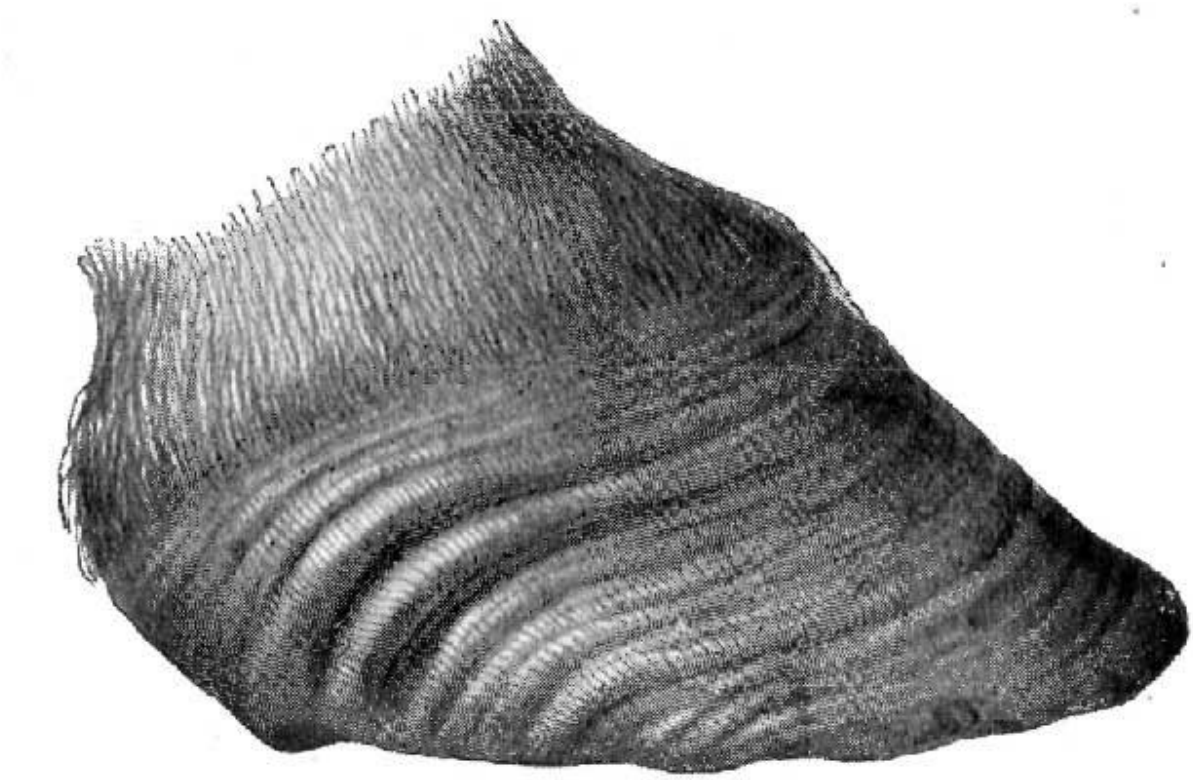
Insulin Resistance and Laminitis in Horses



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Tiivistelmä - Referat – Abstract

Laminitis is a common painful condition in horses that often has a poor outcome. The aetiology of laminitis has been widely studied, but still not completely understood. There is some evidence that pasture associated laminitis is one of the most common forms of laminitis , and this is most likely associated with underlying endocrine dysfunction i.e. insulin resistance. However, what the prevalence of insulin resistance in horses presented with laminitis is, that is currently unknown and it has not been studied previously.

Endocrinopathic laminitis is a term including laminitis developing subsequently to Equine Cushing's Disease, equine metabolic syndrome and iatrogenic corticosteroid-induced laminitis. The term equine metabolic syndrome includes a history of laminitis, insulin resistance and a characteristic phenotype of a cresty neck, bulging supraorbital fat and increased fat deposits. Horses suffering from laminitis and insulin resistance seem to have a slow recovery process, but recovery is more likely if insulin sensitivity is improved. Increased exercise, maintaining optimal body condition, avoidance of high-glycaemic meals and molasses should be incorporated in the treatment of laminitis. Horses with insulin resistance have a higher risk of developing laminitis and to find and treat horses with insulin resistance would be a means of preventing laminitis.

The purpose of the study was to determine the prevalence of insulin resistance in laminitic horses and ponies in Helsinki University Equine Clinic between April 2007 and October 2008. Associations between endocrinopathic laminitis and sex, age, body condition score, breed, season, Obel grade, the presence of laminitic rings, cresty neck, and bulging supraorbital fat were studied in 50 horses. The associations of the same variables with insulin resistance were similarly studied in the 37 laminitic horses. The hypothesis was that a high degree of laminitic horses would be insulin resistant and that age, body condition score, breed, season, Obel grade, the presence of laminitic rings, cresty neck, and bulging supraorbital fat would be associated with endocrinopathic laminitis. The body condition of the horses was scored using the 0-5 graded Carroll & Huntington body condition scoring. Insulin resistance was defined as a basal insulin level of over 30 µIU/ml.

The prevalence of insulin resistance in these 37 laminitic horses/ponies was 84% (95% CI 69-92%). Bulging supraorbital fat, laminitic rings and severe lameness was significantly correlated with endocrinopathic laminitis. The mean age of horses with endocrinopathic laminitis was significantly higher than the mean age of horses without endocrinopathic laminitis. Laminitic rings were statistically more frequent in horses with insulin resistance and laminitis than horses with laminitis and normal insulin level.

The result implies that a high proportion of the horses admitted to the clinic because of laminitis also have insulin resistance. The study results justifies including the presence of laminitic rings and bulging supraorbital fat as a phenotypic indicators of endocrinopathic laminitis and the equine metabolic syndrome. In this study only basal insulin was measured of the horses and it is not the most accurate method of diagnosing insulin resistance. This means that some cases of insulin resistance could have been missed. The lack of controls has propably also affected the results.

Avainsanat – Nyckelord – Keywords

Insulin resistance, laminitis, endocrinopathic laminitis

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Abbreviations

ACTH = Adrenocorticotropic hormone

BCS = Body condition score

ECD = Equine Cushing's disease

EMS = Equine metabolic syndrome

FSIGT = Insulin-modified frequently sampled intravenous glucose tolerance test

GLUT1 = Glucose transporter 1

GLUT4 = Glucose transporter 4

IL-1 = Interleukin-1

IL-6 = Interleukin-6

ITT = Insulin tolerance test

MIRG = The modified insulin response to glucose

OGTT = Oral glucose tolerance test

PPID = Pituitary pars intermedia dysfunction

RISQI = The reciprocal of the insulin square root index

TNF-α = Tumour necrosis factor α

μIU = micro international unit

1 LITERATURE REVIEW

1.1 Structure and effect of insulin

Insulin is a peptide hormone produced by the pancreatic β-cells as a preprohormone, which is converted to proinsulin. Proinsulin is converted to biologically active insulin immediately before secretion. Insulin release is dose dependent on glucose ingestion (Waldhausl, 1989). Increase in serum concentrations of amino acids also stimulates insulin secretion. Hormonal regulation of β-cells is mediated by glucagon stimulating the β-cells and somatostatin inhibiting the β-cells. The nervous system also regulates insulin secretion: the sympathetic system inhibits it and the parasympathetic system stimulates it. Glucocorticoids have insulin-antagonist effects and catecholamines inhibit glucose-stimulated insulin secretion (Masuzaki et al. 2001).

The primary action of insulin is to stimulate cell uptake and metabolism of nutrients, mainly glucose. Insulin binds to cellular membrane receptors that act as a tyrasine kinases and consequently phosphorylation of intracellular proteins occurs (Saltiel & Kahn, 2001). For the regulation of glucose transport into the cell, insulin recruits glucose transport molecules 4 (GLUT4) to the cell membrane. Insulin stimulates glucose uptake in nearly all tissues, but particularly in skeletal muscles and adipose tissue (Saltiel & Kahn, 2001). Glucose uptake is via glucose transport molecules (GLUT1) is insulin independent (Saltiel & Kahn, 2001). The brain, the liver, the intestinal, kidney and mammary epithelial cells are to a great extent independent of insulin for their glucose requirements (Sjaastad et al.). Insulin also regulates fat metabolism, vascular function, inflammation, tissue remodelling and the somatotropic axis of growth. Insulin inhibits the production and release of glucose from the liver (Saltiel & Kahn, 2001).

A circadian rhythm of insulin secretion has been demonstrated in normal horses (Evans et al. 1974). Insulin is influenced by exercise, decreasing after exercise and then returning to

increased concentrations after 30 minutes (Freestone et al. 1991). These fluctuations are however always within the normal range in a normal, healthy individual.

1.2 Insulin resistance

Insulin resistance is the inability of normal insulin concentrations to elicit a normal physiological response. The tissues responsiveness to insulin is decreased and the horse has to rely on the glucose-mediated disposal of glucose that happens independently of insulin (Hoffman et al. 2003). The reason for insulin resistance can be at the level of the insulin receptor or it can be subsequent to insulin binding to the insulin receptor (a post receptor effect). The insulin receptor can be down regulated as a consequence of long lasting hyperglycaemia and hyperlipaemia. Inadequate response to hormonal signalling despite normal insulin binding to the receptor can be caused by disruption of the glucose uptake pathways (Treiber, Kronfeld and Geor 2006, Kronfeld et al. 2005). Most horses are able to compensate for the insulin resistance in tissues by increased release of insulin from the pancreatic β-cells and the blood glucose remains within normal limits. For biologic action to occur a number of receptors have to be occupied and if most receptors are non-functional the body responds by increasing insulin release. With hyperinsulinaemia insulin action in certain resistant pathways is decreased, while in other unaffected pathways insulin signalling is increased. Secondary failure to secrete insulin, with pancreatic exhaustion following decreasing tissue insulin resistance, rarely occurs in horses. This kind of uncompensated insulin resistance has been demonstrated in a pony using the frequent sampling intravenous glucose tolerance test (Hess et al. 2006).

