TITLE: Clinical insights: Diagnosis of laminitis

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Endocrinopathic laminitis encompasses laminitis associated with insulin dysregulation (ID) linked to an underlying endocrine disease, namely equine metabolic syndrome (EMS) and/or pituitary pars intermedia dysfunction (PPID), or corticosteroid therapy. This form of laminitis has been the focus of a considerable amount of research effort recently and improving ways of making a definitive diagnosis has been a particular emphasis.

A diagnosis relies firstly on accurate recognition of the disease by owners, as this then prompts examination and treatment by a veterinarian and/or farrier. By conducting a cross-sectional study involving first opinion veterinary practices in the UK, Pollard et al [1] sought to establish whether cases of owner-suspected laminitis would be confirmed as laminitis by the attending veterinary surgeon. All owners-suspected laminitis cases were confirmed upon veterinary examination (n=51); however 45% of cases diagnosed as laminitis by a veterinary surgeon were not recognised as such by owners. This failure of laminitis recognition by owners highlights the need for evidence-based education to ensure early disease detection. It also validated inclusion of cases of owner-recognised laminitis in prospective and retrospective studies.

It is now universally agreed that insulin dysregulation (ID) is the key, central feature of equine metabolic syndrome (EMS). In addition, ID is a feature of the subset of animals with pituitary pars intermedia dysfunction (PPID) that develop laminitis. Equine ID manifests in three forms, namely basal hyperinsulinaemia, an excessive insulin response to oral carbohydrate and peripheral (tissue) insulin resistance. Thus, detection of ID is essential to identify animals at an increased risk of endocrinopathic laminitis. This in turn allows targeted implementation of preventative management strategies to reduce the risk of disease occurrence. A number of recent publications have sought to determine the ideal test to detect ID in clinical cases.

Basal hyperinsulinaemia can be detected by measuring circulating insulin concentrations. However, the sensitivity of measuring basal insulin concentrations alone to diagnose ID is low and a dynamic test is preferred. Dynamic tests will either detect an excessive insulin response to oral carbohydrate or peripheral (tissue) insulin resistance depending on the individual test. The oral glucose test (OGT), oral sugar test (OST) or a dietary meal challenge test can be used to identify an excessive insulin response to an oral carbohydrate, whilst the euglycaemia hyperinsulinaemic clamp (EHC), frequently sampled intravenous glucose tolerance test (FSIGTT), insulin tolerance test (ITT) and combined insulin glucose test (CGIT) are advocated to detected peripheral insulin resistance.

The oral glucose test was the first test to be promoted for the detection of ID; however, the repeatability of the test had not been reported. A recent study by de Laat and Sillence [2] revealed that the OGT is reasonably repeatable, that a post-glucose sampling point of either 90 or 120 minutes was acceptable and that an equivalent dose of dietary carbohydrate provided in the form if a commercial grain mixture (meal challenge test) was a viable and more palatable option for the test.

More recently, the OST, which uses corn syrup rather than glucose powder to stimulate an insulin response, has been proposed as a field test to identify ID that manifests as an excessive insulin response to oral carbohydrate. It is recommended that the OST be performed after an overnight fast, but fasting is often impractical in animals kept solely at pasture. Knowles et al [3] determined that for dichotomous interpretation (ID or not ID), similar results were obtained using cut-offs of serum insulin concentration >60 µiu/ml at 60 or 90 min post corn syrup if the test is performed in fasted animals and >51 µiu/ml at 30 or 60 min post corn syrup in animals maintained at pasture. Thus, fasting is not essential. However, at any single time point, within-subject coefficients of variation in fasted animals

were 40% and in animals maintained at pasture were 31%. Therefore, clinicians should beware of the limits of interpreting changes in absolute OST results. For example, following implementation of a weight loss program or initiation of a pharmacologic intervention, owing to poor repeatability, small changes in OST results should not be over-interpreted.

