**Dilemmas in diagnosis of EMS: is it the waistline or the carbs?**

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*Update on diagnostic tests and laboratory considerations; basal and dynamic tests for insulin dysregulation, insulin resistance and lipid dysregulation.*

**Introduction**

Equine metabolic syndrome (EMS) is a collection of metabolic and endocrine abnormalities associated with a high risk of laminitis. Although many horses with EMS have general or regional obesity, not all obese horses and ponies have EMS and EMS can occur in the absence of visibly increased adiposity. Previously, a diagnosis of EMS was most commonly investigated after the onset of laminitis. The focus now is increasingly on early detection of horses with EMS before the onset of laminitis, allowing preventive strategies to be implemented.

The key feature of EMS and laminitis risk is **insulin dysregulation (ID**). This term was first introduced by Frank and Tadros in 2014 1, and refers to any combination of 3 abnormalities:

1. Tissue insulin resistance (the inability of tissues to respond appropriately to insulin)

2. Basal hyperinsulinaemia

3. Postprandial (post carbohydrate) hyperinsulinaemia.

The aims of this presentation are to:

1. Discuss how best to diagnose insulin dysregulation, including consideration of whether the test is measuring insulin resistance or hyperinsulinaemia. This will include basal and dynamic testing, taking into account practical considerations and whether testing for diagnosis versus monitoring of the syndrome.
2. Discuss the use of ancillary tests such as adipokines can support the diagnosis of EMS.
3. Discuss interpretation of diagnostic tests including assessment of the accuracy and repeatability of the tests, and how to manage the differences between laboratory assays and the reference ranges produced by them versus published in the literature.

**Diagnosis of EMS**

*It’s just fat ponies, why bother with all the diagnostics?*

Firstly, we are increasingly recognising that EMS is not restricted to obese animals, and that any breed, shape or size of horse can be affected. And although there is certainly an association between obesity and EMS, not all obese horses and ponies have EMS. Secondly, diagnostic testing gives us a baseline by which we can assess the severity of EMS, tailor management and monitor the horse’s response to that management. Thirdly, and most importantly, the detection of early, mild cases of EMS allows identification of horses that are at risk of developing laminitis, allowing preventive strategies to be implemented.

*Identifying risk factors in individuals*

Signalment and history: Breeds thought to have a predisposition to developing ID and EMS include UK and Irish native ponies and cobs, Andalusians, Morgans, PasoFinos and Warmbloods, but further work is required and there is ongoing research investigating a genetic basis for EMS. Onset of clinical signs associated with EMS has been most commonly described in young to middle-aged horses, but any age can be affected. There is evidence of an association between increasing age and ID manifest as hyperinsulinaemia in Australian ponies and cobs. 2

Frequently EMS horses are described as ‘easy keepers’, with owners reporting that a small amount of feed is required to maintain weight. Several studies have shown the beneficial effect of exercise on insulin sensitivity and so horses regularly performing high levels of exercise are at lower risk (but not zero risk) of EMS compared to more sedentary animals. Any evidence of previous or current laminitis will significantly increase the likelihood of a horse having EMS.

Clinical examination: Certain phenotypic features are associated with EMS, however these are not pathognomonic.

*Generalised or regional adiposity*. Obesity is highly prevalent in many populations of horses, and is associated with ID and risk of laminitis. It is important to point out that not every obese horse has EMS, and that lean horses can still have ID and EMS. For assessment of adiposity a body condition score is recommended, with scoring systems of 1-9 or 1-5 most commonly being used. Regional adiposity can occur in multiple sites, with the crest, withers and tail head commonly affected. A cresty neck score has been developed and is associated with EMS in some breeds, 3 but does not translate to all breeds. 4

*Evidence of current or previous laminitis.* Hoof changes such as prominent and divergent growth rings, dropped sole and separation of the white line, and characteristic radiographic changes are indicative of laminitis.

**Laboratory testing for EMS**

Hyperinsulinaemia is central to EMS and laminitis risk, and investigation of insulin dysregulation forms the mainstay of laboratory testing for EMS. When interpreting a diagnostic test for EMS, it is important to consider whether it is testing insulin resistance or hyperinsulinaemia, as they can occur independently.

Hyperinsulinaemia is usually a compensatory response to tissue insulin resistance, but can also occur with normal or near normal insulin sensitivity. In all horses, glucose administered orally results in a greater insulin response compared to equivalent dose administered intravenously. The presence of glucose in the intestine stimulates the secretion of gut-derived hormones called incretins, which stimulate the pancreas to secrete a greater amount of insulin than it would in response to the absorbed glucose alone. This mechanism is termed the enteroinsular axis. Incretin derived hyperinsulinaemia can be exaggerated in some horses with ID.

