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Article type : Review Article

Editorial reference code: EVJ-RA-16-294.R2

The diagnosis of equine insulin dysregulation

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Keywords: horse; insulin; glucose; insulin dysregulation; insulin resistance; hyperinsulinaemia

List of abbreviations

AIR_g: acute insulin response to glucose

CGIT: combined glucose insulin tolerance test

EHC: euglycaemic hyperinsulinaemic clamp

EMS: equine metabolic syndrome

FSIGTT: frequently sampled intravenous glucose tolerance test

GIP: glucose-dependent insulinotropic peptide or gastric inhibitory peptide

GLP-1: glucagon-like peptide-1

HOMA-IS: homeostasis model assessment of insulin sensitivity

IST: insulin sensitivity test

IVGTT: intravenous glucose tolerance test

MIRG: modified insulin to glucose ratio

OGT: oral glucose test

OST: oral sugar test

QUICKI: quantitative insulin sensitivity check index

RISQI: reciprocal inverse square of basal insulin

S_g: glucose effectiveness

SI: insulin sensitivity

S_i: insulin sensitivity index

$T_{1/2}$: glucose half-life

Summary

Insulin dysregulation is the hallmark of Equine Metabolic Syndrome and has received attention because of its direct association with laminitis. In the absence of an adequate treatment for laminitis, a focus on prophylaxis is needed, making early detection of individuals at risk of developing laminitis one of the main challenges in equine endocrinology. Recent studies have shown that insulin dysregulation goes beyond tissue insulin resistance and it is now demonstrated that the equine enteroinsular axis plays a major role in insulin secretion and equine hyperinsulinaemia. In this review, we discuss the different tests currently available to diagnose insulin dysregulation in horses: the ones investigating tissue insulin resistance and the ones investigating the enteroinsular axis, detailing their goals, practicalities and limitations. This review supports the contention that the diagnosis of equine insulin dysregulation should now be based on the investigation of both tissue insulin resistance and the equine enteroinsular axis. Regardless of the tests used many factors of variation, such as breed, diet, fasting state or season, have been identified and could potentially confound the results of a specific test. Therefore, careful interpretation of the results of a given test in each individual situation is required to optimise the detection of horses at risk of laminitis.

Introduction

Equine Metabolic Syndrome (EMS) has been described as a medical condition that groups, insulin dysregulation, altered circulating adipokine concentrations, dyslipidaemia (with or without obesity) and a predisposition to laminitis [1; 2]. Although there is no universally accepted definition of equine obesity, it seems that the world population of overweight or obese horses is increasing and can reach, in some areas, a prevalence as high as 72.2% [3]. This

suggests that, in parallel with what is described in humans, obesity and/or regional adiposity, and its associated endocrine disorders will continue to be a major challenge for the equine industry [4-6].

The pathophysiology of EMS remains unclear. However, it has been shown that infusing insulin can trigger the development of laminitis in healthy horses establishing a link between hyperinsulinaemia and laminitis [7; 8]. The terms insulin dysregulation and insulin resistance have been used interchangeably; however, insulin dysregulation is a broader term as it encompasses both tissue insulin resistance and both persistent or intermittent hyperinsulinaemia [2]. Tissue insulin resistance is the inability of tissues to respond adequately to insulin, whereas hyperinsulinaemia is the circulation of an inappropriate amount of insulin after a given stimulus [9; 10]. Hyperinsulinaemia can be symptomatic of tissue insulin resistance when it occurs as a compensatory response to peripheral tissue insulin resistance. However, recent research on the equine enteroinsular axis has shown that horses can have an excessive insulin response to ingested carbohydrates (without concurrent tissue insulin resistance), which results in transient hyperinsulinaemia and demonstrates that hyperinsulinaemia can be present in the absence of tissue insulin resistance [11]. Therefore, tissue insulin resistance and hyperinsulinaemia may represent two different arms of a pathological process that can precipitate laminitis.

