

Endocrinopathic Laminitis

In recent investigations, Equine Metabolic Syndrome (EMS) and pituitary pars intermedia dysfunction (PPID) have collectively accounted for around 90% of laminitis cases seen in ambulatory practice and therefore account for the overwhelming majority of “pasture-associated” laminitis cases. An interrelationship between EMS and PPID is subject to much debate. Both conditions are associated with hyperinsulinaemia, insulin resistance and laminitis and the 2 conditions may coexist in some animals. Therefore an endocrine investigation of laminitis should always consider both conditions especially in horses in an older age group (e.g. > 10 years). As PPID is progressive in nature then inevitably a spectrum of disease will be present varying between subclinical cases; to those with laminitis but no further signs; to those with extreme end-stage clinical disease with hair coat changes, PU/PD, laminitis, etc.... Although PPID is associated with ageing, that does not mean that it cannot occur in young horses. Many well described cases occur in horses well under 10 years of age. Similarly, not all EMS cases are seen in fat native ponies – other breeds and even apparently lean horses can be affected. ***Thus all laminitis cases are deserving of an endocrine evaluation unless an obvious alternative cause exists*** (e.g. colitis, retained placenta etc..).

Endocrinopathic laminitis investigation can be considered as a multistep procedure as outlined below (the steps can be sequential or simultaneous).

STEP 1: Testing for Pituitary Pars Intermedia Dysfunction (PPID)

The logical starting point with endocrinopathic laminitis investigation should be to rule in or rule out PPID given that there is a specific and effective medication for this disease (pergolide mesylate; Prascend, Boehringer Ingelheim Vetmedica).

Basal plasma ACTH

Recent expert consensus (Durham et al. 2014) is that basal plasma ACTH provides the most appropriate and practical screening test for PPID.

1. Collect EDTA plasma sample
2. Chill sample within 3 hours of collection
3. Centrifuge prior to shipping to laboratory (gravity separated or whole blood samples can be shipped as long as they remain chilled and do not freeze)
4. Ship to laboratory using guaranteed overnight delivery in chilled packaging (freeze centrifuged plasma if delivery is delayed)

PPID testing requires that an equine-validated ACTH assay is used and that local seasonally-adjusted reference ranges are developed by the testing laboratory to take account of the autumnal increase in pituitary activity, as first described at The LEH Laboratory (Copas et al. 2012). Between August and October pituitary activity increases far more in PPID than in normal horses meaning that basal ACTH has greatest sensitivity and specificity at that time (Copas et al. 2012, McGowan et al. 2013). ACTH is not stable for more than about 3 hours at room temperature and therefore chilling is crucial and must be maintained during shipping to the laboratory. Several studies examining the effects of protease inhibitors added to the sample have failed to find

significant benefits. Centrifuged plasma is generally preferred although whole blood or gravity separated plasma can be sent to the laboratory as long as the sample remains chilled and does not freeze. The effects of pain and stress on ACTH concentration are limited and probably don't interfere with diagnosis unless moderate to severe.