1.2.1 Factors influencing insulin sensitivity

Factors influencing insulin sensitivity include age, breed, pregnancy, lactation, inflammation, obesity, fasting, starch versus fat content of concentrates, training,

supplements and drugs, such as chromium, metformin, levothyroxine, corticosteroids and xylazine (Firshmann & Valberg, 2007).

Fetal foals have comparatively low levels of serum insulin and maturation of pancreatic β-cell function occurs over time. The rate of acquired insulin resistance with increasing age in mature horses is dependent upon environment, diet and genetic factors (Firshmann & Valberg, 2007, Murphy et al. 1997).

Temporary physiologic insulin resistance can occur due to inflammatory conditions, infections, injury, starvation, pregnancy and puberty (Firshmann & Valberg, 2007, Fowdean et al. 1984). Insulin resistance is also thought to be involved in pathologic conditions, such as obesity, uraemia, cortisol excess or growth hormone excess (Firshmann & Valberg, 2007). Insulin resistance has been associated with laminitis and obesity (Johnson & Ganjam, 1999, Hoffman et al. 2003). It has been shown to be a risk factor for laminitis and obesity has been shown to be a risk factor for insulin resistance (Johnson & Ganjam, 1999, Hoffman et al. 2003). In the pre-pubertal patient, insulin resistance has been associated with osteochondritis dissecans lesions (Ralston, 1995). It is also thought to be involved in the pathogenesis of pars intermedia dysfunction (Frank et al. 2006), hyperlipaemia, and equine metabolic syndrome (Johnson, 2002). Exertional rhabdomyelisis on the contrary is associated with increased insulin sensitivity (Annandale et al. 2004).

Glucocorticoids oppose insulin actions by promoting gluconeogenesis in the liver and inhibit glucose uptake in the peripheral tissues. A seven day dexamethasone administration showed evidence of insulin resistance and increased pancreatic insulin response compared to a control group (Tiley et al. 2007). Xylazine (α-adrenoreceptor antagonist) inhibits insulin release from pancreatic β-cells and results in hyperglycaemia, which has to be taken into account when evaluating blood samples taken from horses sedated with xylazine (Greene et al. 1987).

1.2.2 Diagnosis of insulin resistance

Current techniques to diagnose insulin intolerance in horses include the basal glucose and insulin measurements, oral glucose tolerance test (OGTT), insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT), insulin tolerance test (ITT), hyperglycaemic and the euglycaemic clamp techniques and the minimal model of glucose and insulin dynamics (Pratt et al. 2005). The hyperglycaemic and euglycaemic clamp techniques were developed for diagnosing insulin resistance in humans, but they have been shown to be useful in horses (Rijnen et al. 2003).

The hyperglycaemic/euglycaemic clamp techniques and the insulin-modified frequently sampled intravenous glucose tolerance test with minimal model analysis are the most accurate methods. Proxy measurements (the reciprocal of the insulin square root index RISQI, the modified insulin response to glucose MIRG) calculated from serum basal insulin have been shown to be predictive (Kronfeld et al. 2005, Kronfeld 2006). RISQI is a proxy used for calculating insulin sensitivity (the capacity of insulin to cause glucose disposal) and MIRG is a proxy used to calculate the pancreatic β-cell response. Glucose effectiveness (the capacity of glucose to mediate its own disposal independent of plasma insulin) can also be calculated.

Basal values of glucose and insulin represent an end-point reached after considerable regulation, but they provide an example of the current chronic state of the patient. There are also potentially large fluctuations in a 24 hour period of the basal insulin level in horses and a single measurement of the patient's basal insulin may not be representative (Evans et al. 1974). They are, however, the most practical test for insulin resistance and insulin response for screening purposes (Kronfeld et al. 2005, Treiber, Kronfeld and Geor 2006, Walsh et al. 2008). Geor and Harris (2009) suggested the following sampling protocol for measurement of the basal insulin: all feed should be withheld for a minimum of 8 hours before sampling and blood should be drawn between 7:00 and 10:00 am. Removal from pasture before sampling is recommended. In laminitic horses, testing should be delayed until after resolution of the acute laminitic episode, because the associated pain and stress can exacerbate hyperinsulinemia (Geor and Harris, 2009).

1.2.3 Pathogenesis of insulin resistance

The equine metabolic syndrome, including insulin resistance and laminitis, has been studied in a closed herd of ponies and the syndrome seems to be at least partly hereditary (Treiber, Kronfeld, Hess et al. 2006). In natural conditions with unreliable food supply, insulin resistance in the muscle would have been an advantage for survival. Insulin resistance could be a metabolic adaptation in these certain breeds (Treiber, Kronfeld, Hess et al. 2006). Beside pony breeds Morgan horses, domesticated Spanish mustangs, European Warmbloods and American Saddlebreds have been implicated to have a higher risk for developing insulin resistance (Johnson et al. 2004).

During pregnancy mares exhibit higher levels of insulin after exogenous and endogenous glucose increase, because of an enhanced β-cell response to glucose. Glucose is not taken up by maternal tissues, in which the insulin sensitivity is reduced, but redirected to nourish the fetus. After 270 days of gestation there's a fall in the insulin concentration, most likely due to exhaustion of the β-cells (Fowdean et al. 1984).

Obesity, inflammatory cytokines and insulin resistance are interrelated (Vick et al. 2007). Insulin sensitivity was reduced in obese compared to non obese geldings, with insulin sensitivity estimated with a minimal model analysis (Hoffmann et al. 2003). In humans and rats obesity resembles an inflammatory state, with increased acute phase proteins and inflammatory cytokines (Tilg and Moschen, 2008). Adipocytes are capable of secreting endocrine signals that cause insulin resistance. Leptin acts as an endocrine signalling molecule affecting hunger, it is secreted by adipose tissue and its concentrations are significantly higher in ponies with insulin resistance (Van Weyenberg et al, 2007). Omental adipocytes also contain an enzyme (11-β hydroxysteroid dehydrogenase) that converts inactive cortisone to active cortisol (Masuzaki et al. 2001). Cortisol opposes insulin activity in carbohydrate metabolism, causing decreased glucose uptake in peripheral tissues and increased hepatic glucose production (Masuzaki et al. 2001). Weight loss by energy restriction on the other hand improved insulin sensitivity in obese

ponies (Van Weyenberg et al, 2008). Insulin resistance developed after adult mares were subjected to a systemic inflammation induced by lipopolysaccharide. During this induced inflammatory state the adipose tissue responded with an upregulation in mRNA expression of the inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α) (Vick et al. 2008).

Even relatively short periods of fasting have caused insulin resistance in horses (Treiber et al. 2005, Hoffmann et al. 2003). The composition of the diet also affect the insulin sensitivity in horses, as horses fed meals high in starch and sugar have an increased risk of developing insulin resistance compared to horses fed meals with a higher content of fat and fibre. In ponies predisposed to laminitis insulin concentrations increased significantly after addition of fructan carbohydrates to the diet (Bailey et al. 2007). Fructan carbohydrates are storage carbohydrates found in some grasses and have been implicated in the pathogenesis of laminitis. The study indicates that fructans could be a factor in the development of insulin resistance in laminitis prone ponies at pasture (Bailey et al. 2007). Increased plasma triglyceride concentrations in nonobese laminitis-prone ponies, may indicate an underlying dyslipidemia associated with insulin resistance (Bailey et al. 2008).

1.2.4 Treatment of insulin resistance

Methods to improve insulin sensitivity in horses include increased exercise, maintaining optimal body condition and avoidance of high-glycaemic meals and molasses.
Agents that counter insulin resistance, for example cinnamon, thiazolidinediones or biguanides, may improve peripheral glucose uptake (Harris et al. 2006). These agents need to be examined for effectiveness in a clinical situation (Harris et al. 2006). Levothyroxine decreases blood lipid concentrations and body weight, and improves insulin sensitivity (Ralston, 2002). Metformin is a biguanide drug used in human medicine to increase insulin sensitivity. Metformin improved insulin sensitivity and decreased pancreatic β-cell secretion of insulin significantly in a study of 18 horses,

although the short-term response (6-14 days) was more favourable than the long-term (23-220 days) (Durham et al. 2008).

Exercise increases glucose uptake in skeletal muscle and thus increases insulin sensitivity as skeletal muscle is the principal site for insulin mediated glucose disposal and therefore also the major site of peripheral insulin resistance. Hyperinsulinaemic obese ponies had improved insulin sensitivity after 2 weeks of increased training (Ralston, 2002). In a study by Pratt et al. (2006) physical conditioning lessened the decrease in insulin sensitivity caused by a diet high in non structural carbohydrates in normal Standard bred horses.

1.3 Laminitis

Laminitis is a common, painful condition in horses. It is broadly defined as an inflammation of the laminae leading to a degeneration of the primary and secondary laminae of the equine hoof. As a consequence the space between the hoof wall and the coffin bone widens which results in a chronic instability of the hoof.

Laminitis can be categorized as acute, subacute or chronic. In acute laminitis there is an acute onset of lameness in one or more limbs, an increased digital pulse and an increase in the temperature of the hoof occurring for less than 72 hours. The pain causes a characteristic wide base stance, inability to lift the hooves and in the worst cases, inability to stand. In acute laminitis the distal phalanx is not displaced. In subacute laminitis the horse shows the same clinical signs as in acute laminitis, except the duration is longer than 72 hours. Horses with chronic laminitis show the signs of chronic pain and lameness and the hoof walls may exhibit laminitic rings. Radiographically the changes in chronic laminitis can be seen as rotation and sinking of the coffin bone, a point at the tip of the coffin bone and a darkened line between the hoof wall and the coffin bone.

The lameness in laminitis can be graded using the Obel grading system (Obel, 1948). Obel Grade 1 is when a horse shows frequent shifting of weight between the feet, but no visible lameness at the walk, and bilateral lameness when trotting. Obel Grade 2 is when a horse does not resist having a foreleg lifted, nor to walk, but it does show lameness at the walk. Obel Grade 3 horses object to the foreleg being lifted, and are reluctant to walk. An Obel Grade 4 horse will only walk if it is forced to.