The direct link between insulin and laminitis remains unknown and whether there is a local or systemic effect is unclear. Several studies have failed to implicate local tissue insulin resistance in laminitis development [12-14]. Some mechanisms to explain insulin-associated laminitis have been proposed, such as a mitogenic effect on the lamellar epithelial cells, obesity-induced

chronic inflammation resulting in neutrophil activation and proinflammatory cytokine production or vascular dysfunction resulting in hypoxia and endothelial activation [15-26]. However, more research is needed to elucidate the finer mechanisms of insulin-associated laminitis.

Although laminitis has been a major burden on the equine industry for many years, no adequate treatment is available and the development of laminitis is still associated with decreased survival after one year of onset [27]. Horses with EMS may have pre-existing radiographic changes or abnormal growth rings on their hooves before the first recognised episode of laminitis [28]. Therefore, prevention of the first painful episode of laminitis is critical when managing horses with EMS. In order to be able to prevent the development of the first episode of laminitis, early detection of the horses at risk is necessary.

As more pieces of the insulin dysregulation puzzle are put together, new tests are developed and the interpretation of older tests changes. The ideal test would be able to detect horses in the early stages of insulin dysregulation and would need to have strong intrinsic value considering that many environmental and individual factors have been shown to confound the results of any given test. In this review, we will detail the different diagnostic tests available for the identification of insulin dysregulation in horses, and consider their advantages and their limits. We will also discuss the confounding factors, and their effect on the interpretation of the tests.

Diagnosis of insulin resistance

The techniques used to assess the degree of tissue sensitivity to insulin in horses and ponies are mostly adapted from human medicine. While many of these tests have been available for some time they continue to be refined for use in the equine species. Further, our appreciation of the need for accurate, flexible and feasible tests is increasing.

Fasting blood glucose concentration

Hyperglycaemia is an indirect measure of tissue insulin resistance. Horses can produce large quantities of insulin and rarely develop pancreatic exhaustion; therefore, hyperglycaemia suggests a defective compensatory insulin response and, if persistent, warrants investigation of *diabetes mellitus* [1; 7]. Although blood glucose concentration may be at the upper end of the reference range in horses with EMS, there are many other possible causes of hyperglycaemia, such as stress, feeding, drug administration or concurrent disease. Additionally, the length of fasting required to consider a sample as fasted is debatable. In studies investigating the effect of fasting on glucose dynamics the length of fast varies from 12 to 72 hours [29-32]. Thus, fasting blood glucose is a poor diagnostic test for tissue insulin resistance with both low specificity and low sensitivity [33-35].

Euglycaemic hyperinsulinaemic clamp

The euglycaemic hyperinsulinaemic clamp (EHC) developed in 1974 by Sherwin *et al*. is still considered as the "gold standard" to diagnose tissue insulin resistance in human medicine [36; 37]. This test, as described by Kronfeld *et al.*, has also been considered as the "gold standard" for the diagnosis of tissue insulin resistance in horses [2; 35; 38; 39]. After a priming dose of insulin, the horse receives a continuous infusion of insulin (3 – 6 mIU/kg/min) and blood glucose is maintained at a constant value (around 5 mmol/L) by adjusting the glucose infusion rate [7; 35; 38]. At steady state, the glucose infusion rate (corrected to account for glucose that had been added or removed from glucose space other than by metabolism) provides the insulin-induced glucose disposal rate. A low glucose infusion rate indicates tissue insulin resistance whereas a high glucose infusion rate indicates effective insulin-induced glucose disposal and insulin sensitivity [38]. Although this test provides a direct measurement of the ability of insulin-sensitive tissues to respond to exogenous insulin, the interpretation of this test may be difficult for a given horse in the absence of breed-specific reference ranges. For example, in a

general population of horses a value lower than 7 µmol/kg/min would indicate tissue insulin resistance but when only Dutch Warmbloods are considered a value lower than 14 µmol/kg/min would indicate tissue insulin resistance [35]. Additionally, the EHC may be too extreme to reflect normal physiologic processes and because it requires several hours with adequate equipment and trained staff to reach a steady state, the use of the EHC may be limited to research settings [35]. Despite its practical issues, the EHC is often used as a reference to compare other tests to, and it is repeatable, with a coefficient of variation of 14% [38; 40].

