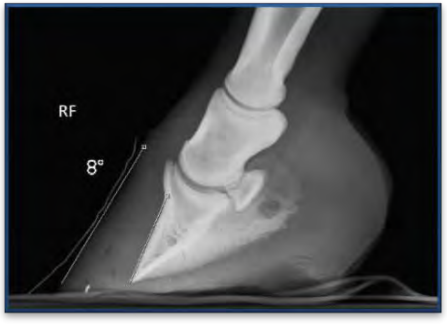
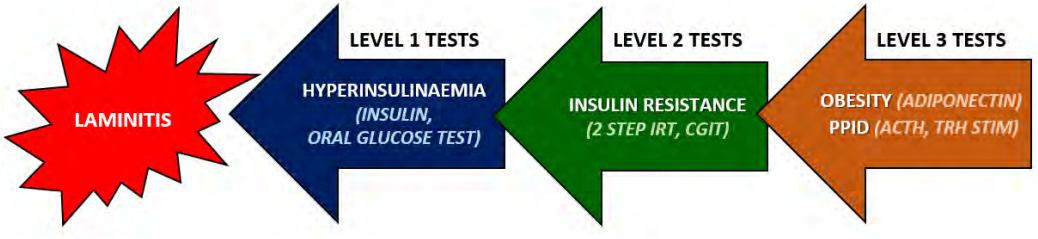
ENDOCRINOPATHIC LAMINITIS

In recent published studies, Equine Metabolic Syndrome (EMS) and pituitary pars intermedia dysfunction (PPID) have collectively accounted for around 90% of laminitis cases seen in ambulatory practice. Certain preconceptions of both diseases have been brought into serious question. Several post-mortem or CT-confirmed PPID cases have been reported as young as 6-7 years of age, often with no clinical signs beyond laminitis. Also “non-obese” EMS cases are reported. Thus, all cases of laminitis deserve endocrine testing unless there is an obvious alternative explanation (e.g. metritis, colitis, excessive load bearing).



A logical approach to investigation and diagnosis mirrors what we know about the pathophysiology of endocrine laminitis. This translates to 3 categories of useful tests, each of which gives us slightly different information about a case which is highly relevant both to diagnosis and to treatment/management.

1) ***Hyperinsulinaemia*** is the direct trigger for laminitis and we can test for this (see below)
2) Horses with ***insulin resistance*** are more likely to be hyperinsulinaemic (and we can test for this)
3) Horses that are ***obese*** and/or have ***PPID*** are more likely to have insulin resistance (and we can test for those)



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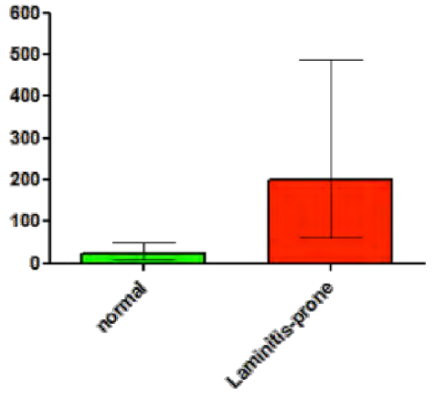
1. LEVEL 1 TESTS - HYPERINSULINAEMIA

Given evidence that hyperinsulinaemia per se is a direct trigger factor for laminitis, then there is a compelling argument for use of tests that examine an individual's inherent tendency towards hyperinsulinaemia. Therefore testing an individual to see if they have a hyperinsulinaemic tendency is helpful to infer increased risk of endocrine laminitis (EMS and/or PPID). Although these tests have the greatest pathophysiologic relevance and are easy to perform, they do not always appear to improve following successful management changes unlike some further tests described later. Nevertheless, continued excessive insulin concentrations or responses indicate the need for ongoing careful dietary control to limit the potential for large insulin spikes.

- ***Fasting hyperinsulinaemia*** – should generally be close to zero or at least <20 mU/L. When high this is suggestive of increased laminitis risk although the test is very insensitive and most laminitis-prone individuals have normal fasting insulin. Additionally, as fasting is not a normal status in horses, this has a doubtful physiologic meaning.

- ***Resting hyperinsulinaemia*** – serum insulin measured during the normal dietary management of the horse gives an impression of current risk on the current ration (should generally be <50-60 mU/L depending on diet). It is best to measure the resting insulin about 2 hours after the most glycaemic intake likely to be experienced by the horse (eg. after grazing or hard feed). However, this is only really meaningful if the dietary quality never changes (eg. non-grazed horses).