Age, sex, breed, seasonality, obesity, insulin resistance, gut disturbance and endotoxaemia are known risk factors for developing laminitis **(**Polzer & Slater, 1996, Alford et al. 2001, Snook Parsons et al. 2007). Age, breed and seasonality were not shown to be statistically significant risk factors for acute laminitis, but age and seasonality were statistically significant risk factors for chronic laminitis (Polzer & Slater, 1996). Obesity and a cresty neck seem to be a risk factor for both acute and chronic laminitis. Mares had a higher risk to develop laminitis than geldings and ponies are generally more prone to develop laminitis than horses (Alford et al. 2001).

The aetiology of equine laminitis is still unknown. Most research has focused on inflammatory laminitis associated with severe systemic inflammation secondary to gastrointestinal disease, especially the carbohydrate overload model, oligofructose overload model and the Black Walnut toxicity model. Overfeeding of cereals (Obel, 1948) lush pasture, bolus feeding of oligofructose (French & Pollitt, 2004) and black walnut extract (Eaton et al. 1995) can reproducibly cause laminitis. Theories on the pathogenic/aetiologic mechanisms of these forms of laminitis include: 1) disruption in the vascularization due to increased venous resistance leading to a period of ischaemia, followed by reperfusion injury, 2) haematogenous spread of inflammatory, toxic, metabolic or enzymatic trigger factors or 3) trauma to vascular endothelium or peripheral nerves resulting in blood pooling in the hoof. Lately, studies have focused on finding trigger factors involved in inflammatory states, which cause vasoconstriction and decreased vascularization, thus combining the theories of disruption of vascularization and inflammatory trigger factors. Trigger factors studied include matrix metalloproteinase enzymes, nitric oxide, 5-hydroxy-tryptamine, cyclo-oxygenase and interleukin (Bailey et al. 2004).

There has been less research on the pathogenesis of endocrine laminitis. However, the general mechanisms explored included glucose deprivation and hyperinsulinaemia. Hoof tissue has a high demand of glucose and it uses glucose at much faster rate than other tissues (Wattle & Pollitt, 2004). Glucose deprivation was shown in vitro to result in separation of connective tissue between coffin bone and hoof (Pass et al. 1998).

1.4 Relationship between insulin resistance and laminitis

Insulin resistance was suggested to play a role in the pathogenesis of laminitis for the first time in the 1980's. A relative glucose intolerance and resistance to exogenous insulin was found in ponies, with a history of laminitis, compared to ponies with no history of laminitis (Jeffcott et al. 1986, Coffman & Colles, 1983). In 1985 Jeffcott and Field demonstrated diminished oral glucose tolerance and intravenous insulin tolerance in obese and laminitic ponies compared to normal Standardbred horses. Their theory was that the ponies had a clinical condition with stress and insulin intolerance associated hyperlipaemia and laminitis. This would be due to an innate peripheral insensitivity to insulin in ponies, causing poorly regulated mobilization of fatty acids (Field & Jeffcott, 1989). This metabolic state may be manifested seasonally, possibly linked to the nutrients in pastures (Bailey et al. 2008). Plasma insulin concentrations were significantly correlated to laminitis grade and a decrease in plasma insulin concentration was significantly correlated to a decrease in laminitis grade in a study by Walsh et al. (Walsh et al. 2009).

The term equine metabolic syndrome (EMS, insulin resistance syndrome) was first used by Johnson in 2002, before that the syndrome had been described by Johnson and Ganjam in 1999 and named a peripheral cushingoid syndrome. The syndrome includes a history of laminitis, insulin resistance and a characteristic phenotype of a cresty neck, bulging supraorbital fat and increased fat deposits in the withers and the dorsal back (Johnson,

2002). Especially a cresty neck score and a high body condition score serve as phenotypic indicators associated with insulin resistance (Walsh et al. 2008, Frank et al. 2006).

There are three main theories of the mechanisms by which insulin resistance would cause laminitis. The first theory is that the connection lies in impairment of glucose uptake, the second in vascular effects caused in the hoof and the third in pro-inflammatory effects caused by inflammatory agents **(**Bailey et al. 2008, Vick et al. 2008)**.**

Impaired glucose uptake by lamellar epithelial cells, the first theory, has been suggested as a possible link between insulin resistance and laminitis (Bailey et al. 2008). As mentioned before, the high demand of glucose and fast rate of glucose use of the hoof tissue, because of the lack of tissue with glycogen storage capacity in the hoof, could implicate that glucose deprivation could be a factor in the laminitis aetiology (Wattle & Pollitt, 2004). When hoof tissue was subjected to glucose deprivation in vitro, it resulted in separation of connective tissue between coffin bone and hoof (Pass et al.1998). However, in a later study glucose uptake to hoof lamellae were shown in vitro to happen via glucose transporter molecules 1 (GLUT1) that are independent of insulin and not via insulin dependent glucose transporter molecules 4 (GLUT4) (Asplin, McGowan et al. 2007, Wattle & Pollitt, 2004).

Endothelial dysfunction, the second theory, may be a cause for the increased risk of developing laminitis in insulin resistant ponies, as endothelial dysfunction would cause vasoconstriction and hypertension. Ponies with recurrent laminitis have previously been found to be hypertensive compared to control ponies (Bailey et al. 2008). Glucocorticoids and catecholamines are elevated during the endotoxaemic phase of the carbohydrate overload. Glucocorticoids may potentate the vasoconstrictive action of catecholamine and cause laminitis by reducing blood flow through lamellar connective tissues (Johnson & Ganjam, 1999).

Obesity, inflammatory cytokines and insulin sensitivity has been shown to be interrelated and this could be the third possible connection between laminitis and insulin resistance. Obesity in association with tissue resistance to insulin could cause an inflammatory state

in the patient. This inflammatory state, with the release of inflammatory cytokines, could predispose to laminitis. The inflammatory cytokines TNF-α, TNF-α-protein, IL-1 and IL-6 have been found in increased numbers in obese ponies (Vick et al. 2007, Vick et al. 2008).

When prolonged hyperinsulinaemia was caused in clinically normal ponies they all developed laminitis in all four hooves (Asplin, Sillence et al. 2007). As none of the ponies had insulin resistance and their blood glucose was maintained within normal values, the conclusion was that prolonged hyperinsulinaemia induces laminitis independent of changes in blood glucose concentration or insulin sensitivity.

Equine Cushing's disease (ECD, Pituitary pars intermedia dysfunction) is a disease caused by loss of tonic inhibition by dopamine of the pars intermedia of the pituitary gland (Schott, 2002). This causes a markedly increased adrenocorticotropic hormone-level (ACTH) and the clinical signs are mainly attributed to its effects. The clinical signs are changes in fat distribution (pot belly, muscle wasting and bulging supraorbital fat), polyuria and polydipsia, susceptibility to infections and laminitis (Schott, 2002). Equine Cushing's disease is frequently associated with both insulin resistance and laminitis (Keen et al. 2004). Insulin resistance could be a cause of increased levels of glucocorticoids in ECD, but it is also likely that many horses are genetically affected with insulin resistance and later develop Equine Cushing's disease (Johnson, 2002). Although there is a clear relationship between EMS and ECD, they are two different syndromes as EMS horses have been tested negative for ECD (Reeves et al. 2001). Measuring the basal insulin value in diagnosed ECD cases has been proved to be of prognostic value. Horses with a marked hyperinsulinaemia have a poorer prognosis than those with a normal or mildly increased basal insulin value (McGowan et al. 2004). Basal serum insulin has also been used as a diagnostic indicator of ECD, but research has indicated that it is not a specific indicator of the disease (Reeves et al. 2001). Laminitis occurring subsequently to changes in the hormonal status of the horse has been defined as endocrinopathic laminitis (Johnson, 2004). This includes laminitis developing subsequently to ECD, EMS and iatrogenic corticosteroid-induced laminitis. Insulin has been shown to be the final triggering event in the development of endocrinopathic laminitis (Asplin, Sillence et al.

2007). The induction of laminitis in this study happened independently of diet and gastrointestinal disturbances. In the study the horses were subjected to high levels of insulin, so whether long-term exposure to lower insulin levels would cause the same effect still needs to be established.

The significance of the connection between laminitis and insulin resistance is the possibility of determining the risk of laminitis in the patient and reducing this risk. Endocrinopathic laminitis seems to have a slow recovery process and an ideal clinical outcome is more likely if hyperinsulinaemia is reversed early in the process (Walsh et al. 2008). Baseline concentration of plasma insulin is a useful tool for predicting the risk of laminitis to occur and also for predicting prognosis of laminitis. Plasma leptin concentrations could also be useful for predicting total fat mass and diagnosing insulin resistance in ponies, because of the high correlation with insulin resistance (Van Weyenberg et al. 2007).

Factors influencing insulin resistance and the connections between insulin resistance, laminitis and ECD are collected into figure 1.

Methods to improve insulin sensitivity in horses, such as increased exercise, maintaining optimal body condition, weight loss in obese horses, avoidance of high-glycaemic meals and molasses could also reduce the risk of laminitis and the clinical signs of laminitis. The diet should be based on mature grass hay, with supplements of vitamins and minerals. Grains and sweet feeds as well as pasture should be avoided. (Geor and Harris, 2009). Agents that counteract insulin resistance could also become a part of the treatment of laminitis.