Insulin sensitivity test

The insulin sensitivity test (IST) directly assesses the ability of insulin-sensitive tissues, such as skeletal muscles, adipose tissue and liver, to take up glucose. Different versions of this test have been described, a complete test and a short test [41; 42]. The complete test, as described by Caltabilota *et al*. assesses insulin sensitivity by measuring blood glucose after intravenous injection of variable doses of insulin (20 – 125 mIU/kg) over 5 hours [41]. The short test (2-step test), as described by Bertin *et al*. assesses insulin sensitivity by measuring blood glucose 30 minutes after intravenous injection of 100 mIU/kg of insulin [42]. For both tests, a 50% decrease in blood glucose indicates insulin sensitivity. The repeatability of the complete IST has been shown to be equal to, or better than, most currently used methods of assessing tissue insulin resistance in horses, with a coefficient of variation of 9% [41]. Additionally, the agreement between the complete and the short version of the tests has been shown to be excellent suggesting that the short version of the insulin tolerance test could be used in the field [42]. The test carries a small risk of inducing marked hypoglycaemia. However, clinical signs of hypoglycaemia rarely require treatment [43].

Frequently sampled intravenous glucose tolerance test

Two versions of the frequently sampled intravenous glucose tolerance test have been described. The first is based on an intravenous glucose injection (FSIGTT, sometimes called intravenous glucose tolerance test or IVGTT) and the other one consists of an intravenous glucose injection followed by intravenous insulin injection (modified FSIGTT). In the FSIGTT described by Pratt *et al.*, 500 mg/kg of dextrose is injected and up to 36 blood samples are collected over 6 hours to measure glucose and insulin [38]. The computerised mathematical models used to describe the glucose and insulin responses to intravenous glucose injection (minimal model analysis and area under the glucose and insulin curves) allow the calculation of the insulin sensitivity index (S_i, reflecting insulin-dependent glucose clearance), the glucose effectiveness (S_g, reflecting insulin-independent glucose clearance), the acute insulin response to glucose (AIR_g, reflecting the glucose-induced insulin secretion or β cell responsiveness) and the glucose half-life ($T_{1/2g}$, reflecting overall glucose clearance) [38]. This FSIGTT has been shown to have poor repeatability with a within-horse coefficient of variability between 24 and 30%, but to correlate well with the EHC [38; 40]. Some minor changes have been made to the test in order to limit glucose urinary spilling resulting in the injection of smaller doses of dextrose (100 – 300 mg/kg) [44-47]. The modified FSIGTT, as described by Hoffman *et al*. or Bailey *et al.*, differs from the FSIGTT by the intravenous injection of 20 – 30 mIU/kg of insulin 20 minutes after dextrose injection allowing the calculation of insulin sensitivity (SI, reflecting the efficiency of insulin to accelerate glucose uptake) [48; 49]. A diagnosis of tissue insulin resistance is made when SI is low (<1 L/min/mIU) whereas a horse is classified as insulin sensitive with a high SI (>1.5 L/min/mIU) [50]. This modified FSIGTT has been widely used in research settings and is often considered as a new "gold standard" for the assessment of tissue insulin resistance in horses [1; 40; 45; 50-54].