- ***Insulin measured after a test-meal of sugars*** – A test-meal of sugars can be used to simulate rich grazing and a worst-case scenario in a turned out horse or pony in the spring when grass sugars are at a peak. There are essentially 2 choices of test-meal: glucose/dextrose or Karo Light corn syrup which differ in their ease of use and sensitivity.
    - ***Oral Glucose Challenge*** – after an overnight (5-6 hours) fast, a test-meal of chaff with 1 g/kg bodyweight glucose or dextrose powder is fed. Alternatively the glucose/dextrose can be mixed with boiling water at a rate of about 30 mL water to 100 g glucose/dextrose and this "syrup" can then be given orally by dosing syringe (typically 200-400 mL total volume). Both serum insulin and plasma glucose are then measured 2 hours later. Peak glucose >8 mmol/L confirms adequate absorption and a valid test. Normal horses and ponies peak insulin should be < 85 mU/L. Horses that do not rapidly eat the test meal should be given the glucose/dextrose by syringe as described above, or the Karo Light Corn Syrup test is used as described below. This test is a more sensitive test than the corn syrup test (which consists of a lower dose of sugar).
    - ***Karo Light Corn Syrup Test*** (Oral Sugar Test) – after an overnight fast, the horse is given a 15 mL/100 kg dose of syrup by dosing syringe (typically 30-60 mL total). Insulin is measured between 60-90 minutes after dosing. Insulin values >60 mU/L are regarded as abnormal with values >45 mU/L being "suspicious". The advantage of this test is the low volume of syrup which is easy to administer, although the test is not as sensitive as the glucose/dextrose test due to the lower amount of sugar in the test dose. Therefore some horses will test negative using corn syrup but positive using glucose/dextrose.



Insulin responses to 1 g/kg glucose in normal and laminitis-prone horses (median and interquartile ranges)

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2. LEVEL 2 TESTS – INSULIN RESISTANCE

Although insulin resistance does not appear to be a direct cause of laminitis (hyperinsulinaemia is the direct cause), there are at least 2 advantages to measuring insulin resistance in EMS and PPID cases. Firstly, insulin resistance is still relevant to laminitis as it augments hyperinsulinaemia as a compensatory response; and secondly, tests for insulin resistance tend to show more consistent improvements with successful management/dietary changes and are useful for monitoring.

By definition, tests for insulin resistance must evaluate the function of insulin and therefore require measuring the response to intravenous insulin. Two main options exist:

The 2-step Insulin Response Test (IRT)

This is probably the simplest test for insulin resistance in practice and involves measuring plasma glucose before and 30 minutes after a bolus of 0.1IU/kg neutral insulin. Normal horses will show a >50% decrease in their baseline plasma glucose 30 minutes following this insulin bolus. Insulin has a lessened effect on plasma glucose in insulin resistant 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 insulin resistance. Glucose (150 mg/kg) is injected as 50% dextrose IV (30 mL per 100 kg), followed by neutral insulin (0.1 IU/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).

3. LEVEL 3 TESTS – CAUSES OF INSULIN RESISTANCE

Aside from inherent genetic factors, acquired insulin resistance is likely to be as a result of obesity/inactivity or PPID and therefore indicates targeted treatment/management of these 2 conditions as appropriate. Therefore testing and evaluation of obesity and PPID are also important in a proper evaluation of laminitis cases.

Tests for Obesity

Obesity is defined as "*abnormal or excessive fat accumulation that presents a risk to health"* and it is well recognised both in humans and in horses that there is more to obesity than simply the quantity of adipose tissue. Thus obesity is a functional and pathophysiological term rather than simply a quantitative and morphologic term; and in order to strictly establish the presence of obesity, it needs to be shown that the adipose deposits within the individual are harmful and not just simply above a threshold measure of mass (e.g. body condition score). Conversely to describe a laminitis-prone individual as "non-obese" is a bold statement without examination of the potential functionality of the individual's fat deposits, irrespective of how little fat they appear to carry.