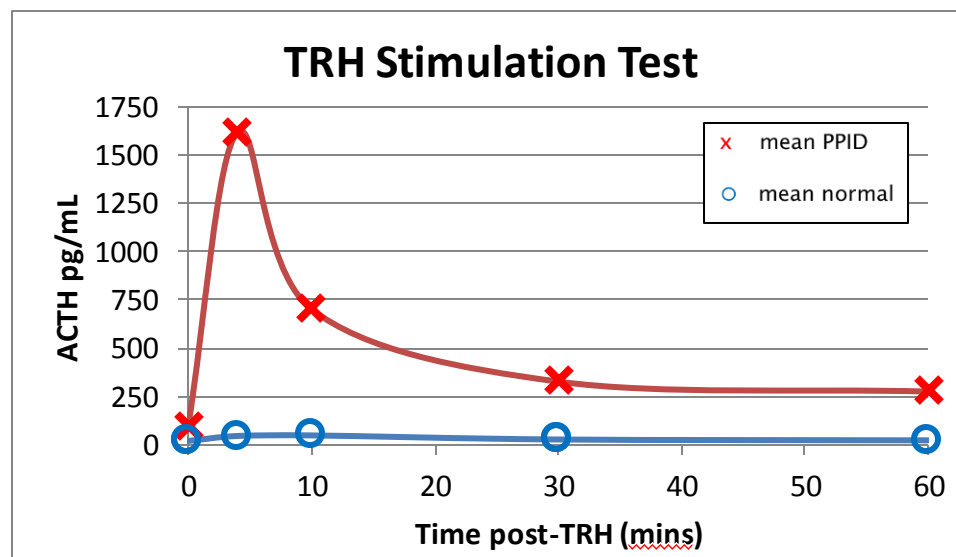
When *borderline or unexpected* results are obtained then 2 further choices exist: either retesting using basal ACTH between August and October inclusive when test accuracy is maximal; or using the TRH stimulation test between December and June inclusive:

TRH Stimulation Test

1. Collect baseline EDTA plasma sample for ACTH analysis
2. Inject 1 mg TRH iv
3. Collect a further EDTA plasma sample at 10 minutes following TRH.
4. Process plasma samples as per measurement of basal plasma ACTH procedure above

Measurement of ACTH following stimulation with TRH has been shown to have higher diagnostic accuracy than measurement of resting ACTH (Beech et al 2007). There are clearly seasonal effects on the TRH stimulation test also and these have not been fully translated into seasonal reference intervals. **Therefore this test cannot be properly applied between July and November.** TRH is currently unlicensed in UK but can be supplied by The LEH laboratory on request. Horses sometimes yawn, cough, show mild tremors or demonstrate flehmen response following TRH injection but no serious adverse effects have been reported.

PPID is indicated by a post stimulation plasma ACTH value greater than 120 pg/mL at 10 minutes post TRH or a 30 minute test can be performed using a cutoff of 65 pg/mL (N.B. as greater experience of this test is acquired, expect further refinement of reference intervals).



STEP 2: Assessment of insulin status

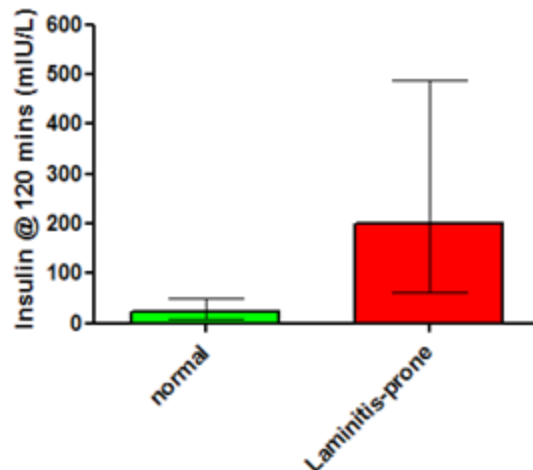
When a diagnosis of PPID has been investigated, consideration should then be given to further endocrine factors relevant to both PPID+ and PPID- cases. Given evidence that hyperinsulinaemia *per se* is the probable direct trigger factor for laminitis, then there is a compelling argument for use of tests that examine an individual's inherent tendency towards hyperinsulinaemia. Hyperinsulinaemia of sufficient magnitude/duration to precipitate laminitis may be associated with PPID and/or EMS.