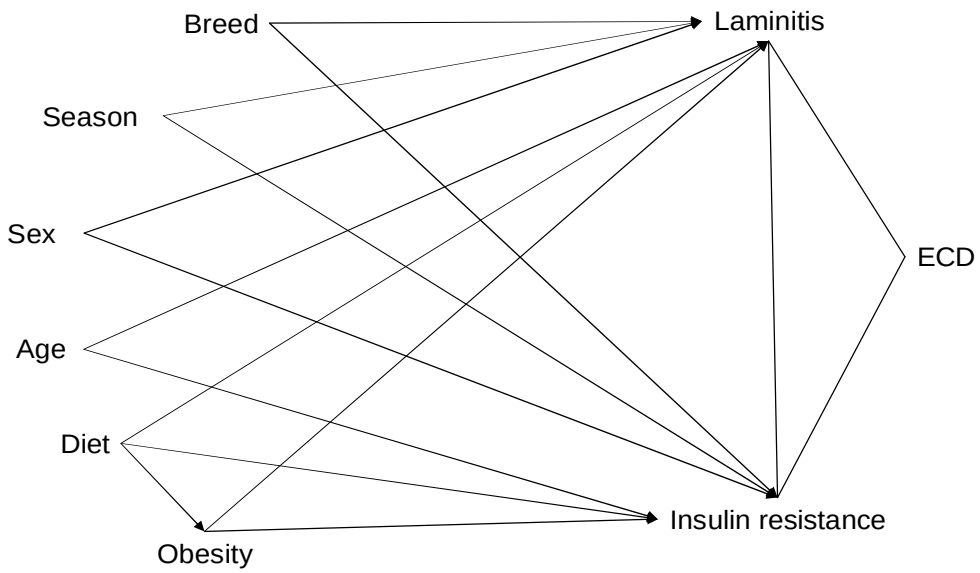


Figure 1. Causality of risk factors for laminitis and insulin resistance and associations between insulin resistance, laminitis and Equine Cushing's Disease (ECD) as composed by the author.

2 ENDOCRINOPATHIC LAMINITIS IN EQUINE CLINIC PATIENTS IN FINLAND

2.1 Introduction

Laminitis is a common painful condition in horses that often has a poor outcome. The painfulness, poor prognosis and frequency of occurrence of the disease make it important both in an animal welfare and economical point of view. The aetiology of laminitis has been widely studied, but still not completely understood.

The association between insulin resistance and laminitis has been demonstrated in multiple studies (Jeffcott et al. 1986, Coffman & Colles, 1983, Bailey et al. 2008, Field & Jeffcott, 1989). Plasma insulin concentrations were significantly correlated to laminitis grade and a decrease in plasma insulin concentration was significantly correlated to a decrease in laminitis grade in a study by Walsh et al. (2009). Prolonged hyperinsulinaemia caused laminitis in horses in a study by Asplin et al (2007).

Endocrinopathic laminitis is a term including laminitis developing subsequently to Equine Cushing's Disease, equine metabolic syndrome and iatrogenic corticosteroid-induced laminitis (McGowan, 2008). The term equine metabolic syndrome (EMS, insulin resistance syndrome) was first used by Johnson in 2002. The syndrome includes a history of laminitis, insulin resistance and a characteristic phenotype of a cresty neck, bulging supraorbital fat and increased fat deposits in the withers and the dorsal back (Johnson, 2002). A cresty neck and a high body condition score can serve as phenotypic indicators associated with insulin resistance (Walsh et al. 2009, Frank et al. 2006).

The reasons for separating endocrinopathic laminitis as an entity of its own as opposed to laminitis developing of other causes are the poorer prognosis, the importance of exercise and diet in the treatment, the phenotypic indicators and the strong breed and age association.

Horses suffering from laminitis and insulin resistance seem to have a slow recovery process, but recovery is more likely if insulin sensitivity is improved (Walsh et al. 2009). Increased exercise, maintaining optimal body condition, avoidance of high-glycaemic meals and molasses should be incorporated in the treatment of laminitis. Agents that improve insulin sensitivity could also become a part of the treatment of laminitis. Horses with insulin resistance have a higher risk of developing laminitis and to find and treat horses with insulin resistance would be a means of preventing laminitis.

There is some evidence that pasture associated laminitis is one of the most common forms of laminitis (NAHMS 2005), and this is most likely associated with underlying endocrine dysfunction i.e. insulin resistance. Further, from extrapolation, it is possible that the majority of cases of laminitis not associated with severe systemic illness, severe trauma or exposure to Black Walnut are associated with insulin resistance. However, what the prevalence of insulin resistance in horses presented with laminitis is, that is currently unknown and it has not been studied previously.

The purpose of the study was to determine the prevalence of insulin resistance in laminitic horses and ponies in Helsinki University Equine Clinic between April 2007 and October 2008. This kind of study has not - to our knowledge - previously been conducted. Cases of laminitis associated with systemic illness or trauma were excluded. Associations between endocrinopathic laminitis and sex, age, body condition score, breed, season, Obel grade, the presence of laminitic rings, cresty neck, and bulging supraorbital fat were studied in 50 horses.

2.2 Materials and Methods

2.2.1 Study design and population

This was a cross-sectional study and the study population consisted of 50 horses/ponies that were screened for insulin resistance between April 1st 2007 and October 1st 2008. All laminitic horses/ponies that were admitted (37) during this period were included in this material. All horses and ponies were admitted at the Helsinki University Equine Clinic as patients or for corrective shoeing. Breed, age and sex of the patients were recorded. Insulin was measured from all laminitic patients, patients with a high body condition score, patients undergoing prolonged corticosteroid treatment and patients suspected of having Equine Cushing's disease. Inclusion criteria were testing for insulin resistance because of obesity, laminitis or endocrinopathy and laminitis. Exclusion criteria were insulin testing prior to or after a long course of corticosteroid treatment. The body condition of the horses was scored using the 0-5 graded Carroll & Huntington body condition scoring (Carroll & Huntington, 1988). Signs of Equine Metabolic Syndrome, bulging supraorbital fat, cresty necks and laminitic rings were recorded. When laminitis was diagnosed, X-ray findings and Obel grade of 0-5 (Obel N, 1948) were recorded.

2.2.2 Laboratory examination

Blood samples were collected in 6 ml serum sample tubes (Vacuette®, Greiner bio one, 2 serum clot activator) using a vacutainer (Vacutainer®, Becton & Dickinson, Plymouth, United Kingdom). All horses were fasted at least an hour before taking the blood sample. The samples were centrifuged and the serum was separated within three hours, frozen to -20 ˚C and sent to Cambridge Specialist Laboratories by courier to arrive in seven days. The samples were analysed using the Pharmacia Insulin radioimmunoassay 100[1] validated for use in horses. A reference range of 5-30 μIU/ml was used based on published ranges (McGowan et al. 2004, Ralston, 2002) and the type of assay used.

2.2.3 Definitions

Insulin resistance was defined as a basal insulin level of over 30 μIU/ml, based on published results (McGowan et al. 2004, Frank 2008). Horses with laminitis and insulin resistance were defined as having endocrinopathic laminitis.

The 14 original breeds were categorized into four groups of breeds. The first group included 11 Warmblood (the warmblood group), the second included 13 riding ponies, one Connemara, one New forest, two Shetland ponies, one Welsh cob (the pony group). The third group included nine Finnhorses, three Norwegian fjord horses, three Icelandic horses and two Estonian draft horses (the coldblood and Icelandic horses group). Finally, the fourth group included one Arabian, one American Quarterhorse, one Standardbred and one mixed breed (the other breed group).

The horses were divided into two groups, the nonobese group and the obese group, based on their body condition score. Horses with a body condition score higher than 3 were grouped as obese and horses with a body condition score of three or lower were grouped as non obese. Season of testing basal insulin value was obtained by categorizing the testing date into summer (1^{st} of June-31^{st} of August) and winter (1^{st} of September-30^{th} of May). To further determine any significant differences of Obel grade between the horses with and without endocrinopathic laminitis, the horses were divided into one group with mild lameness (Obel grade 0 or 1) and another group with severe lameness (Obel grade 2, 3 or 4).

2.2.4 Statistical methods

All statistical analyses were performed using SPSS® 16.0 for Windows® (SPSS Inc, Chicago, IL, USA). For descriptive purposes the procedures Cross tabs, Frequency and Logistic regression were used. The 95% confidence intervals (95% CI) based on binomial distribution for obtained percentages (such as prevalence) were calculated using the approximation in Casella and Berger (1990), with continuity correction. Confidence intervals were calculated, because the horses/ponies included in the study were only a

sample of all possible horses/ponies that could have been included during the period (at another period or at another clinic the material would be different even if the same inclusion criteria were used).

The age distribution between horses with endocrinopathic laminitis and horses without endocrinopathic laminitis was compared using the T-test procedure, because the age was normally distributed.

The potential risk factors for endocrinopathic laminitis (Figure 2) were studied preliminary using the Cross Tabs procedure (with Pearson Chi-square testing) and univariable Logistic Regression procedure regressing endocrinopathic laminitis on the following variables: sex, body condition score, breed, season, Obel grade, the presence of laminitic rings, cresty neck and bulging supraorbital fat, one at a time. In the regression analyses the group of 32 horses with endocrinopathic laminitis and the group of 18 horses without laminitis were compared. The category of horses without endocrinopathic laminitis included horses with insulin resistance, but no laminitis (N=6), horses with laminitis, but no insulin resistance (N=4) and horses with neither complaint (N=8). The associations of the same variables with insulin resistance were similarly studied in the 37 laminitic horses/ponies.

Multivariable logistic regression analyses for endocrinopathic laminitis were not conducted, as all statistically significant (Wald p-value < 0.20) variables were interrelated. The relationship between the variables was studied with the Cross Tabs procedure. Body condition score was assumed to be a potential risk factor both for endocrinopathic laminitis (dependent variable) and the presence of bulging supraorbital fat (independent variable); being a potential confounder it was included in the logistic regression analyses with the bulging supraorbital fat.

The prevalence of insulin resistance in the laminitic group was calculated. Basal insulin levels increase logarithmically (Figure 3) and therefore the geometric mean was calculated for horses with endocrinopathic laminitis and horses without endocrinopathic

laminitis. The arithmetic mean of the logarithm transformed values of the basal insulin values (i.e. the arithmetic mean on the log scale) was computed and then it was returned to the original scale using the exponentiation (Cochran & Snedecor, 1989). The geometric mean is related to the log-normal distribution.

If the odds ratios (OR) obtained from the logistic regression analyses were greater than one, the studied independent variable was considered as a potential risk factor; if OR was between zero and one, the studied independent variable was considered a potential protective factor. The ORs were transformed into ratios of probabilities using the fact that odds equals the probability of being diseased divided by the probability of being non-diseased (as described for example in Toft et al. 2004): Odds = P/(100-P), where P = probability of disease given in %, and P = odds/(100+odds).