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Adiponectin is a 244 aminoacid protein produced by adipocytes. In the circulation adiponectin tends to form trimers, hexamers or high molecular weight (HMW) multimers. The HMW forms of adiponectin appear to be most biologically active, having anti-inflammatory effects and increasing insulin sensitivity. HMW adiponectin has been found to be abnormally low in obese horses and those prone to laminitis and is emerging as a diagnostic target for the assessment and monitoring of EMS cases as it may be a key factor associating obesity with insulin dysregulation. HMW adiponectin is diagnostically attractive in that it is largely unaffected by diet, gender or age, has no discernible circadian rhythm and appears reasonably stable *in vitro*. The Liphook Equine Hospital is the first diagnostic laboratory in the world to have fully validated a HMW adiponectin assay for use in practice. HMW adiponectin is best measured in serum (plain red top tubes) and values < 3.2µg/mL suggest metabolic obesity and are generally seen to increase with effective management changes.

Tests for PPID

It is very important 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). As previously mentioned, cases are well recognised at a surprisingly young age in the absence of other clinically suggestive features of PPID (eg. hair coat, polydipsia) and therefore most laminitis cases merit testing.

Basal plasma ACTH – is still regarded as providing the most appropriate and practical screening test for PPID. Valid testing requires that seasonally-adjusted reference ranges are developed by the testing laboratory to take account of the summer/autumn increase in pituitary activity which takes effect from late June until mid-November. The LEH Laboratory remains the only UK laboratory to have performed and published this (Copas and Durham 2012).

The effects of pain and stress on ACTH concentration are limited and probably don't interfere with diagnosis unless moderate to severe. Nevertheless, as an indeterminate effect on ACTH concentration may occur, it is preferable to test in the absence of pain/stress so that further tests can be validly compared.

Important aspects of pre-analytical handling are:

- Collect EDTA plasma sample
- Chill sample within 3 hours of collection – otherwise slow degradation will progressively occur making it impossible to compare the result with future tests
- 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*)
- Ship to laboratory using guaranteed overnight delivery in chilled packaging (freeze centrifuged plasma if delivery is delayed)

As long as valid, seasonally adjusted reference ranges are used, testing basal ACTH has greatest accuracy for diagnosis of PPID during the period of the summer/autumn increase in pituitary activity. Thus when borderline or unexpected results are obtained then retesting between August and October maximises sensitivity and specificity of testing (Copas *et al*. 2012, McGowan *et al*. 2013). Alternatively, between December and June inclusive, the thyrotropin releasing hormone (TRH) stimulation test can be used as described below.

TRH Stimulation Test - has been shown to have higher diagnostic accuracy than measurement of resting ACTH (Beech *et al* 2007). However, due to poorly-determined seasonal effects on this test, it should not be used between July and November. TRH is currently unlicensed in the UK but can be supplied by 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.

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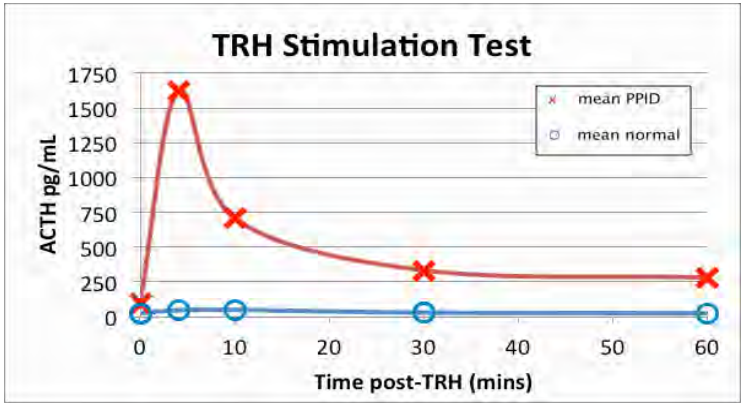
PPID is indicated by a post stimulation plasma ACTH value greater than 110 pg/mL at 10 minutes post TRH or a 30 minute test can be performed using a cutoff of 65 pg/mL (N.B. as greater experience of this test is acquired, expect further refinement of reference intervals).

	TRH Stimulation test for PPID
•	Collect baseline EDTA plasma sample for ACTH analysis
•	Inject 1 mg TRH IV (please contact us for details on obtaining TRH)
•	Collect a further EDTA plasma sample at 10 minutes and/or 30 minutes later for assessment for post-TRH ACTH concentration.
•	Process plasma samples as per previous section on basal ACTH measurement



INVESTIGATING SUSPECTED THYROID DISORDERS

Although thyroid enlargement due to adenomas and C-cell tumours (or occasionally adenocarcinomas) is seen not infrequently in older horses, functional thyroid disease resulting in hypo- or hyper-thyroidism is an extremely rare condition. Reduced thyroid secretory activity, when it does occur, is likely to be secondary to other factors, such as co-existing non-thyroidal illness or drug treatment (e.g. phenylbutazone). Evaluation of thyroid function is difficult as problems could potentially arise anywhere between the hypothalamic secretion of TRH, the pars distalis secretion of TSH, thyroid gland secretion of T4 and T3 and the peripheral action of free T3 and free T4. Firm establishment of an abnormality at any of these levels is however very difficult. Measurement of resting levels of total or free T3 and T4 is often the starting point for assessment of thyroid secretory activity though in reality it provides very little useful information.