Combined glucose insulin tolerance test

The combined glucose insulin tolerance test (CGIT) was developed as a simplified combination of the IST and the FSIGTT. The CGIT, as described by Eiler *et al.*, is based on the almost simultaneous intravenous injection of 150 mg/kg of dextrose and 100 mIU/kg of insulin [43]. Over 150 minutes, 14 blood samples are collected to measure glucose and insulin. The almost simultaneous injection of glucose and insulin results in a biphasic curve with a positive phase (relative hyperglycaemia) and a negative phase (relative hypoglycaemia). The interpretation of the test is either based on the duration of the positive phase (tissue insulin resistance if the relative hyperglycaemia lasts more than 45 min) or on the insulin concentration after 45 min (tissue insulin resistance if >100 μIU/mL), or both [43; 45; 55]. However, these cut-off values are yet to be fully validated in the horse. The repeatability of the test is similar to other tests with a coefficient of variation around 15%, with insulin data being more repeatable than glucose data [43]. However, using the duration of the positive phase, the sensitivity and specificity of the CGIT have been shown to be 85.7% and 40% respectively (using the FSIGTT as reference), and using the insulin value at 45 min, the sensitivity and specificity of the CGIT have been shown to be 28.5% and 100% respectively (using the FSIGTT as reference) [45].

Proxies and surrogate tests

In order to simplify the diagnosis of tissue insulin resistance in horses, a few surrogate tests have been developed. These tests seem to be widely used in human medicine and are attractive to equine practitioners aiming to make a diagnosis of tissue insulin resistance based on limited tests in a field environment. However, it is important to remember that none of these tests have been fully validated in equine patients [56; 57]. In horses, the glucose/insulin ratio has been shown to be a good estimate of insulin sensitivity and the insulin/glucose ratio has been shown to correlate with insulin secretion as measured by the EHC [35]. A glucose/insulin ratio <10 suggests insulin resistance whereas a glucose/insulin ratio <4.5 indicates severe or

decompensated insulin resistance [34]. However, in another study, this ratio failed to identify horses diagnosed as insulin resistant by other tests [29]. In a study by Kronefeld *et al*. the homeostasis model assessment of insulin sensitivity (HOMA-IS: 22.5/(glucose x insulin)) and the quantitative insulin sensitivity check index (QUICKI: 1/(log(glucose) + log(insulin)) correlated well with the results of the EHC [35] and in a study by Treiber *et al.*, the reciprocal inverse square of basal insulin (RISQI: $insulin^{-0.5}$) and the modified insulin to glucose ratio (MIRG: $(800 – 0.3 (insulin – 50)^2)/(glucose – 30)$) correlated well with the SI and AIR_g results of the modified FSIGTT in 46 healthy Thoroughbreds (coefficient of correlation of 0.774 and 0.754 respectively) [58]. However, the total predictive values of the proxies in the assessment of insulin sensitivity in healthy horses was about 80% and whilst both proxies have a high specificity (85% using SI as a reference or 88% using AIR_g as a reference), their sensitivity was low (45% using SI as a reference and 50% using AIR_g as a reference) [58]. Unfortunately, very few studies have evaluated the relevance and the validity of these surrogate tests in equine diseases, which limits their potential clinical application [34].

Diagnosis of hyperinsulinaemia

Basal serum insulin concentration

Hyperinsulinaemia (>20 µIU/mL if fasted or >60 µIU/mL if fed) in the presence of clinical suspicion of EMS is supportive of insulin dysregulation and warrants the use of a dynamic test [1; 59; 60]. Conversely, a serum insulin concentration within the reference range (this varies slightly between laboratories) in the presence of clinical suspicion of EMS is non-diagnostic [1]. In a few studies, horses diagnosed with insulin dysregulation by other tests were not found to be hyperinsulinaemic, and when specifically compared to the FSIGTT, the sensitivity of basal serum insulin was 0% [45]. Although single point in time hyperinsulinaemia is not sensitive to diagnose insulin dysregulation, spontaneous hyperinsulinaemia is associated with the development of laminitis and decreased survival. In a group of horses suffering from

endocrinopathies, a basal serum insulin >188 μIU/mL had a sensitivity and a specificity >90% to predict laminitis and nonsurvival after one year [7]. In another study in horses with no history of laminitis, a basal serum insulin >21.8 μIU/mL had a sensitivity and a specificity >70% to predict the occurrence of laminitis within 3 years [3].

