments are associated with ID, resulting from decreased arginine and spermidine

concentrations [94]. However, *in vitro* studies on isolated digital vessels and arteries show inconsistent results [95,96].

Due to its similarity with the insulin receptor (InsR), the insulin-like growth factor-1 receptor (IGF-1-R) has been found as a potential target of circulating insulin within the equine hoof in states of HI. Although laminar endothelial cells express InsR, laminar keratinocytes do not express InsR. These findings suggest an alternative pathway of insulin signaling in laminar epithelial cells by the IGF-1-R [97,98]. Additionally, dietary carbohydrate challenges as well as insulin infusion promote upregulation of intracellular signaling downstream from the IGF-1-R and the downstream target ribosomal protein S6. This metabolite is known to cause disruption of cytoskeletal regulation, which results in a loss of laminar integrity and stability [99].

1.5 Diagnosis of insulin dysregulation

1.5.1 Measurement of equine insulin

Accurate measurement of hormones in clinical endocrinology is essential to making accurate diagnoses. However, due to very low concentrations of most hormones, specific hormonal assays have to be highly sensitive to produce reliable results [100]. Equine insulin is a peptide hormone, partially different from human insulin [101,102]. Since quantification of equine insulin is mostly done using assays designed for diagnostic purposes in human medicine, results have to be interpreted carefully. Numerous studies have evaluated different commercially available immunoassays and tested their agreement regarding the insulin concentrations obtained. In short, agreement between different assays is highly variable and especially high insulin concentrations have to be interpreted with caution [103–108]. This is important, since different cut-off values and reference ranges are published to define ID in horses. Therefore, the diagnosis of ID and IR should always be based on the horses' history as well as clinical and laboratory findings [109].

1.5.2 Basal testing protocols

Fasting HI is one aspect of ID in horses. Since ID is a complex composition of different disturbances of the insulin homeostasis, it may not be fully represented by measuring basal insulin and glucose concentrations [3,110,111]. However, several studies investigated basal insulin measurement to predict ID and the risk of laminitis in horses [36,40,112]. Assay-dependent insulin concentrations below 20 µIU/mL are considered physiologic, while insulin concentrations between 20 and 50 µIU/mL are considered ID suspicious and higher than 50 µIU/mL indicate ID [113]. It should be emphasized that both, fasting and feeding grain, is no longer recommended before assessment of basal insulin concentrations as it may influence IS [114]. Other metabolites, including glucose, triglycerides (TRG), adipokines and incretins, are less reliable biomarkers and are currently not recommended to safely diagnose ID in equids [115].

1.5.3 Intravenous testing protocols for assessment of insulin resistance

In relation to human medicine, where IR is a central hub of diabetes and associated with obesity and the metabolic syndrome, various intravenous (IV) tests have been adopted to equine medicine. These tests differ considerably in their complexity and some are primarily designed for research purposes. With all these tests either insulin, glucose, or both are administered. Amongst them, the euglycemic-hyperinsulinemic clamp (EHC) [116] and the frequently sampled intravenous glucose tolerance test (FSIGTT) [34] are appropriate and reproducible methods for assessment of IS in horses [117]. However, both tests are time-consuming, complex, and expensive to perform. Hence, modified tests have been established for the use in clinical settings.

The combined glucose-insulin test (CGIT) is a simplified method to assess IS as, in contrast to a constant rate infusion (EHC) or frequently administered insulin and glucose boluses (FSIGTT), only a single, parallel injection of insulin and glucose is necessary. For implementation of the CGIT, 150 mg/kg BW glucose IV, followed by 0.1 IU/kg BW regular insulin IV are administered. Basal concentrations of glucose and insulin as well as their concentrations after 45 and 75 minutes are measured. Decreased IS can be assumed if insulin concentrations are higher than 20 µIU/mL at

baseline and 75 minutes and exceed 100 µIU/mL at 45 minutes. Furthermore, healthy horses show a biphasic glucose curve (hyperglycemia followed by hypoglycemia) and glucose concentrations should return to baseline values after 45 minutes. Less insulin-sensitive horses show alterations of the glucose curve and a delayed return to baseline values [118,119].

A direct assessment of the insulin-dependent glucose uptake and thus IS can be made by an insulin response test (IRT). Two different protocols for the implementation of an IRT under clinical conditions are available. For the complete IRT, 0.1 IU/kg BW regular human recombinant insulin is injected rapidly IV and blood samples have to be collected at nine different time-points up to three hours after injection of insulin. A horse is considered insulin-sensitive, if plasma glucose concentrations decrease ≥ 50% during this time in comparison to baseline values [120].

In a shortened protocol, the 2-step IRT, 0.1 IU/kg BW regular human recombinant insulin is administered IV and plasma glucose concentrations are determined at baseline and 30 minutes after IV insulin injection. Afterward, 150 mg/kg glucose solution is injected IV to avoid hypoglycemia. Insulin-sensitive horses show a plasma glucose reduction of ≥ 50% after 30 minutes [121].

1.5.4 Oral testing protocols

A pathological high postprandial HI following carbohydrate intake is considered the most consistent feature of ID. Since the GIT seems to play a major role in the development of ID, dynamic oral challenge tests mimic the relationship of glucose and insulin dynamics following food intake more closely and thus, are recommended to diagnose ID [9].

A variety of protocols, including different sources of carbohydrates, is available to test for ID. Initially, the oral glucose tolerance test (OGTT) was designed to assess the glucose tolerance and IS of ponies in comparison to Standardbed horses. After administration of 1 g/kg BW glucose, plasma glucose levels peaked at 90 to 120 minutes after glucose administration and returned to baseline values within four to six hours in insulin-sensitive horses. Prolonged hyperglycemia was considered to be