HYPOTHYROIDISM

Suspected hypothyroid subjects are best assessed using the TRH stimulation test (please contact us for details on obtaining TRH). Following injection of 1 mg TRH, T3 peaks approximately 2 hrs later at 2-5 x baseline. T4 peaks at around 4 hrs and generally has a lower peak of 1 ½ - 3 x baseline (Figure 1). Responses to TRH comprising a less than 50% increase in either hormone would be consistent with hypothyroidism but would not differentiate pituitary from thyroid disease. Hypothyroidism in horses is extremely rare (and possibly non-existent).

TRH stimulation test for the diagnosis of hypothyroidism
• Collect baseline serum sample for measurement of T3 and T4
• Inject 1 mg of TRH IV
• Collect further serum samples at 2 and 4 hours after TRH injection for measurement of T3 and T4

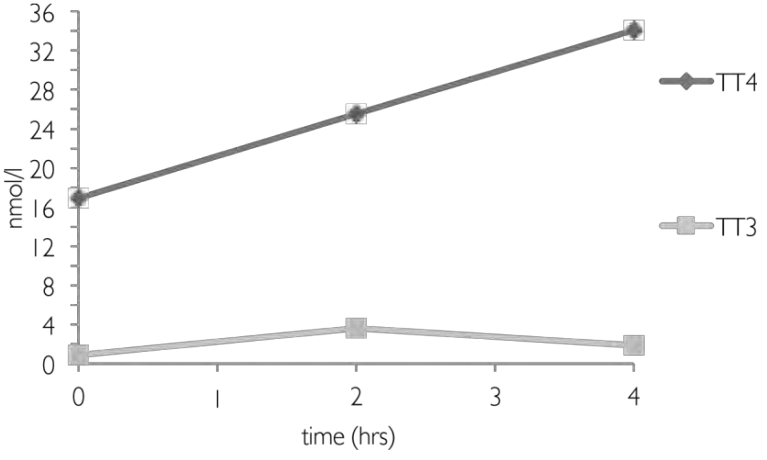


Figure 1. Expected T3 and T4 responses in a horse with normal thyroid function following administration of TRH

INVESTIGATING SUSPECTED THYROID DISORDERS

HYPERTHYROIDISM

Few reports exist of endocrinologically active thyroid tumours (Figure 2). Hyperthyroidism is best diagnosed by using a T3 suppression test.

T3 Suppression Test
• Inject 2.5 mg of exogenous T3 at 0830 and 1800 for 7 doses
• Collect serum for measurement of T4 at 0830 every day for 4 days during the administration.

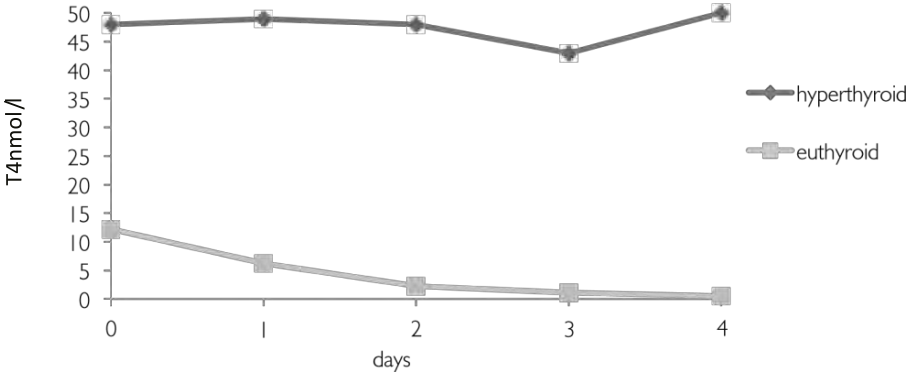


Figure 2. Responses to a T3 suppression test in euthyroid and hyperthyroid horses

Normal horses are expected to show a progressive depression of T4 following negative feedback of the exogenous T3, however an autonomously active hyperthyroid gland will continue to produce high levels of T4 irrespective of the exogenous T3 administered.