Confounding the problems associated with the use of basal serum insulin concentration is the variety of laboratory assays currently used to determine equine insulin concentration [61-65]. All of the currently available tests suffer limitations and this is an area in desperate need of further research.

Oral sugar test

The oral sugar test (OST) investigates the glucose-induced insulin response to the oral administration of non-structural carbohydrates [46; 66]. A diagnosis of hyperinsulinaemia with the OST, as described by Schuver *et al*., is based on a serum insulin >60 μIU/mL 60 or 90 minutes after oral administration of 0.15 mL/kg of corn syrup to horses fasted overnight [67]. This test measures the insulin response to an oral dose of carbohydrate and account for the enteroinsular axis [11]. The interpretation of the results of this test may be difficult as a few studies have found the test to have poor repeatability, with a coefficient of variation as high as 40% [68; 69]. Additionally, when compared to the FSIGTT, the OST had a sensitivity of 0% which would tremendously limit the potential clinical value of the test if this value is correct [45].

Oral glucose test

In areas of the world where corn syrup is not as readily available, an oral glucose test (OGT) has been developed [70]. A diagnosis of hyperinsulinaemia with the OGT, as described by de Laat *et al*., is based on a serum insulin >80 μIU/mL 120 minutes after oral administration of 0.75 – 1 g/kg of glucose [71]. The method of administration of glucose varies; in some studies, powdered dextrose is mixed with bran and/or chaff and spontaneously eaten by the horse and in others, glucose is administered through a nasogastric tube as a solution [40; 69]. Regardless of the method of administration, the OGT has better repeatability than the OST, with a coefficient of variability around 20% and the two tests agreed with respect to dichotomous outcome in 85% of cases [69; 71]. This test has also been shown to correlate with the results of the EHC and the modified FSIGTT in healthy Standardbreds [40]. The ease of this test, and the ability to accurately determine the dose of glucose administered (the glucose content of corn syrup is proprietary information) may positively impact on its' repeatability. Further, it has been suggested that the glucose could be substituted for grain or sweet feed, which may further increase the popularity and uptake of the test [71]. However, more research in this area is required.

Diagnosis of insulin dysregulation

Combination of tests

The diagnosis of insulin dysregulation can be achieved by demonstration of tissue insulin resistance or hyperinsulinaemia (basal or after evocative testing). It appears that some horses develop tissue insulin resistance in the absence of compensatory hyperinsulinaemia (decreased peripheral tissue uptake), and others can develop transient hyperinsulinaemia in the absence of tissue insulin resistance (inappropriate intestinal absorption effect), while some horses develop both hyperinsulinaemia and tissue insulin resistance [11]. The problem with current tests is

that they only investigate one arm of the disease and therefore may lack sensitivity and fail to diagnose patients that would benefit from earlier therapeutic measures. Additionally, since the intrinsic value of the different tests described in this review are often calculated based on the results of the EHC or the modified FSIGTT, it is possible that the apparent low sensitivity of some tests is explained by the fact that the tests were not investigating the same arm of insulin dysregulation [40; 45]. Therefore, it may be recommended that the diagnosis of insulin dysregulation be done in a stepwise manner, starting with baseline values of insulin and glucose and finishing with a combination of tests that diagnose tissue insulin resistance as well as tests that assess the enteroinsular axis.

