Equine: how to test for and manage insulin dysregulation in horses

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What is equine insulin dysregulation and why does it matter

The equine endocrine disorder 'insulin dysregulation' (ID) is a central feature of 'equine metabolic syndrome' (EMS)(Durham *et al.* 2019). The presently accepted definition of EMS is that it is a collection of pathologic conditions (namely obesity, regional adiposity, insulin dysregulation) that increase a horse's risk of developing endocrinopathic laminitis (Durham *et al.* 2019). Sustained hyperinsulinaemia is the key pathophysiologic mechanism as to why these predisposed horses develop laminitis, so much so, the term 'hyperinsulinaemia-associated laminitis' (HAL) now supersedes the term endocrinopathic laminitis in current literature (Asplin *et al.* 2007, Frank *et al.* 2022).

There are multiple reasons as to why a horse might have hyperinsulinaemia. The term ID encompasses resting hyperinsulinaemia, post-prandial hyperinsulinaemia and insulin resistance (Durham *et al.* 2019). Post-prandial hyperinsulinaemia is a result of exaggerated insulin release from the pancreas in response to oral intake of non-structural carbohydrates (simply put, NSC = simple sugars + starch), i.e. this response is glucose-stimulated. The tendency for post-prandial hyperinsulinaemia in individual equids is thought to have a genetic predisposition, whereby breeds with a higher genetic risk include pony and cob breeds, Warmbloods, Spanish breeds and miniature horses (Durham *et al.* 2019). When these individuals continuously ingest high-NSC feed, hyperinsulinaemia sustained overtime can induce HAL which can be insidious in development (Meier *et al.* 2018b), where early indicators might be missed and only noticed by owners once critical clinical laminitis is reached. There also appears to be a spectrum of severity in ID-affected individuals, where some horses are more exquisitely susceptible to post-prandial hyperinsulinaemia than others (Macon *et al.* 2022).

Insulin resistance on the other hand is a result of reduced tissue sensitivity to the hormone insulin or reduced hepatic metabolism of insulin – both these pathways can also lead to hyperinsulinaemia at rest (McFarlane 2019). The relevance of insulin resistance in EMS is that the metabolic outcomes of obesity and regional adiposity can lead to reduced insulin sensitivity (Carter *et al.* 2009). To overcome insulin resistance of tissues the pancreas releases more insulin, thus, exacerbating hyperinsulinaemia in horses already susceptible to post-prandial hyperinsulinaemia (Carter *et al.* 2009). As horses have a large pancreatic reserve they rarely develop diabetes mellitus as a sequalae, although equine case reports do exist (Durham *et al.* 2009). It is important to note that horses can have post-prandial hyperinsulinaemia without being 'insulin resistant', in turn, there are conditions which can induce peripheral tissue insulin resistance in non-EMS horses (e.g. systemic inflammatory response syndrome, sepsis, stress, starvation, pregnancy) (Frank *et al.* 2022). Additionally, insulin sensitivity appears to decrease with age (Rapson *et al.* 2018).

With laminitis being such a debilitating condition with significant welfare implications, the saying 'prevention is better than cure' certainly holds true. In a cohort of 374 non-laminitic ponies that were followed over four years, serum insulin concentration was the best predictor of future laminitis development (Knowles *et al.* 2023). Whereas, body condition score and cresty neck score (means of identifying horses with obesity and regional adiposity respectively) in the multivariable time-dependent models were not statistically significant factors associated with laminitis development (Knowles *et al.* 2023). Not all obese horses have post-prandial

hyperinsulinaemia, and not all horses with post-prandial hyperinsulinaemia are phenotypically obese (Frank *et al.* 2022). These points place onus on insulin testing in clinical practice to identify horses with ID, thereby identifying those at risk of HAL (Knowles *et al.* 2023).

Indications for insulin testing in horses

As with any equine laboratory testing, it is important that we select tests when they are indicated and interpret the test results in light of the horse's signalment (age, breed, sex), reason for presentation, history and clinical signs. Although breed, age, history, phenotypic traits and clinical signs will raise a clinician's suspicion of ID, ID in horses can only be confirmed through diagnostic testing. Understandably if a clinician has a high suspicion and an owner cannot afford insulin testing, appropriate management changes (see below) should still be made.