*Basal Insulin concentration*

This is a convenient test as it only requires a single visit. The previously-recommended prolonged fast prior to sampling and cut-off of 20 µIU/ml for diagnosis of EMS 5 results in a very low sensitivity and is no longer recommended by the author. More recent work has shown that 75% of ponies with a 12 h fasted serum insulin >8.5 mIU/mL developed laminitis when subjected to a high non-structural carbohydrate (NSC) dietary challenge. 4 In our hands prolonged fasting results in poor owner compliance so the following protocol is used. Basal glucose concentration is rarely abnormal in horses with EMS, as most insulin resistance is compensated.

Protocol:

1. Feed only low NSC (<12% DM) hay (soak if nec.) overnight or for a minimum of 4 hours before the test. If grazing can’t be avoided it should be restricted/poor quality.
2. Collect blood into serum / EDTA tube (check with laboratory) for measurement of insulin.

Interpretation (Cut-off according to laboratory and assay):

On Immulite analyser <15 µIU/ml = low risk/normal

15-20 µIU/ml = weak positive

> 20 µIU/ml increasing severity of ID

For all negative, ‘low risk’ and weak positive results further dynamic testing is recommended.

**Dynamic tests for insulin regulation**

These tests measure response to orally or intravenously administered glucose +/- exogenous insulin. They are more sensitive for detection of ID compared to basal insulin. There are very few validated reference ranges for these tests, and so recommendations here are taken from a combination of published data and personal communication/experience.

The oral tests predominantly measure the postprandial insulinaemic response to a measured amount of carbohydrate, are generally simpler to perform than intravenous tests and include the contribution of the enteroinsular axis to ID. In addition, as they reflect more closely the insulinaemic response to ingested carbohydrate, they have a greater relevance to pasture associated laminitis.

For assessment of the tissue insulin resistance element of insulin dysregulation, intravenous tests are more standardised and repeatable, as serum glucose and insulin concentrations can be more accurately regulated when administered IV. They are of minimal use for assessing the enteroinsular axis however.

*Oral Glucose test (OGT)*

Protocol:

1. Stable the horse and feed only 1 slice of hay the evening before (or fast for 6 hours before the test).
2. The following morning the owner feeds 1 g/kg bodyweight (BWT) powdered glucose/dextrose, mixed with 1g/kg BWT unmolassed low NSC chaff (e.g. Happy Hoof) and 1g/kg water.
3. Owner records how long the horse takes to consume the feed. If the feed is not completely consumed, weigh the residual feed to allow an estimate of how much glucose has been consumed.
4. Collect a blood sample for insulin and glucose 2 hours after the feed was given.

Interpretation (Cut-off according to laboratory and assay):

Insulin < 85 µIU/ml = normal

85-125 µIU/ml = borderline ID

>125 µIU/ml = Positive ID

If the feed was incompletely consumed, inform the laboratory how much was consumed and they can adjust their interpretation accordingly.

Recent work from Australia looking at the development of laminitis following an oral high NSC feed challenge compared to the OGT has supported a lower cut off of < 65 µIU/ml where resting basal insulin is < 8.5 µIU/ml or 50 µIU/ml based on 2 h insulin concentration only (although the latter may result in some false positives). 6

*Oral Sugar test (OST)*

This uses Karo Light corn syrup (not Karo Lite), which is available from online supermarkets, administered by dosing syringe. The advantage of OST over the OGT is that you don’t rely on the horse eating it, and the sugar is all given at once, producing a more consistently timed peak glucose peak. Conventionally, a dose of 0.15ml of Karo/ kg BWT has been recommended, however this dose results in a low and poorly discriminating insulin peak, and so higher doses of Karo are now being used.

Protocol:

1. Feed only 1 slice/biscuit of hay the evening before (or remove access to feed for 6 hours before the test).
2. Owner administers Karo Light syrup by dosing syringe, either:
	1. Protocol 1: 0.15ml/kg bwt
	2. Protocol 2: 0.45ml/Kg bwt
3. Measure serum insulin concentration at 75 and 90 minutes

Interpretation (cut-off according to laboratory and assay):

Protocol 1: Insulin <30 = negative, 30-45 = borderline, >45 µIU/ml = positive in either sample

Protocol 2: Insulin <30 = negative, 30-55 = borderline, >55 µIU/ml = positive in either sample

*Combined Intravenous Glucose- insulin tolerance test (CGIT)*

This assesses tissue insulin sensitivity. It is more time consuming than oral tests and requires the horse to have a catheter placed the previous evening, making it less practical for use on the yard and in practice. It is useful for borderline cases, or those in which ID is suspected but which are negative with oral tests. The standard CGIT protocol is as described by Eiler et al. 7 A shortened version of the test can be performed, with glucose and insulin being measured only at baseline, 45 minutes and 75 minutes.