For the OST, only a dose of 0.1 or 0.15 mL/kg bwt corn syrup has been evaluated previously [4]. Thus, Jocelyn et al (2018)[5] sought to determine the effect of varying the dose of corn syrup (0.15 vs 0.30 vs 0.45 mL/kg bwt) on the insulin and glucose response to the OST and the test's ability to distinguish between ponies with a history of laminitis and those without. The insulin response following 0.15 mL/kg bwt corn syrup was not significantly different from that following 0.3 mL/kg bwt at any time point. However, the insulin response following 0.45 mL/kg bwt corn syrup significantly differed from both lower doses at all time points apart from 0 min. The repeatability of the test was fair and there was no difference between the resulted obtained when the ponies were fasted for 6 hours or maintained at pasture prior to the test. Using the serum insulin concentration at 60 min, a cut-off value of 110 μiu/ml (measured using a radioimmunoassay) was able to differentiate between previously laminitic and non-laminitic ponies. Thus, using a dose of 0.45ml/kg bwt corn syrup improves the ability of the OST to detect ID that manifests as an excessive insulin response to oral carbohydrate.

Whilst both the OGT and OST are advocated as tests suitable for the detection of ID, the two tests have not been directly compared. Smith et al [6] compared the two tests in horses (n=5) and ponies (n=8) of unknown insulin sensitivity. Using previously defined criteria of ID, the OGT identified 7/13 animals as ID, whereas the OST identified 5/13 animals as ID. Thus, the OGT and OST agreed in 85% of equine subjects, but the results of the two tests are not comparable in all cases. Therefore, if repeated

tests are carried out, care should be taken to ensure that the same test is undertaken each time and that the two tests are not used interchangeably.

Finally, Bertin and de Laat [7] reviewed the different tests currently available to diagnose ID that manifests as peripheral (tissue) insulin resistance. An ideal test would be able to detect horses in the early stages of ID and would have a strong intrinsic value considering the many environmental and individual animal factors that have been shown to confound the results of any given test. The authors concluded that with respect to the possible tests advocated for the diagnosis of peripheral insulin resistance, fasting blood glucose concentration and proxies derived from glucose and insulin concentrations are poor diagnostic tests; that the euglycaemia hyperinsulinaemic clamp (EHC) and the frequently sampled intravenous glucose tolerance test (FSIGTT) were only suitable in the research setting; that the insulin tolerance test (ITT) is suitable for assessing tissue IR; and that the combined insulin glucose test (CGIT) requires further validation. In addition, it should be acknowledged that regardless of the test 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.

When undertaking any test to detect any of the manifestations of ID, it should be remembered that a number of innate (breed, sex, adiposity, genetics) and environmental (diet, exercise) factors affect insulin dynamics in equids. Jacob et al [8] sought to evaluate the effect of age and dietary carbohydrate profile on insulin and glucose dynamics. The effect of adaptation to diets containing varying amounts of starch, fibre and sugar on the glucose and insulin dynamics in two breeds of healthy non obese adult and aged horses was determined using minimal model analysis of an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT) as well as the glucose and insulin responses to

an OST and a dietary meal challenge test. The study found that the effect of age, breed and diet on glucose and insulin dynamics was variable depending on the assessment. The responses at the tissue level (FSIGTT) revealed that age influences the acute response insulin to glucose (AIRg), regardless of diet, whilst adaptation to starch and sugar improves tissue insulin sensitivity (SI) in both adult and aged horses. In contrast, at the enteral level (assessed using OST), minimal changes in glucose and insulin parameters due to dietary adaptation were detected. In contrast, the dietary meal challenge (a single meal of the diet) demonstrated enhanced postprandial hyperinsulinaemia in both adult and aged horses, following adaptation to both starch- and sugar-rich diets compared to the control and high fibre diets. Thus, this study highlights the need to consider age and diet when evaluating glucose and insulin dynamics using certain tests.

Whilst ID is the central feature of endocrinopathic laminitis, there are other additional risk factors. Previous studies have evaluated a variety of risk factors in groups of animals after disease occurrence only and any differences detected may reflect the disease rather than a predisposition. Identifying risk factors prior to disease occurrence would allow the targeting of preventive management strategies. Potential risk factors other than ID that require investigation include obesity, inflammatory cytokines and markers of endothelial dysfunction. Therefore, a prospective cohort study was undertaken to investigate these risk factors in animals prior to disease occurrence [9]. Various phenotypic and metabolic markers were evaluated in a cohort of 446 animals with no history of laminitis. After 1, 2 and 3 years, respectively, 18 (4.0%), 30 (6.7%) and 44 (9.9%) animals were reported to have had laminitis. Plasma adiponectin, and serum basal insulin and insulin post-dexamethasone concentrations were significantly associated with laminitis occurrence cumulatively after 1, 2 and 3 years. Combinations of these biomarkers did not improve their predictive value and surprisingly, the development of laminitis was not associated with regional or generalised obesity, hyperleptinaemia or hypertriglyceridaemia. Thus, risk factors for future laminitis prior to disease occurrence include low