The following are situations where insulin testing in horses is indicated, these might be the reason for presentation or findings identified on wellness or prepurchase examinations (Durham *et al.* 2019):

- Horses of breeds with higher risk for ID
- Horses with regional adiposity (e.g. cresty neck, preputial or mammary gland enlargement)
- Obese horses (body condition score >7/9)
- Horses diagnosed with clinical laminitis
- Horses with a history of chronic/episodic laminitis
- Horses with signs of subclinical laminitis (e.g. divergent hoof rings, lamellar wedge formation)
- Horses diagnosed with pituitary pars intermedia dysfunction (PPID)
- Guiding management of horses previously diagnosed with EMS

How to approach insulin testing in horses

Resting insulin – non-fasting

One way to identify hyperinsulinaemia in horses is via measuring a resting 'basal' insulin concentration. Contrary to earlier recommendations, current recommendations are to perform this test in a non-fasted state. This approach is to test horses under their normal dietary conditions, whether that be hay or grass; however, hard feeding within 4 hours of this test should be avoided (Frank *et al.* 2022). In ambulatory practice this is a far more convenient insulin test than dynamic testing, and being a simpler test, it is also more affordable. However, its major disadvantage is that although this is a highly specific test (low risk of false positives), it is not a particularly sensitive test (high risk of false negatives) (McFarlane 2019). This means horses with severe ID will likely be identified via this test (high resting insulin concentration), yet mild-moderately affected horses could be missed (low/normal resting insulin concentration). Thus, for the initial diagnosis of ID it is prudent to start with dynamic testing (see the oral sugar test below). If resting insulin test results are not consistent with your clinical suspicion, dynamic insulin testing should be performed.

An additional application of this test is monitoring horses following management changes once a diagnosis of ID/EMS has previously been made (Frank *et al.* 2022). As mentioned, severity of susceptibility to post-prandial hyperinsulinaemia varies amongst individuals as does resistance to weight loss protocols – management changes that are effective for one ID horse might not be adequate for another. Thus, the resting insulin test can be repeated at revisit appointments to 'assess' the effectiveness of an owner's management changes of a horse with EMS. Determining what the resting insulin concentration is under the new management/feeding conditions, can reflect where management changes have been adequate (low/normal result) or inadequate (high result). Should resting insulin concentration remain high despite management changes, either

the management recommendations have not been followed (poor compliance), the horse is exquisitely susceptible requiring more stringent management changes, or the horse might benefit from the addition of pharmacological intervention.

Dynamic insulin testing – the oral sugar test

Best practice for identifying ID in the context of EMS diagnosis and risk for laminitis is to assess the postprandial insulin response via the dynamic insulin test 'oral sugar test' (OST), or alternatively the in-feed 'oral glucose tolerance test' (OGTT) (Meier *et al.* 2018b; Durham *et al.* 2019, Knowles *et al.* 2023). Dynamic insulin testing reduces the false negative rate compared to measuring resting insulin (McFarlane 2019). The entero-insular axis of horses is involved, therefore, physiologically it might be considered the most clinically relevant equine insulin test. All horses are expected to have an increase in insulin concentration in a given time following an oral sugar stimulation, assuming normal gastric emptying and normal small intestinal function. Whereas horses with ID have an excessive pancreatic release of insulin in response to oral sugar stimulation – whereby cut off values have been designed experimentally for clinical application to detect these excessive responders (Schuver *et al.* 2014, Jocelyn *et al.* 2018; Meier *et al.* 2018b). The advantages of using the OST and why it is becoming the more favourable dynamic test for ID in practice is that it does not rely on the voluntary intake of sugar. Whereas the OGTT requires horses to voluntarily ingest glucose powder on a small amount of chaff which might involve palatability issues. In order to improve test performance, the recommended OST sugar dose and respective insulin cut-off values used have been recently revised (within the past five years).

The oral sugar test protocol (Jocelyn *et al.* 2018): all feed is withheld for at least 3-6 hours prior to commencing this test, water is allowed. Then 0.45ml/kg corn syrup (e.g. Karo® Light, in New Zealand this can be ordered online) is administered via a 60ml catheter-tip syringe (in cold weather it helps to put the corn syrup bottle in a warm water bath prior, and/or cut the syringe tip off for easier administration). Following administration of the sugar dose, blood should be sampled at 60-minutes and/or 90-minutes after and submitted for insulin testing. Sampling at both time points might increase the likelihood of capturing those with peak insulin concentrations occurring later than 60-minutes, however, this approach costs more to the client. Some advocate for sampling once between 60- and 90-minutes (i.e. 75-minutes) (Frank *et al.* 2017). Although taking a blood sample prior to administering sugar also used to be recommended, it is no longer essential as a 'time zero' sample is akin to a fasted resting insulin. With the development of stall-side insulin testing (see below), a time zero sample could be included as a screening test because if the insulin results are high the veterinarian might not need to proceed with an OST. Another application of stall-side insulin testing is if results are below the cut-off at 60-minutes, the veterinarian could proceed with sampling at 90-minutes in case of delayed peak insulin concentrations.