Shortened protocol:

1. Place jugular catheter the previous evening.
2. Horse is offered a single slice/biscuit of hay overnight
3. The following morning, baseline insulin and glucose are measured.
4. 150mg/kg glucose (150ml of 50% glucose solution for a 500kg horse) is administered IV, followed immediately by 0.1 unit/kg regular insulin (e.g. 0.5ml of 100IU/ml Humulin S for a 500kg horse)
5. Blood glucose and insulin are measured at 45 and 75 minutes after infusion of glucose and insulin.

Occasionally horses can show signs of hypoglycaemia (normally after 45 minutes). While administration of IV 50% glucose has been described, in the author’s experience, offering mixed concentrate feed or even hay will quickly alleviate clinical signs of hypoglycaemia.

Interpretation (cut-off according to laboratory and assay):

CGIT is considered positive for EMS if any of the following occur:

1. Glucose concentration at 45 minutes > baseline
2. Insulin >100 µIU/ml at 45 minutes
3. Insulin > 20 µIU/ml at 75 minutes

Our research has shown that the insulin component of the GCIT is more useful than the glucose, being both better correlated with basal insulin and less affected by stress.

*Intravenous Insulin tolerance test (ITT)*

This assesses tissue insulin resistance. A simplified, 2-step version of the full insulin response test is recommended, which assesses insulin sensitivity by measuring blood glucose 30 min after i.v. injection of 100 mIU/kg of insulin. A 50% decrease in blood

glucose indicates insulin sensitivity. 8 Hypoglycaemia can occur in this test and if noted, feeding a starchy meal, hay or intravenous or oral glucose can be administered as for the CGIT.

Interpretation:

ITT is considered positive for EMS if the glucose concentration at 30 minutes is >50% of the basal glucose concentration.

**Adipokines**

Adipose-derived cytokines (adipokines) have also been investigated as markers of EMS and are described below.

*Leptin* is derived from adipocytes, and blood concentration increases proportionally with body fat. Leptin has been significantly correlated with insulin concentration in ponies. 2 However, leptin is not commonly available as a diagnostic test, and also may reflect of the size of adipose deposits as well as predict hyperinsulinaemia, potentially confusing the results, so is not commonly used.

*Adiponectin* has insulin sensitising and anti-inflammatory functions, is secreted by fat and circulates in three main forms: trimers, hexamers or high molecular weight. Counterintuitively, adiponectin concentration is lower with increasing levels of adiposity. The high molecular weight form of adiponectin is negatively correlated with BCS and insulin concentration and in a recent longitudinal study 9 total adiponectin was a significant predictor of laminitis occurrence in subsequent years (along with basal and dynamic insulin measurements). Unfortunately, the assays used in these experimental studies have been changed or discontinued, and have correlated poorly with the commercial assays currently available. The longitudinal study above showed basal insulin to have a similar predictive value to adiponectin for occurrence of laminitis. Given the well-established role of hyperinsulinemia and insulin dysregulation in the pathogenesis of laminitis, insulin is still recommended over adiponectin for diagnosis of EMS and assessment of laminitis risk. Adiponectin remains an interesting area of research and may have a role for diagnosis of EMS and monitoring weight loss in the future. Further research and assay validation are required before it is recommended.

**References**

1. Frank N, Tadros EM. Insulin dysregulation. Equine vet J. 2014;46(1):103-12.
2. Morgan RA, McGowan TW, McGowan CM. Prevalence and risk factors for hyperinsulinaemia in ponies in Queensland, Australia. *Aust Vet J.* 2014;92(4):101-6.
3. Carter RA, Treiber KH, Geor RJ, et al. Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. *Equine Vet J.* 2009;41(2):171-8.
4. Dugdale AH, Curtis GC, Cripps P, et al. Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Vet J*. 2010;42(7):600-10.
5. Frank N, Geor RJ, Bailey SR, et al. Equine metabolic syndrome. *J Vet Intern Med.* 2010;24(3):467-75.
6. Meier AD, de Laat MA, Reiche DB, et al. The oral glucose test predicts laminitis risk in ponies fed a diet high in nonstructural carbohydrates*. Domest Anim Endocrinol*. 2017;63:1-9.
7. Eiler H, Frank N, Andrews FM, Oliver JW, Fecteau KA. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. *Am J Vet Res*. 2005;66(9):1598-604.
8. Bertin, F.R. and M.A. de Laat, The diagnosis of equine insulin dysregulation*.* *Equine Vet J.* 2017;49(5):570-576.
9. Menzies-Gow NJ, Harris PA, Elliott J. Prospective cohort study evaluating risk factors for the development of pasture-associated laminitis in the United Kingdom. *Equine Vet J.* 2017;49(3):300-306.