Incretins

With a better understanding of insulin dysregulation, new targets, beyond insulin and glucose, have been studied to facilitate early diagnosis. Studies from the human literature have demonstrated the role that incretins released by the intestine after carbohydrate ingestion play in stimulating the release of insulin, and this effect has now also been demonstrated in horses [11; 72]. The two principal incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP, previously gastric inhibitory peptide) have been investigated in horses [11; 73]. GLP-1 is secreted by pancreatic L cells after ingestion of carbohydrate and increases glucose-stimulated insulin secretion by pancreatic β cells [74]. Plasma GLP-1 has been shown to strongly correlate with insulin secretion in horses [11; 73; 75]. However, in one study, GLP-1 plasma concentrations did not differ between control horses and horses diagnosed with EMS, even when horses with EMS were fed a diet rich in carbohydrates [73]. In other studies, horse/ponies diagnosed as insulin dysregulated on the results of an OGT had a significant increase in post-glucose GLP-1 [11; 73; 75]. The role of GIP has been investigated less frequently in horses but it is also secreted after the ingestion of carbohydrates. It appears to be secreted in larger amounts overall; however, no difference in plasma GIP was found following a

dose of oral glucose in control ponies and ponies diagnosed as insulin dysregulated using an OGT [11]. The stimulation of post-prandial insulin secretion by incretins is important, as this pathway may be a potential target for the treatment of EMS. However, the incretin effect appears to be smaller in horses than in humans, with glucose remaining the primary stimulus for insulin release [11].

C peptide

Proinsulin is cleaved into C peptide and insulin and, unlike insulin, C peptide does not undergo first-passage through the liver increasing its half-life to six times that of insulin [76]. In horses, it has been shown that exaggerated insulin responses to an OGT also result in significantly elevated C peptide concentrations suggesting that C peptide could be used to assess pancreatic secretion and dysfunction in this species [77; 78].

Adiponectin

Adiponectin is an anti-inflammatory cytokine produced by adipocytes that has a role in glucose regulation [79]. While two early studies failed to correlate plasma adiponectin with insulin, even after intravenous or oral glucose challenges [80; 81], more recent studies have demonstrated that plasma adiponectin could be more useful than initially expected [3; 82]. Adiponectin concentration and body mass index are negatively correlated in horses [83]. It has also been shown that feeding horses a diet high in carbohydrates further decreases plasma adiponectin compared to feeding horses an isocaloric diet high in fat [82], which demonstrates that diet can be more important than body weight in lowering plasma adiponectin concentration [82]. Additionally, in a study looking at horses with no previous history of laminitis, a plasma adiponectin concentration <2.5 μg/mL was found to have a sensitivity and a specificity of 80% to predict the occurrence of pasture-associated laminitis within 3 years [3].

In that study, there was no correlation between morphometric markers and plasma adiponectin, suggesting that adiponectin could be valuable in the diagnosis of insulin dysregulation beyond obesity. To date, studies have reported values for both total and high molecular weight adiponectin using a number of different assay types. More recent data suggests that measurement of high molecular weight adiponectin is more relevant with respect to the identification of laminitis risk [3; 82].

Leptin

In some studies, leptin has been associated with decreased insulin sensitivity [41; 60; 84]. However, rather than insulin sensitivity, increased leptin may be associated with increased body condition score [83]. Nevertheless, a leptin concentration of >7.3 ng/mL has been shown to have a sensitivity of 63 – 83% in predicting the occurrence of laminitis [85].

Triglycerides

Hypertriglyceridaemia has been reported to be a component of EMS in horses, and therefore a risk factor for laminitis [2; 60; 86; 87]. However, more recent data show that not all obese horses have EMS and that a metabolically healthy, obese state can exist [88]. As serum triglyceride concentration is often correlated with body condition score, triglycerides may not be a very specific marker of insulin dysregulation and therefore not a valuable predictor of laminitis [3; 89].