Dynamic insulin testing – insulin tolerance test

In contrast to testing for post-prandial hyperinsulinaemia, a clinical test for detecting tissue insulin resistance is the 'intravenous insulin tolerance test' (ITT). The ITT is a test of insulin sensitivity and does not involve the entero-insular axis (Bertin and Sojka-Kritchevsky 2013). Because this test involves intravenous administration of insulin, it is less practical in ambulatory practice and runs the risk of iatrogenic hypoglycaemia should the horse not be insulin resistant. Additionally, as this test involves the measurement of glucose over time it is important that confounding physiologic variables (e.g. stress, excitement, recent exercise) are avoided prior to testing (Frank *et al.* 2022). An alternative test for assessing insulin sensitivity is the 'combined glucose-insulin test', the design of this test reduces the risk of hypoglycaemia but is more labour intensive making it less practical in practice (Eiler *et al.* 2005).

The insulin tolerance test (Bertin and Sojka-Kritchevsky 2013): The horse can have access to feed prior to this test, in fact it is important that horses are not fasted for this test. Blood is collected at time zero for glucose measurement, then a 0.1mg/kg dose of regular (soluble) insulin is administered intravenously. Blood glucose measurement is repeated 30-minutes after insulin administration. Glucose can be measured using a handheld device stall-side (more convenient), or can be collected into a fluoride-oxalate (grey) tube for submission to a commercial laboratory for measurement. Once testing is completed it is important the horse is fed. Insulin resistance is suggested if the decrease in blood glucose across the two time points is <50%. If a horse does not demonstrate insulin resistance on the ITT, it is still important to perform the OST, as discussed earlier, not all horses with ID are insulin resistant and the ITT is not a test that assesses for post-prandial hyperinsulinaemia.

Insulin testing in New Zealand and interpreting test results

Once it has been decided insulin testing is indicated for a horse, the owner has consented to the expense of testing and testing is performed, the subsequent decision is the type of insulin assay. In New Zealand, sending equine blood to commercial laboratories for serum insulin measurement (plain tube) is no longer the only means of measuring insulin concentration in horses. The lateral-flow stall-side test 'Wellness Ready™ Insulin Test' is also available in New Zealand, this test uses whole equine EDTA-coagulated blood and provides an insulin concentration in 15-minutes (Berryhill *et al.* 2023). Although highly convenient by allowing for real-time management recommendations to be made based on immediate results, and likely a more affordable way for measuring insulin for clinics and clients long-term, the reporting range is limited to 20-99.9µIU/ml. Low (normal) insulin values are denoted as <20µIU/ml, and exceedingly high values are reported as >99.9µIU/ml. Despite this, the binary changes as to whether a result is above or below a given cut-off value can still be determined, and the test's validation study showed acceptable precision and good association with radioimmunoassay (RIA) as the 'gold standard' (Berryhill *et al.* 2023). Commercial laboratories in New Zealand do not disclose the assay used for equine insulin testing when results are released, but can be sought if requested. For example, IDEXX provide cut-off values published by the Equine Endocrinology Group (EEG) for interpretation of test results that correspond with the cut-offs for RIA and chemiluminescent assay using the Immulite 1000 analyser (Frank *et al.* 2022).

The overarching principle of how equine insulin results are interpreted is that increasing insulin concentrations are associated with increasing risk of HAL, i.e. the higher the concentration, the higher the risk (Frank *et al.* 2022). When receiving insulin results, it is important that the correct 'cut-off' values are applied as there are different values for interpreting resting insulin results and insulin concentrations following the OST. These cut-offs are published open-access via the EEG website, the recommendation are based on peer-reviewed literature and are updated regularly. When comparing insulin test results of an individual over time, it is important to ensure the same assay is used (i.e. 'apples-to-apples') if you are to draw conclusions from trends over time. This is because reference intervals and 'cut-offs' are specific to the assay type (McFarlane 2019). It is also worth noting that insulin test results can be reported using different units of measurement i.e. mU/L or pmol/L, thus, when interpreting your test result against recommended cut-off values ensure that the units match.

Managing horses with insulin dysregulation

Reviewing the key pathologic components of EMS – obesity, regional adiposity, insulin dysregulation – it is logical that weight loss through caloric restriction and regular exercise, in conjunction with minimising postprandial hyperinsulinaemia through reducing dietary NSC, are necessary management changes to combat these problems (Durham *et al.* 2019). Thus, through these specific management tactics of horses with ID, the goal is to prevent the development of HAL in at-risk individuals. The degree to which dietary NSC is restricted should correspond to

the severity of hyperinsulinaemia identified via insulin testing. For horses that are already presenting with laminitis, regular exercise to promote weight loss and increase insulin sensitivity is no longer an available tactic, placing onus on careful elimination of NSCs in the diet of these horses.