STEP 2a: Testing for hyperinsulinaemia

At its most simple this might involve measuring basal serum insulin concentration with or without withholding of food. However, this is a very insensitive test with the majority of laminitis-prone individuals returning normal basal insulin concentrations (typically < 50 or <20 mIU/L depending on whether fed or fasted). Greater test sensitivity, and therefore better identification of laminitis prone individuals, is achieved by provocative testing. As laminitis is frequently triggered by oral consumption of grass, these provocative tests make most pathophysiologic sense when they involve oral sugar dosing with subsequent measurement of endogenous insulin response; tests intended to mimic the effects of grazing high-sugar grasses (or eating starch-rich cereal feeds). This is usually performed after an overnight fast. There are 2 main choices:

The Oral Glucose Test

Measure serum insulin 2 hours after a horse consumes a feed of chaff containing 1 g/kg BWT glucose or dextrose powder (normal response < 85 mU/L insulin, see chart below). Overnight fasting encourages rapid consumption of the glucose meal and it is always worth checking plasma glucose also at 2 hours to see that the glucose meal was properly absorbed and the test is valid (expect to see glucose >8 mmol/L).



The Corn Syrup Test ("Oral Sugar Test")

This involves oral dosing with 15 mL per 100 kg BWT Karo Light Corn Syrup (usually readily obtainable via internet shops), again after an overnight fast. Using this test insulin should be measured 75 minutes after being dosed with the syrup (normal response <60mU/L insulin). The advantage of this test is that the sugar can be dosed straight into the horse rather than relying on

rapid voluntary consumption.

STEP 2b: Testing for Insulin Resistance (IR)

Although IR does not appear to be a direct cause of laminitis, its presence predisposes to hyperinsulinaemia and therefore assessment of IR is useful diagnostically in EMS and PPID. Additionally, experience suggests that improvement in tests for IR more commonly follow successful management of EMS than do improvements in Oral Sugar Challenge Tests above, meaning that IR tests may be better for monitoring success. Tests for IR necessarily evaluate the function of insulin and therefore require intravenous challenge tests. Two main options exist:

The 2-step Insulin Tolerance Test (ITT)

This is probably the simplest test for IR in practice and involves measuring plasma glucose before and 30 minutes after a bolus of 0.1 U/kg neutral insulin. Insulin fails to suppress glucose in IR horses which then have a 30 minute plasma glucose >50% of the baseline (NB. Normal horses might experience hypoglycaemia 30-90 minutes after insulin and therefore close monitoring is important).

The Combined Insulin-Glucose Test (CGIT)

This test can also be used to indicate the presence of IR. Glucose (150 mg/kg) is injected as 50% dextrose iv (30 mL per 100 kg), followed by neutral insulin (0.1 U/kg). Frequent samples for glucose and insulin can be taken over 2-3 hours although a simplified version of the test involves only measuring plasma glucose at 45 mins (normal response should be back to baseline) and insulin at 75 minutes (normal response < 100 mU/L).

STEP 3: Further related tests

The third tier of test involves examination for factors that predispose to IR (and therefore indirectly promote hyperinsulinaemia, which in turn promotes laminitis). These include clinical markers such as obesity (body condition score, cresty neck score, girth:height ratio etc..) and also laboratory markers such as hypertriglyceridaemia, hyperleptinaemia and hypoadiponectinaemia. These latter 2 analytes are known as adipokines and derive from fat depots and may interfere with insulin action. Further research is still needed to establish their diagnostic usefulness, but adiponectin appears to most useful at this stage, and we hope to offer adiponectin testing in the next few months.

Beech, J. et al. (2007) Adrenocorticotropin concentration following administration of thyrotropin-releasing hormone in healthy horses and those with pituitary pars intermedia dysfunction and pituitary gland hyperplasia. *J Am Vet Med Assoc.* 231, 417-26.

Copas, V.E. and Durham, A.E. (2012) Circannual variation in plasma adrenocorticotrophic hormone concentrations in the UK in normal horses and ponies, and those with pituitary pars intermedia dysfunction. *Equine Vet J.* 44, 440-3.

Durham, A.E. et al. (2014) The diagnosis and treatment of PPID in the horse. *Equine Veterinary Education* 26(4), 216-223.

McGowan, T.W. et al. (2013) Evaluation of basal plasma α -melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. *Equine Vet J.* 45, 66-73.