The ORs cannot be interpreted as ratios of two probabilities (i.e. risk ratios), if the probability of the disease is greater than 5% (Dohoo et al. 2003). The OR is the odds of those exposed divided by the odds of those not exposed (OR = odds(exposed)/odds(not exposed)). First we assumed that the probability of disease in those not exposed was 50% giving odds(not exposed) = 1. Therefore, the ORs obtained could be considered representing odds(exposed), since odds(exposed)/1 = odds(exposed). Then we could calculate the probability of disease in exposed being odds(exposed)/(100+odds(exposed)). However, since we knew the probability of disease in the not exposed group was not 50%, we calculated the appropriate probability of disease in the exposed group with the following formula derived from the previous formulas:

$$P_{exp}=100*(P_{exp50\%}*(100-P_{notexp50\%})/(P_{notexp50\%}*(100-P_{exp50\%}))*P_{notexp})/(100-P_{notexp}*(1-(P_{exp50\%}*(100-P_{notexp50\%})/(P_{notexp50\%}*(100-P_{exp50\%})))))$$

where

P_{exp} = actual probability of disease in exposed

$P_{exp50\%}$= probability of disease in exposed when the probability of disease in not exposed is 50%

$P_{notexp50\%}$= 50% (set probability of disease in not exposed)

P_{notexp} = actual probability of disease in not exposed

Finally, the ratio of these probabilities was calculated as follows: P_{exp}/ P_{notexp}

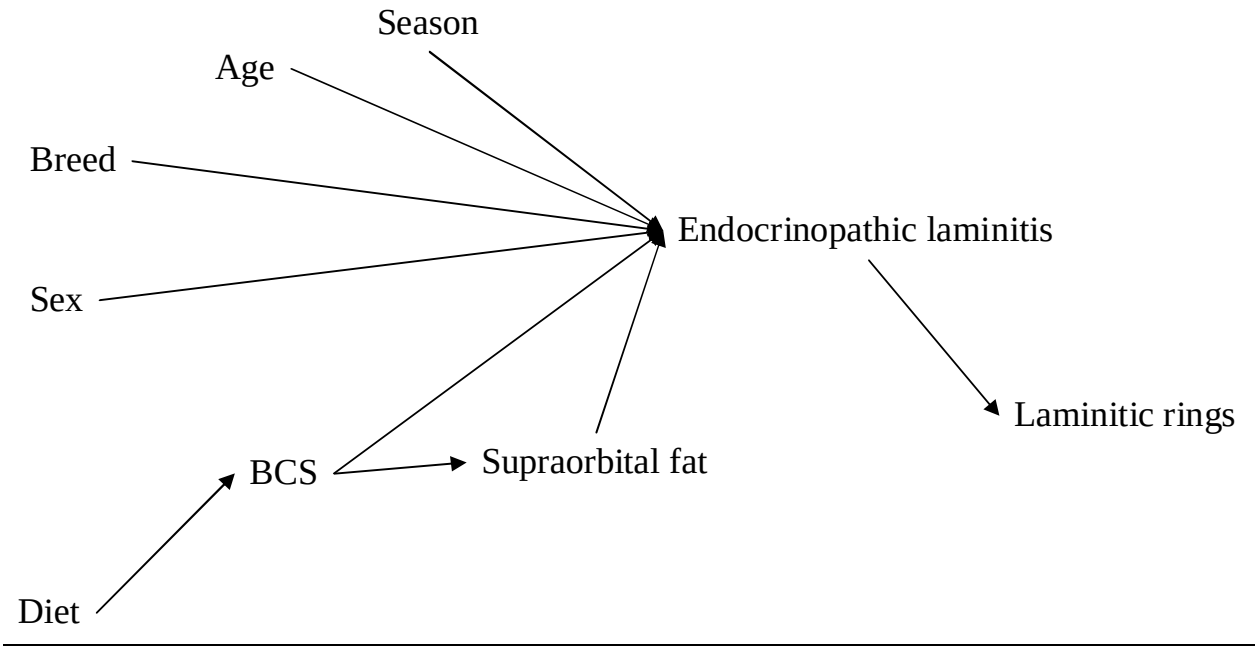


Figure 2. The potential risk factors for endocrinopathic laminitis as composed by author.

2.3 Results

All together 32 horses of the 50 horses studied had endocrinopathic laminitis of which 31 had insulin resistance and laminitis. One horse had Equine Cushing's disease and

laminitis but a normal basal insulin level. This horse was included in the group of horses with endocrinopathic laminitis. All results were checked without this horse and no important changes in the results were recognized.

The material included 37 horses and ponies with laminitis. The prevalence of insulin resistance in these 37 laminitic horses/ponies was 84% (95% CI 69-92%: Table 1). As measured with 95% confidence intervals frequencies for laminitic rings, cresty neck, and bulging supraorbital fat were statistically almost significantly higher in the group with laminitis and insulin resistance than in the group with laminitis but no insulin resistance (Table 1). Frequencies of laminitic rings and bulging supraorbital fat were significantly higher at 95% confidence level in the group with endocrinopathic laminitis compared to the group without endocrinopathic laminitis (Table 2).

The mean age of the endocrinopathic laminitis group was 16 years and that of the group without endocrinopathic laminitis was 13 years (Table 3), this difference was statistically significantly different (t-test p-value < 0.001). The mean age of all 50 horses was relatively high (15 years). The geometric mean basal insulin value of all the horses was 53.47 μIU/ml, the geometric mean basal insulin level of horses with endocrinopathic laminitis was 96.02 μIU/ml (arithmetic distribution in Figure 4), and the geometric mean basal insulin level of horses without endocrinopathic laminitis was 18.92 μIU/ml (arithmetic distribution in Figure 4).

Horses/ponies with laminitic rings had 34.67 times higher odds of having endocrinopathic laminitis than horses without laminitic rings (Table 4, Wald p-value <0.001). This means that horses/ponies with laminitic rings were almost five (4.73) times more likely to have endocrinopathic laminitis than horses without laminitic rings. The association between obesity and endocrinopathic laminitis was almost statistically significant at 95% confidence level (p-value 0.069). Obel grade three was associated with endocrinopathic laminitis with a p-value of 0.045 (Wald) and when the horses were divided into two groups based on the Obel grade (mild and severe lameness), severe lameness was significantly correlated with endocrinopathic laminitis (Wald p-value 0.007). Horses/ponies with bulging supraorbital fat had 6.09 times higher odds of having

endocrinopathic laminitis than horses without bulging supraorbital fat (Table 4). This means that horses/ponies with bulging supraorbital fat were almost two (1.99) times more likely to have endocrinopathic laminitis than horses without bulging supraorbital fat.

When studying the associations of these same independent variables with insulin resistance in the horses with laminitis (Table 5) the presence of a cresty neck was almost significantly associated with insulin resistance (Wald p-values 0.057). The presence of laminitic rings was significantly different between horses with insulin resistance and horses without insulin resistance (Wald p-value 0.019).

Table 1. Description of 37 laminitic horses/ponies in Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008. Statistically almost significant differences between horses/ponies with or without insulin resistance are indicated with italics.

	All laminitic horses		Laminitic horses with insulin resistance		Laminitic horses without insulinresistance	
n	37		31		6	
Factor	%	CI 95 % [1]	%	CI 95 %	%	CI 95 %
Gender:						
Gelding	48.6	33.4 - 64.06	48.4	31.98 - 65.17	50	18.76 - 81.24
Mare	51.4	35.94 - 66.6	51.6	34.83 - 68.02	50	18.76 - 81.24
Laminitic rings	75.7	59.91 - 86.66	*83.9*	*67.4 - 92.93*	*33.3*	*9.66 - 69.98*
Cresty neck	56.8	40.95 - 71.37	*64.5*	*46.93 - 78.87*	*16.7*	*3.02 - 56.38*
Bulging suprarbital fat	54.1	38.43 - 69	*61.3*	*43.83 - 76.28*	*16.7*	*3.02 - 56.38*
Breed:						
Warmblood	18.9	9.47 - 34.18	19.4	9.22 - 36.33	16.7	3.02 - 56.38
Pony breeds	37.8	24.03 - 53.86	38.7	23.72 - 56.17	33.3	9.66 - 69.98
Draft breeds	32.4	19.61 - 48.5	32.3	18.6 - 49.9	33.3	9.66 - 69.98
Other breeds	10.8	4.28 - 24.7	9.7	3.36 - 24.93	16.7	3.02 - 56.38
Season:						
Summer	32.4	19.61 - 48.5	32.3	18.6 - 49.9	33.3	9.66 - 69.98
Winter	67.6	51.5 - 80.39	67.7	50.1 - 81.4	66.7	30.02 - 90.34
Insulin resistance[2]	83.8	68.88 - 92.36	100	NA	0	NA
Laminitis[2]	100	NA	100	NA	100	NA
ECD[3]	29.7	17.47 - 45.75	32.3	18.6 - 49.9	16.7	3.02 - 56.38
BCS[4]:						
2	2.7	0.48 - 13.82	3.2	0.56 - 16.16	0	0 – 39.03
3	16.2	7.64 - 31.12	12.9	5.13 - 28.85	33.3	9.66 - 69.98
4	54.1	35.94 - 66.6	51.6	34.83 - 68.02	66.7	30.02 - 90.34
5	27	15.38 - 42.95	32.3	18.6 - 49.9	0	0 – 39.03
Obesity:						
Obese	81.1	65.82 – 90.53	83.9	67.4 – 92.93	66.7	30.02 - 90.34
Nonobese	18.9	9.47 – 34.18	16.1	7.07 – 32.6	33.3	9.66 - 69.98
Obel grade:						
0	32.4	19.61 - 48.5	25.8	13.7 - 43.24	66.7	30.02 - 90.34
1	18.9	9.47 - 34.18	19.4	9.22 - 36.33	16.7	3.02 - 56.38
2	27	15.38 - 42.95	32.3	18.6 - 49.9	0	0 – 39.03
3	16.2	7.64 - 31.12	16.1	7.07 - 32.6	16.7	3.02 - 56.38
4	5.4	1.49 - 17.7	6.5	1.81 - 20.78	0	0 – 39.03

[1]CI 95 % = 95 % Confidence interval
[2] = Criteria for inclusion/exclusion of horses into the groups
[3]ECD = Equine Cushing's disease
[4]BCS = Body condition score

Table 2. Description of 50 horses/ponies in Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008. Statistically significant differences between horses/ponies with or without ECPL[1] are indicated with italics.