Morphometric markers

The physical appearance of the horse has been associated with insulin dysregulation and extensive research had been undertaken to elaborate an equine body condition score [90]. In several studies, an increased body condition score or an increased cresty neck score have been

found to correlate with metabolic status and the occurrence of laminitis [22; 84; 86; 91-94]. In a study by Carter et *al.*, a body condition score >7/9 had a sensitivity of 100% and a specificity of 29 – 44% to predict laminitis whereas a cresty neck score >4 had a sensitivity of 50 – 83% and a specificity of 78 – 80% to predict laminitis [85]. The relatively low sensitivity and specificity of these tests confirms the clinical findings that some obese horses do not have insulin dysregulation and some lean horses can have severely insulin dysregulation and contrary to what has been thought for a long time, increased body condition score may not alter insulin regulation *per se*. In a recent study by Bamford *et al*., horses that gained weight on a high fat diet did not show any decrease in insulin sensitivity whereas horses that gained weight on a carbohydrate diet did, suggesting that the type of diet fed to horses to obtain a significant weight gain is more important than the weight itself [82].

Confounding factors

Beyond the obvious variability induced by the assay used to measure glucose, insulin or other metabolites, the interpretation of different tests may also be altered by horse-related and environmental factors.

Horse factors

The main factor of variability during testing is the horse itself. In almost all studies, there is a significant effect of the individual with frequent outliers limiting the generalisation of the data and underlying the importance of monitoring horses with equivocal results [66; 69; 75; 95]. Another factor of variation, as described above with the EHC, is breed [35]. A significant effect of breed was observed for insulin and GLP-1 responses to either an OGT or a modified FSIGTT with ponies and Andalusian horses showing greater evidence of insulin dysregulation than Standardbred horses [51; 75]. It is anticipated that more breed-associated specificities will be

discovered as genome-wide association studies help to establish a genetic basis for insulin dysregulation [96]. Finally, conflicting evidence regarding age and the association of ageing with hyperinsulinaemia, which may increase the likelihood of older animals developing laminitis, suggests that more research is needed before considering the adaptation of reference ranges [18; 60; 82].

Environmental factors

Several studies have found seasonal variation in the evaluation of insulin dysregulation, with spring and autumn being times of relatively poor insulin regulation [66; 95; 97]. Beyond the increased non-structural carbohydrate content in grass during these seasons, that might explain relative hyperinsulinaemia, the potential relationship between EMS and pituitary *pars intermedia* dysfunction (which is also seasonally affected) may warrant further research, especially in older horses [2]. The effect of diet is also amplified by a circadian rhythm as the same diet fed in the morning or in the evening did not have the same effect on the GLP-1 response [75]. Another important factor of variability during testing for insulin dysregulation is the fed state of the horse. In fasted horses, a resting insulin >20 μIU/mL is suggestive of insulin dysregulation whereas in fed conditions, a resting insulin >60 IU/mL would be suggestive of insulin dysregulation [59]. Similarly, for the OST, different results are to be expected whether the horse is fasted or fed. In a fed horse, a serum insulin >51 μIU/mL between 30 and 60 min post corn syrup is suggestive of insulin dysregulation whereas in a fasted horse, serum insulin >60 μIU/mL between 60 and 90 min post corn syrup is suggestive of insulin dysregulation [68]. In the short IST, it has been shown that fasted horses were at an increased likelihood of being diagnosed as insulin resistant [29]. Fasting may not always be practical and other environmental factors are frequently beyond the control of the clinician so the interpretation of results needs to be undertaken in an informed manner.

Conclusions

There are still many questions to be answered about the pathophysiology of insulin dysregulation in horses, and its links to laminitis. Hopefully, recent advances in the understanding of the biology of the equine enteroinsular axis, as well as the initiation of large scale studies investigating the genetic basis for insulin dysregulation, will improve our diagnostic capabilities and improve the detection of horses at risk before their first episode of laminitis.

There is mounting evidence that the diagnosis of insulin dysregulation should include an investigation of both tissue insulin resistance and the enteroinsular axis. Regardless of the tests used, clinicians should be aware of the potential for test failure or confounding factors and, in cases with equivocal results, the repetition of the same test or initiation of another test may be warranted.

Authors' declaration of interests

No competing interests have been declared.

Source of funding

None

Ethical animal research

Not applicable.

Authorship

Manuscript prepared by F.R. Bertin with sections contributed by M.A. de Laat.

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