EMS feeding recommendations for reducing NSC intake were originally adapted from management recommendations for horses with polysaccharide storage myopathy. Researchers found that forage with NSC <12% per dry matter (DM) basis would reduce post-prandial pancreatic insulin responses (Borgia *et al.* 2009). However, such a recommendation does not provide guidance on the total amount of NSC tolerated per meal by horses susceptible to post-prandial hyperinsulinaemia; indeed, 12% NSC can be too much for horses with severe ID. A randomized crossover experiment using six horses with ID and eight control horses identified a NSC threshold of approximately 0.1g/kg bwt/meal, above which insulin responses significantly increased compared with non-insulin dysregulated horses (Macon *et al.* 2022). This investigation also revealed the variability in insulin response between individual horses with ID, highlighting the need to individually tailor the dietary plan to each individual patient and to monitor non-fasted insulin levels to ensure that NSC restrictions are adequate on the prescribed diet. This can be a challenge in the exquisitely NSC-sensitive horse: grass access should be eliminated, hay should be weighed (1.5% of bwt provided per day) then soaked for at least 60-minutes, and commercial feed products must be closely scrutinised as labelling is not always fulling transparent regarding NSC content (e.g. reporting the sugar percentage but not the starch percentage and vice versa). In otherwise healthy horses, commercial pelleted feeds could be eliminated entirely whereby daily vitamin/mineral supplementation can be provided through powdered formulations mixed in a small amount of low-NSC products such as beet pulp, soy hulls or fermented lucerne products available in NZ (Fiber Protect® or Fiber Ezy®). In highly susceptible horses, as well as those resistant to weight loss strategies, evidence suggests gradually transitioning to a diet containing up to 50% straw might be beneficial (Jansson *et al.* 2021). In lean horses (e.g. BCS 4-5/9) with ID, care with NSC intake remains essential and calories to maintain ideal body weight should be provided through good quality fibre and oil. In ID horses where weight-gain is required, it is less important to restrict the amount of hay permitted, and adding oil up to 1ml/kg/d (e.g. divided over 2-3 hard feeds per day) can be of assistance.

Feeding strategies for obese equids and tactics on addressing obesity with horse owners was covered in detail by David Rendle's 'How To' in the 2023 NZVA Conference Proceedings. Additionally, the indications for pharmacological management of horses with ID, obesity and laminitis was also covered adeptly in this paper and is also discussed in detail in a recent open-access review (Sundra *et al.* 2024a). Notably is the growing interest in the preliminary reports suggesting sodium-glucose-cotransporter-2-inhibitors (SGLT2i) can assist in reducing insulin concentrations, promote weight loss and accelerate laminitis recovery (Meier *et al.* 2018a; Lindåse *et al.* 2023, Sundra *et al.* 2023). Reports discussed in last year's NZVA proceedings are extremely encouraging. However, there is emphasis in the literature that this class of drug is to be reserved only for HAL cases and cases of severe ID that appear to be refractory to appropriate dietary changes (i.e. persistent hyperinsulinaemia on repeated insulin testing over time despite appropriate dietary NSC reduction), as these drugs are not without side effects (Frank *et al.* 2022). Through the mechanism of urinary loss of glucose through the inhibition of renal glucose reuptake, resting insulin concentrations are reduced but lipid mobilisation (hypertriglyceridaemia) can develop, as well as polyuria and polydipsia (Sundra *et al.* 2024b). Thus, triglyceride (TG) monitoring is also recommended while horses are treated with SGLT2i drugs. Although elevated TG concentrations are often asymptomatic, feed intake should be monitored and if clinical signs of inappetence and/or dullness develop treatment should be stopped (Kellon and Gustafson 2023). Until there is more research on long-term effects in horses of this drug class, it might be prudent to limit the use of this drug to <3 months (Frank *et*

al. 2022). In New Zealand, this class of drug is off-label in horses and unfortunately some of the medications studied in horses are not readily available. Ertugliflozin capsules (0.05 mg/kg PO SID) can be sourced through a NZ veterinary compounding pharmacy, alternatively empagliflozin tablets can be prescribed off-label through human pharmacies using an anecdotal dose 0.02-0.05mg/kg PO SID, noting that the later has not be formally studied in horses and the dose rate was obtained through expert personal communication.

Conclusion

Identifying horses that have insulin dysregulation through insulin testing and implementing exercise and dietary changes in order to reduce periods of hyperinsulinemia, should be our primary goals to ensure optimal well-being and prevent endocrinopathic laminitis (HAL) in these at-risk horses. Pharmacological intervention with SGLT2i for severe ID and HAL cases refractory to appropriate management changes is gaining enormous attention in equine practice due to promising clinical benefits, improving the welfare of affected horses.

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