	All horses		Horses with ECPL		Horses without ECPL	
n	50		32		18	
Factor	%	CI 95 %[2]	%	CI 95 %	%	CI 95 %
Gender:						
Gelding	52	38.51 - 65.2	46.9	30.89 - 63.57	61.1	38.61 – 79.69
Mare	48	34.8 - 61.49	53.1	36.43 - 69.11	38.9	20.31 – 61.39
Laminitic rings	56	42.31 - 68.84	*81.2*	*64.63 -91.08*	*11.1*	*3.1 – 32.79*
Cresty neck	58	44.23 - 70.63	62.5	45.25 - 77.07	50	29.03 – 70.97
Bulging suprarbital fat	44	31.16 - 57.69	*59.4*	*42.28 - 74.5*	*16.7*	*5.85 – 39.26*
Breed:						
Warmblood	22	12.75 - 35.24	21.9	11.04 - 38.78	22.2	8.99 – 45.19
Pony breeds	36	24.14 - 49.86	37.5	22.93 - 54.75	33.3	16.26 – 56.22
Draft breeds	34	22.44 - 47.85	31.2	17.91 - 48.52	38.9	20.31 – 61.39
Other breeds	8	3.15 - 18.84	9.4	3.25 - 24.25	5.6	1 – 25.82
Season:						
Summer	32	20.76 - 45.81	31.2	17.91 - 48.52	33.3	16.26 – 56.22
Winter	68	54.19 - 79.24	68.0	54.19 - 79.24	66.7	43.78 – 83.74
Insulin resistance[3]	72	58.33 - 82.3	96.9	84.29 - 99.45	27.8	12.51 – 50.9
Laminitis[3]	74	60.45 - 84.13	100	NA	27.8	12.51 – 50.9
Equine Cushing's disease	30	19.1 - 43.75	34.4	20.43 - 51.71	22.2	8.99 – 45.19
BCS[4]:						
2	2	0.35 - 10.5	3.1	0.55 - 15.71	0	0 – 17.59
3	22	12.75 - 35.24	12.5	4.97 - 28.07	38.9	20.31 – 61.39
4	46	32.97 - 59.6	53.1	36.43 - 69.11	33.3	16.26 – 56.22
5	30	19.1 - 43.75	32.1	18.61 - 49.42	27.8	12.51 – 50.9
Obesity:						
Obese	76	62.59 – 85.7	84.4	68.27 – 93.15	61.1	38.61 – 79.69
Nonobese	24	14.3 – 37.41	15.6	6.85 – 31.73	38.9	20.31 – 61.39
Obel grade:						
0	50	36.64 - 63.36	25	*13.25 - 42.11*	*94.4*	*74.18 – 99*
1	14	6.95 - 26.19	21.9	11.04 - 38.78	0	0 – 17.59
2	20	11.24 - 33.04	31.2	17.91 - 48.52	0	0 – 17.59
3	12	5.62 - 23.81	15.6	6.85 - 31.73	5.6	1 – 25.82
4	4	1.1 -13.46	6.2	1.71 - 20.08	0	0 – 17.59

[1]ECPL = Endocrinopathic laminitis
[2]CI 95 % = 95 % Confidence interval
[3] Criteria for inclusion/exclusion of horses into the groups
[4]BCS = Body condition score

Table 3. Results of basal insulin level and age distribution in 50 horse/ponies in Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008.

	All	ECPL[1] (n[2] = 32)	Non ECPL (n = 18)
Age (yrs):			
Mean	15	16	13
Median	15	15	13
Minimum	7	7	7
Maximum	27	27	20
Insulin results (μIU/ml):			
Geometric mean	53.47	96.02	18.92
Median	47	98.5	21.25
Minimum	3	15	3
Maximum	600	600	54

[1] ECPL = Endocrinopathic laminitis
[2]n = number of horses

Table 4. Results of nine univariable and one bivariable logistic regression analyses for endocrinopathic laminitis in 50 horse/ponies in Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008.

Risk factor	b[1]	p-value (Wald)	OR[2]	CI 95 %[3]
Gender:				
Mare* [4]	NA[5]	1	0	NA
Gelding	0.577	0,336	1.781	0.550 – 5.766
Breed:				
Warmblood*	NA	1	0	NA
Pony breeds	0.134	0,868	1.143	0.237 – 5.501
Draft breeds	-0.203	0,799	0.816	0.171 – 3.895
Other breeds	0.539	0,682	1.714	0.131 – 22.513
Season				
Summer*	NA	1	0	NA
Winter	0.095	0.880	1.100	0.321 – 3.773
Cresty neck	0.511	0.392	1.667	0.518 – 5.363
Laminitic rings	3.546	0.000	34.667	6.225 – 193.58
BCS[6]:				
2*	NA	1	0	NA
3	-21.763	1.000	0.000	
4	-20.161	1.000	0.000	
5	-20.510	1.000	0.000	
Obesity:				
Nonobese*	NA	1	0	NA
Obese	1.234	0.069	3.436	0.895 – 13.187
Obel grade				
0 *	NA	1	0	NA
1	21.957	0.999	3.433E9	0
2	21.957	0.999	3.433E9	0
3	2.363	0.045	10.625	1.059 – 106.573
4	21.957	0.999	3.433E9	0
Lameness				
mild	NA	1	0	NA
severe	2.958	0.007	19.297	2.283 – 162.606
Bulging supraorbital fat (when controlled for BCS)[7]	1.807 (0.547)	0.019 (0.467)	6.094 (1.727)	1.353 – 27.436 (0.395 – 7.547)

[1]b = Regression coefficient
[2]OR = Odds ratio
[3]CI 95 % = 95 % Confidence interval of odds ratio
[4]* = Reference group
[5]NA = Not applicable
[6]BCS = Body condition score
[7]Coefficient, p-value and odds ratio for BCS in brackets; BCS had here two categories similar to obesity

Table 5. Results of eight univariable and one bivariable logistic regression analyses for insulin resistance in 37 laminitic horse/ponies in Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008.

Risk factor	b[1]	p-value (Wald)	OR[2]	CI 95 %[3]
Gender:				
Gelding*[4]	NA[5]	1	0	NA
Mare	0.065	0.942	1.067	0.186 – 6.129
Breed:				
Warmblood*	NA	1	0	NA
Pony breeds	0.000	1.000	1.000	0.75 – 13.367
Draft breeds	-0.182	0.891	0.833	0.062 – 11.277
Other breeds	-0.693	0.661	0.500	0.023 – 11.088
Season:				
Summer*	NA	1	0	NA
Winter	0.049	0.959	1.050	0.164 – 6.724
Cresty neck	2.207	0.057	9.091	0.940 - 87.959
Laminitic rings	2.342	0.019	10.400	1.482 – 72.998
BCS:				
2*	NA	1	0	NA
3	-20.510	1	0	0
4	-19.817	1	0	0
5	0.000	1	1	0
Obesity:				
Nonobese*	NA	1	0	NA
Obese	0.956	0.337	2.6	0.370 - 18.249
Obel grade				
0	NA	0.9	0	NA
1	1.099	0.376	3	0.263 - 34.198
2	20.510	0.999	8.077E8	0
3	0.916	0.465	2.5	0.214 - 29.254
4	20.510	0.258	8.077E8	0
Bulging supraorbital fat (when controlled for BCS)[7]	2.003 (0.214)	0.096 (0.841)	7.411 (1.239)	0.701 - 78.390 (0.153 - 10.049)

[1]b = Regression coefficient
[2]OR = Odds ratio
[3]CI 95 % = 95 % Confidence interval of odds ratio
[4]* = Reference group
[5]NA = Not applicable
[6]BCS = Body condition score
[7]Coefficient, p-value and odds ratio for BCS in brackets; BCS had here two categories similar to obesity

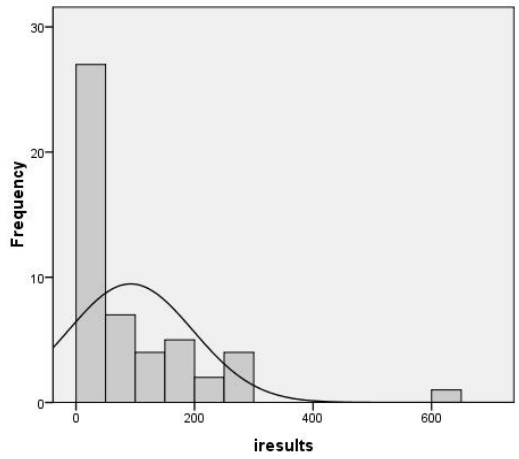
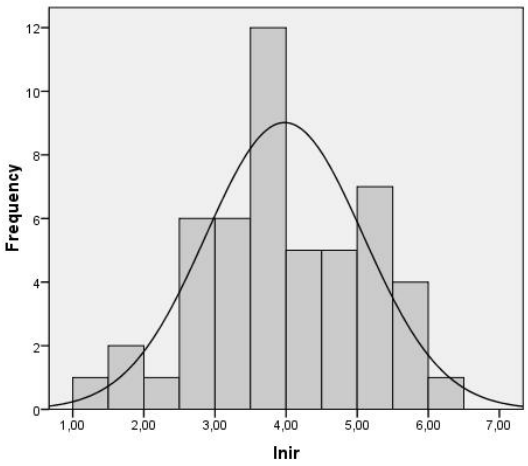
A.	B.
	

Figure 3. Arithmetic insulin results (A) and logarithmic insulin results (B) of 50 horses admitted to Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008.