plasma adiponectin and high serum basal insulin or insulin post-dexamethasone concentrations. It is possible that measurement of these in animals could be used to identify animals at an increased risk of endocrinopathic laminitis before the disease occurs for the first time. Since the radioimmunoassay used in this prospective study to measure plasma total adiponectin concentrations is no longer valid, samples from this study were used to validate an immunoturbidimetric assay [10].

Finally, analysis of plasma adrenocorticotropic hormone (ACTH) concentration aids diagnosis of pituitary pars intermedia dysfunction (PPID), the second endocrine disease associated with an increased risk of laminitis. Concentrations are most commonly measured using a validated chemiluminescent-immunoassay (CI). However, an automated immunofluorescence assay (IF) has newly been validated in the horse and comparison of the validated chemiluminescent-immunoassay (CI) and immunofluorescent (IF) assays was limited [11]. Thus, Knowles et al [12] sought to compare the assays using blood samples collected from a cohort of ponies in autumn (n=99) and spring (n=88) and additionally to assess assay cross-reactivity to ACTH fragments. The study found that the results obtained with the IF assay were proportional to, but lower than, those obtained using the CI assay, such that the results cannot be used interchangeably. However, using appropriate cut-off values specific to the assay, agreement for binary classification was good. A different relationship was found between ACTH concentration results generated by the two methods when measuring concentrations in samples collected from the same animals in the autumn compared with the spring. A probable explanation for this finding is cross-reactivity to or interference by a substance present in equine plasma at greater concentrations in the autumn than the spring, such as other POMC derived peptides. This was investigated by the addition of commercially available synthetic ACTH fragments to equine plasma samples. Cross-reactivity or interference was demonstrated with both assays, but the most marked effect was of corticotropin-like intermedia peptide (CLIP) on the CI assay. Thus, the authors concluded that a naturally occurring autumnal increase in endogenous CLIP production could be

148 responsible for the differences between ACTH assays in the present study. Finally, of the 88 ponies
149 with both spring and autumn samples, 56 (64%) exceeded a published autumn CI threshold (>47
150 pg/ml), of which 39 (70%) were below the equivalent threshold (<29 pg/ml) the following spring
151 without treatment. Thus, clinicians should interpret the results of basal ACTH testing with caution and
152 with reference to clinical signs and in horses that are diagnosed with PPID during the autumn, begin
153 pergolide treatment and are then retested the following spring, clinicians should be very cautious
154 before attributing a return of ACTH concentration to reference range to pergolide treatment.

155

156 In conclusion, these nine recent publications have highlighted a number of important advances in
157 knowledge relating to the diagnosis of endocrinopathic laminitis. Firstly, there is a need for further
158 evidence-based education to address the under-recognition of laminitis by owners. Secondly, the oral
159 glucose test (OGT), the OST (OST) using a higher dose of corn syrup (0.45 ml/kg bwt) or a meal
160 challenge test can all be used to detect an excessive insulin response to carbohydrate, but the tests
161 do not always agree and have only fair repeatability. Thirdly, the insulin tolerance test (ITT) is the most
162 suitable test currently available for assessing peripheral (tissue) insulin resistance and that regardless
163 of the test used to detect ID, many factors of variation, such as breed, diet and age could potentially
164 confound the results. Fourthly, whilst ID is the central feature of endocrinopathic laminitis, there are
165 other additional risk factors including low circulating concentrations of the adipokine adiponectin.
166 Measurement of these could be used to identify animals at an increased risk of endocrinopathic
167 laminitis before the disease occurs for the first time. Finally, the results obtained using two assay
168 available for the measurement of ACTH concentrations in animals suspected of having PPID are not
169 interchangeable; ACTH fragments cross-react with the CI assay; and ACTH concentrations above the
170 seasonally adjusted reference range in autumn frequently return to normal the following spring and
171 should be interpreted with caution. All of these papers can be read in full in this special laminitis online
172 edition.

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