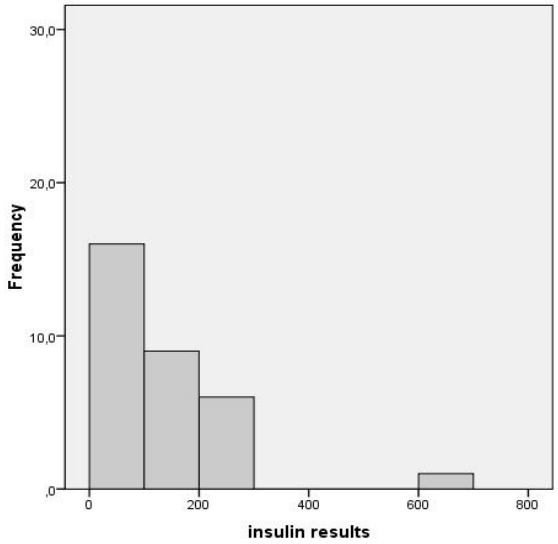
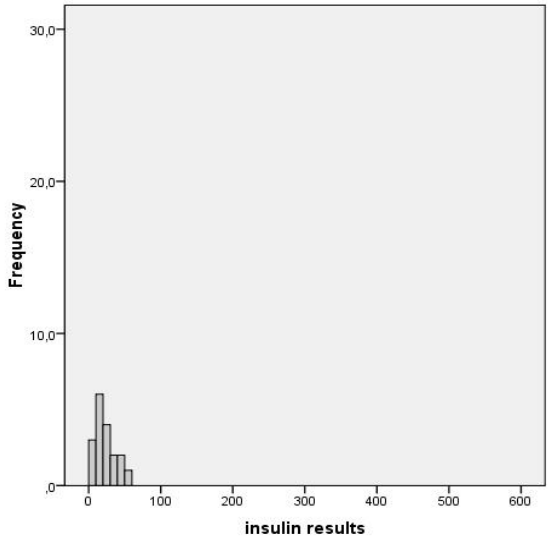
A.	B.
	

Figure 4. Arithmetic insulin results of 32 horses with endocrinopathic laminitis (A) and 18 horses without endocrinopathic laminitis (B) at Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008.

2.4 Discussion

The high prevalence of insulin resistance in the laminitic horses in Helsinki University Equine Clinic (between April 1st 2007 and October 1st 2008) shows that it is an important feature to acknowledge. However, with this material the result tells us nothing about the prevalence of insulin resistance in laminitic horses in Finland in general. As the material was collected from a referral hospital and a farrier, it could be assumed that there was a higher degree of cases with chronic and complicated laminitis. This would give a higher prevalence than in general, because insulin resistance is associated with a poorer prognosis and complications of laminitis. The result implies that a high proportion of the horses admitted to the clinic because of laminitis also have insulin resistance.

No significant differences in the distribution of sex, season, Obel grade or obesity between insulin resistant horses and the horses without insulin resistance were found in the group of laminitic horses. The presence of laminitic rings, cresty neck and bulging supraorbital fat had Wald p-values of 0.019, 0.06 and 0.096, respectively. The lack of significance at 95% confidence level of the two last variables is probably due to the small amount of laminitic horses that did not have insulin resistance.

One horse had laminitis and equine Cushing's disease but a normal basal insulin level. This horse was grouped as having endocrinopathic laminitis, as it may have had stable insulin but chronic hoof changes from previous high insulin. The insulin result could also have been lower for some reason e.g. he may have been fasted on his way to the hospital. The statistics were done with and without this individual to ensure it did not alter any results.

No significant differences in the distribution of sex, breed, season, Obel grade, body condition score or the presence of cresty neck between endocrinopathic and nonendocrinopathic horses were found (Table 4).

Previous studies have shown a breed predilection for both laminitis and insulin resistance, with pony breeds being prone to both insulin resistance and laminitis (Treiber, Kronfeld, Hess et al. 2006, Alford et al. 2001). The result in this study is propably due to the fact that there were no matched controls in this study.

Mares have been shown to have a higher risk to develop laminitis than geldings, but insulin resistance has not been shown to have a sex predilection, although physiologic insulin resistance occurs during pregnancy and lactation (Alford et al. 2001, Fowdean et al. 1984). In this study the gender was not a risk factor for endocrinopathic laminitis or insulin resistance in laminitic horses.

The presumption was that seasonality would affect the occurrence of endocrinopathic laminitis. Laminitis occurs more often during the summer, when horses are out on pasture and a diet high in starch, such as lush pasture, decreases insulin sensitivity. The even distribution of cases between summer and winter months could be due to relapses of the disease. Some horses in the endocrinopathic laminitis group did not suffer from laminitis at the time of blood sampling, but had chronic changes in the hooves.

The age distribution was higher in horses with endocrinopathic laminitis than in horses without endocrinopathic laminitis. The incidence of chronic laminitis is higher in older horses and also the incidence of ECD increases with age (Polzer et al.1996, van der Kolk et al.1993). Whether the age affects the incidence of insulin resistance is not known, although there seem to be some age influence on insulin sensitivity in the horses (Murpy et al. 1997). In this study the high mean age of the material in general may have had an influence on the result.

A high Obel grade (2-4) was significantly correlated with endocrinopathic laminitis in this material (Table 4, severe lameness). Previous reports have shown significant correlation between plasma insulin and laminitis grade (Walsh et al. 2008).

The body condition score was not significantly associated with endocrinopathic laminitis. However, the association between obesity (horses/ponies with body condition score

above 3 were considered as obese) and endocrinopathic laminitis almost reached statistical significance at 95% confidence level. Despite the nonsignificance at this level, this result can be seen as being consistent with the results of previous studies. Some horses were tested only because they were obese and this increased the amount of obese horses in the nonlaminitic group. In a case-control study the difference in obesity between horses with endocrinopathic laminitis and horses without it would probably be greater.

In a study by Frank et al. (2006) the neck circumference was significantly correlated with area under curve for insulin. Their conclusion was that measurements of neck circumference should be taken, in addition to body condition score and insulin to identify horses at risk for insulin resistance. In this study no significant correlation between endocrinopathic laminitis and a cresty neck was found. However, the correlation between insulin resistance and cresty neck was almost statistically significant (Wald p-value 0.06). The neck circumference was not measured in this study. The horses were subjectively evaluated by the treating veterinarian as having or not having a cresty neck. The reason for this difference in results is most likely the same as for the lack of correlation between endocrinopathic laminitis and a high body condition score, i.e. the screening of some obese, but otherwise healthy horses for insulin resistance.

The horses with bulging supraorbital fat were approximately two times more likely to also have endocrinopathic laminitis. This association has been reported previously and it is one of the features of equine metabolic syndrome (Johnson, 2002).

Horses with laminitic rings were almost five times more likely to also have endocrinopathic laminitis. Partially this is self-evident, as horses without laminitis would not have laminitic rings and most of the laminitic horses were included in the group of endocrinopathic laminitis. The association could also be due to the chronic nature of endocrinopathic laminitis, as laminitic rings are a sign of chronic changes in the hoof. Because of the lack of information for some horses the distribution between acute and chronic laminitis between the groups could not be calculated.

In this study only basal insulin was measured of the horses and it is not the most accurate method of diagnosing insulin resistance. This means that some cases of insulin resistance could have been missed, either because the laminitis had been caused by a previous period of high insulin or the insulin was momentarily lower for some reason (fasting for example). Horses with laminitis but a normal basal insulin value were included in the group of horses with nonendocrinopathic laminitis and the laminitic horses were divided into insulin resistant and non insulin resistant based on their basal insulin value. This could mean that some horses might have been grouped into a wrong group.

The results in this study were obtained using several univariable logistic regression analyses (except with bulging supraorbital fat) and therefore possible confounding effects of other variables have not been taken into account.

Due to the cross sectional study design it was not possible to conclude the time sequence, i.e. whether the variables (bulging supraorbital fat and laminitic rings) precedes the disease or are caused by the disease. However, the correlation justifies including the presence of laminitic rings and bulging supraorbital fat as a phenotypic indicators of endocrinopathic laminitis and the equine metabolic syndrome.

2.5 Conclusions

1. The prevalence of insulin resistance in the 37 laminitic horses at Helsinki University Equine Clinic between April 1st 2007 and October 1st 2008 was 84%, so the majority of admitted laminitic horses were insulin resistant.

2. The horses with bulging supraorbital fat were approximately two times more likely to also have endocrinopathic laminitis and horses with laminitic rings were almost five times more likely to also have endocrinopathic laminitis in this material. Severe lameness was significantly correlated with endocrinopathic laminitis.

3. The mean age of horses with endocrinopathic laminitis was significantly higher than the mean age of horses without endocrinopathic laminitis.

4. There was a statistically almost significant difference at 95% confidence level in the distribution of cresty neck and bulging supraorbital fat between the groups (Wald p-values 0.06 and 0.096, respectively). Laminitic rings were statistically more frequent in horses with insulin resistance and laminitis than horses with laminitis and normal insulin level (Wald p-value 0.019).

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2.7 References

1. Alford P, Gellar S, Richardson B, et al. A multicenter, matched case-control study of risk factors for equine laminitis. Prev Vet Med 2001, 49: 209–222.

2. Annandale EJ, Valberg SJ, Mickelson JR, Seaquist ER. Insulin sensitivity and skeletal muscle glucose transport in horses with equine polysaccharide storage myopathy. Neuromuscular Disorders 2004, 14: 666-674.

3. Asplin KE, McGowan CM, Pollitt CC, Curlewis J, Sillence MN. Role of insulin in glucose uptake in the equine hoof. Am College Vet Int Med Forum Proceedings, Seattle, Washington, 2007.

4. Asplin KE, Sillence MN, Pollitt CC, McGowan CM. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. The Vet J 2007, 174: 530-535.

5. Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ. Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. Am J Vet Res 2008, 69: 122-129.

6. Bailey SR, Marr CM, Elliott J. Current research and theories on the pathogenesis of acute laminitis in the horse. Vet J 2004, 167: 129-142.

7. Bailey SR, Menzies-Gow NJ, Harris PA, Habershon-Butcher JL, Crawford C, Berhane Y, Boston RC, Elliott J. Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis. J Am Vet Med Ass 2007, 231: 1365-1373.

8. Carroll CL, Huntington PJ. Body condition scoring and weight estimation in horses. Equine Vet J 1988, 20: 41-45.

9. Casella G, Berger RL. Statistical inference. Wadsworth & Brooks/ Cole Publishing Co, Pacific Grove, California 1990: 444-445.

10. Cochran WG, Snedecor GW. Statistical methods. Iowa State University Press, Ames, Iowa 1989.

11. Coffman JR, Colles CM. Insulin tolerance in laminitic ponies. Can J Comp Med 1983, 47: 347-51.

12. Durham AE, Rendle DI, Newton JR. The effect of metformin on measurements of insulin sensitivity and β cell response in 18 horses and ponies with insulin resistance. Equine Vet J 2008, 40: 493-500.

13. Eaton SA, Allen D, Eades SC, Schneider DA. Digital Starling forces and haemodynamics during early laminitis induced by an aqueous extract of black walnut (Jugulans nigra) in horses. Am J Vet Res 1995, 56: 1338-44.

14. Evans JW, Thompson PG, Winget GM. Glucose and insulin biorhythms in the horse. J S Afr Vet Ass 1974, 45: 317-329.

15. Field Jr, Jeffcott LB. Equine laminitis – another hypothesis for pathogenesis. Med Hypotheses 1989, 30: 203-210.

16. Firshmann AM, Valberg SJ. Factors affecting clinical assessment of insulin sensitivity in horses. Equine Vet J 2007, 39: 567-575.

17. Fowdean AL, Comline RS, Silver M. Insulin secretion and carbohydrate metabolism during pregnancy in the mare. Equine Vet J 1984, 16: 239-246.

18. Frank N. Endocrinopathic laminitis, obesity-associated laminitis, and pasture-associated laminitis. Proceedings of 54th Annual Convention of the American Association of Equine Practitioners. San Diego, CA 2008.

19. Frank N, Elliott SB, Brandt LE, Keisler DH. Physical characteristics, blood hormone concentrations and plasma lipid concentrations in obese horses with insulin resistance. J Am Vet Med 2006, 229: 1383-1390.

20. Freestone JF, Wolfsheimer KJ, Kamerling SG, Church G, Hamra J, Bagwell C. Exercise induced hormonal and metabolic changes in Thoroughbred horses: Effects of conditioning and Acepromazine. Equine Vet J 1991, 23: 219-223.

21. French KR, Pollitt CC. Equine laminitis: loss of hemidesmosomes in hoof secondary epidermal lamellae correlates to dose in an oligofructose induction model: an ultrastructural study. Equine Vet J 2004, 36: 230-235.

22. Geor RJ, Harris P. Dietary management of obesity and insulin resistance: countering risk for laminitis. Vet Clin North Am Equine Pract 2009, 25: 51-65.

23. Greene SA, Thurmon JC, Tranquilli WJ, Benson GJ. Effect of yohimbine on xylazine-induced hypoinsulinemia and hyperglycemia in mares. Am J Vet Res 1987, 48: 676- 678.

24. Harris P, Bailey SR, Elliott J, Longland A. Countermeasures for Pasture-Associated Laminitis in Ponies and Horses. American Soc Nutrition J Nutr 2006 136:2114S-2121S.

25. Hess TM, Treiber KH, Kronfeld DS, Furr MO. Insulin resistance demonstrated by a specific quantitative method in a hyperlipemic laminitic pony. J Equine Vet Science 2006, 26: 271-274.

26. Hoffman RM, Boston RC, Stefanovski D, et al. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred gelding. J Anim Sci 2003, 81: 2333–2342.

27. Jeffcott LB, Field JR, McLean JG, O'Dea K. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Vet J 1986, 18: 97-101.

28. Johnson PJ. The equine metabolic syndrome peripheral Cushing's syndrome. Vet Clin North Am Equine Pract 2002, 18: 271-293.

29. Johnson PJ, Ganjam VK. Laminitis, "hypothyroidism", and obesity: A peripheral cushingoid syndrome in horses? Proc 17th ACVIM, Chicago, 1999: 192-194.

30. Johnson PJ, Messer NT, Ganjam VK. Cushing's syndromes, insulin resistance and endocrinopathic laminitis. Equine Vet J 2004, 36: 194–198.

31. Keen JA, McLaren M, Chandler KJ, McGorum BC. Biochemical indices of vascular function, glucose metabolism and oxidative stress in horses with equine Cushing's disease. Equine Vet J 2004, 36: 226-229.

32. Kronfeld D. Insulin resistance predicted by specific proxies. J Equine Vet Science 2006, 26: 281 – 284.

33. Kronfeld DS, Treiber KH, Geor RJ. Comparison of nonspecific indications and quantitative methods for the assessment of insulin resistance in horses and ponies. J Am Vet Med Assoc 2005, 226: 712–719.

34. Masuzaki H, Paterson J, Shinyama H, Morton MN, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001, 294: 2166-2170.

35. McGowan C.M. Role of insulin in endocrinopathic laminitis. Journal of Equine Veterinary Science 2008, 28: 603-607.

36. McGowan CM, Geor R, McGowan TW. Prevalence and risk factors for hyperinsulinemia in ponies.

37. McGowan CM, Frost R, Pfeiffer DU, Neiger R. Serum insulin concentrations in horses with equine Cushing´s syndrome: response to a cortisol inhibitor and prognostic value. Equine Vet J 2004, 36: 295-298.

38. Murphy D, Reid SWJ, Love S. The effect of age and diet on the oral glucose tolerance test in ponies. Equine Vet J 1997, 29: 467-470.

39. NAHMS, National Animal Health Monitoring System 2005 http://nahms.aphis.usda.gov/equine/equine05/equine05_part1.pdf

40.Obel N. Studies on the histopathology of acute laminitis (dissertation). Almqvists & Wiksells Boktryckeri AB, Uppsala 1948.

41. Pass MA, Pollitt S, Pollitt CC. Decreased glucose metabolism causes separation of hoof lamellae in vitro : a trigger for laminitis ? Equine Vet J Suppl 1998, 26: 329-340.

42. Pratt SE, Geor RJ, McCutcheon LJ. Repeatability of 2 methods for assessment of insulin sensitivity and glucose dynamics in horses. J Vet Int Med 2005, 19: 883-888.

43. Pratt SE, Geor RJ, McCutcheon LJ. Effects of dietary energy source and physiological conditioning on insulin sensitivity and glucose tolerance in standardbred horses. Equine Vet J Suppl 2006, 36: 579-584.

44. Polzer J, Slater MR. Age, breed, sex and seasonality as risk factors for equine laminitis. Prev Vet Med 1996, 29: 179–184.

45. Ralston SL. Insulin and glucose regulation. Vet Clin N Am: Equine Pract 2002, 18: 320-322.

46. Ralston SL. Postprandial hyperglycemia/insulinemia in young horses with osteochondritis dissecans lesions. J Anim Sci 1995, 73:184.

47. Reeves HJ, Lees R, McGowan CM. Measurement of basal serum insulin concentration in the diagnosis of Cushing's disease in ponies. Vet Rec 2001, 149: 449-452.

48. Rijnen KEPM, van der Kolk JH. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. Am J Vet Res 2003, 64: 1260-1264.

49. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001, 414: 799-806.

50. Sjaastad ØV, Hove K, Sand O. Physiology of domestic animals 1st ed. Scandinavian Veterinary Press, Oslo 2003.

51. Schott II HC. Pituitary pars intermedia dysfunction: equine Cushing's disease. Vet Clin North Am Equine Pract 2002, 18: 237-270.

52. Snook Parsons C, Orsini JA, Krafty R, Capewell L, Boston R. Risk factors for development of acute laminitis in horses during hospitalization: 73 cases (1997-2004). J Am Vet Med Ass 2007, 230: 885-889.

53. Tiley HA, Geor RJ, McCutcheon LJ. Effects of dexamethasone on glucose dynamics and insulin sensitivity in healthy horses. Am J Vet Res 2007, 68: 753-759.

54. Tilg H, Moschen AR. Inflammatory Mechanisms in the regulation of insulin resistance. Mol Med 2008, 14: 222-231.

55. Toft N, Agger JF & Bruun J: Measures of association and effect in H Houe, AK Ersbøll & N Toft (editors): Introduction to Veterinary Epidemiology, Frederiksberg, Denmark, Biofolia, 2004: 99

56. Treiber KH, Boston RC, Kronfeld DS, et al. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic meals. J Anim Sci 2005, 83: 2357–2364.

57. Treiber KH, Kronfeld DS, Geor R. Insulin resistance in Equids: Possible role in laminitis. J of Nutrition 2006, 136: 2094S-2098S.

58. Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. J Am Vet Med Assoc 2006, 228: 1538-1545.

59. Waldhausl W. Circadian rhythms of insulin needs and actions. Diabetes Res Clin Pract 1989, 6: S17-S24.

60. Walsh DM, McGowan CM, McGowan TW, Lamb SV, Schanbacher BJ and Place NJ. Correlation of plasma insulin concentration and laminitis score in a field study of equine Cushing's disease and equine metabolic syndrome. Journal of Equine Veterinary Science J Eq Vet Sci 2009, 29: 87-94.

61. Van Weyenberg S, Hesta M, Buyse J, Janssens GPJ. The effect of weight loss by energy restriction on metabolic profile and glucose tolerance in ponies. J Anim Phys Anim Nutr 2007, 92: 538-545.

62. van der Kolk JH, Kalsbeek HC, van Garderen E, Wensing Th, Breukink HJ. Equine pituitary neoplasia: a clinical report of 21 cases (1990-1992). Vet Rec 1993, 133: 594-597.

63. Wattle O, Pollitt CC. Lamellar metabolism. Clin Tech Equine Pract 2004, 13: 133-138.

64. Vick MM, Adams AA, Murphy BA, Sessions DR, Horohov DW, Cook RF, Shelton BJ, Fitzgerald, BP. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. J Anim Science 2007, 85: 1144-1155.

65. Vick MM, Murphy BA, Sessions DR, Reedy SE, Kennedy EL, Horohov DW, Cook RF, Fitzgerald BP. Effects of systemic inflammation on insulin sensitivity and inflammatory cytokine expression in adipose tissue. Am J Vet Res 2008, 69: 130-139.