

DIAGNOSIS OF THE EQUINE METABOLIC SYNDROME

Introduction and definitions

Metabolic syndrome in humans is defined as **“a collection of risk factors that are associated with increased susceptibility to type 2 diabetes mellitus and cardiovascular disease”**. Different groups have included slightly different components in human Metabolic Syndrome and this is still a topic of considerable debate more than 20 years after its original recognition. However, all definitions essentially condense down to the parameters listed in table 1. As an example, the International Diabetes Federation definition is illustrated in table 2.

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| <ul style="list-style-type: none"> • Insulin resistance • Obesity • Hypertension • Dyslipidaemia |
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Table 1: General components defining the Metabolic Syndrome in humans

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| <ul style="list-style-type: none"> • Central obesity (waist circumference >94cm (men) or 80 cm (women)) <p style="margin-left: 20px;">Plus any 2 of the following:</p> <ul style="list-style-type: none"> • Triglyceride >1.7 mmol/L • HDL cholesterol <1.3 mmol/L (men) or <1.29 mmol/L (women) • Systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg |
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Table 2. International Diabetes Federation definition of metabolic syndrome in European men and women (www.idf.org).

Recognising the analogy with Metabolic Syndrome in humans, Johnson (2002) first proposed the term “Equine Metabolic Syndrome” (EMS) to describe a condition characterised by laminitis, obesity and insulin resistance (IR) in horses and ponies. Essentially EMS may be defined as **“a collection of risk factors that are associated with an increased susceptibility to laminitis”**. The terms “equine peripheral Cushing’s syndrome” and “pre-laminitic metabolic syndrome” have also been used to refer to this same condition. EMS is still a relatively new concept and it seems inevitable that it will evolve and develop over years to come. Already several equine studies have proposed further defining components including hypertension, dyslipidaemia and hyperleptinaemia. EMS has recently been subject to a consensus statement formulated by a panel appointed by The American College of Veterinary Internal Medicine (Frank *et al* 2009).

Thus far the only published working definition of EMS has come from investigation of a closed herd of in-bred Welsh and Dartmoor ponies in Virginia (Treiber *et al* 2006) (table 3). However, as a strong genetic influence on occurrence of the disease was evident then it is unlikely that this precise definition will apply exactly to all populations of horses.

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| <ul style="list-style-type: none"> • Reverse inverse square of insulin (RISQI*) $<0.32 \text{ (mU/L)}^{-0.5}$ • Modified insulin response to glucose (MIRG*) $>5.6 \text{ mU}_{\text{Ins}}^2 / 10. \text{L.mg}_{\text{Glu}}$ • Triglyceride > 0.64 mmol/L • Body condition score > 6/9 |
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Table 3. Definition of EMS proposed by Treiber *et al* (2006). When 3 of the 4 listed criteria were present a 10 fold increased risk of laminitis was predicted (*see later for method of calculation)

Currently there is no widely applicable working definition of EMS. The only readily measurable components of EMS (and therefore an increased susceptibility to laminitis) comprise **obesity, IR and dyslipidaemia** although addition of other factors including **blood pressure** and serum concentrations of **adipokines such as leptin** may be considered if testing becomes more widely available. Both researcher- and practitioner-interest has concentrated generally on the relationship between IR and laminitis although it is important to remember that EMS includes other risk factors in addition to IR and also that it may define increased risk of other problems such as type 2 *diabetes mellitus*, infertility and lethargy.

1. Estimates of obesity

The working definition of EMS proposed by Treiber *et al* (2006) (above) included a general body condition scoring system that was published following a study of Quarterhorse mares. Two problems exist with this: firstly the scoring system might not be applied validly to other breeds; and secondly, it is recognised that measures of regional (rather than generalized) obesity might better reflect laminitis-susceptibility. Fat deposited in the neck (“crest”) has received most attention in the context of regional obesity and laminitis due to the widely recognised phenotype of laminitis-prone individuals.

Carter *et al* (2009) found an objective measure of crestiness, the ratio of mid-neck circumference to height at the withers, to predict laminitis when ratios were >0.71. Also a subjective measure of crestiness, the “Cresty Neck Score” was also proposed (table 4) which found that scores >3/5 were associated with IR and risk of laminitis.

Score 0	No visible or palpable tissue dorsal to ligamentum nuchae
Score 1	No visible crest but slight filling palpable
Score 2	Noticeable visible crest. Fat evenly distributed from poll to withers. Crest easily cupped in one hand and bent from side to side
Score 3	Crest enlarged and thickened. More fat deposited in mid-crest than near poll or withers leading to a mounded appearance. Crest fills cupped hand and is losing side to side flexibility
Score 4	Crest grossly enlarged and thickened. Cannot be cupped in one hand or moved from side to side. May have wrinkles/creases across dorsum.
Score 5	Crest so large that it droops to one side

Table 4. “Cresty Neck Score” (adapted from Carter *et al* (2009)).

2. Estimates of IR

Practical measurement of IR is compromised for 2 main reasons. Firstly the ‘gold standard’ methods for investigating insulin-glucose dynamics are the clamp techniques (hyperinsulinaemic-euglycaemic and hyperglycaemic) and minimal model analysis of the frequently sampled intravenous glucose tolerance test. Neither is practical for routine clinical use. Secondly many other influences on IR exist in clinical cases related to pain, stress, illness, age, diet, exercise and other endocrinopathies (e.g. Cushing’s disease).

Nevertheless several measures can be used to **estimate** IR (or sensitivity) in practice and also the expected compensatory pancreatic secretory response when IR occurs.

a. Estimates based on single blood samples

In practice, single spot samples are clearly highly attractive in a practical context and several tests have been shown to correlate reasonably well with “gold standard” methodology. Such single tests are referred to as surrogates or proxies and can be used to estimate IR, insulin sensitivity (the converse of IR) or pancreatic β cell secretory activity.

i. Resting hyperinsulinaemia

High serum insulin in a single blood sample is strongly suggestive of IR as long as potential confounding factors such as pain, stress and recent feeding are controlled. Standardisation of sampling is desirable to minimise the effects of these factors although protocols and reference ranges are still poorly defined. Grazing certainly has a marked hyperinsulinaemic effect and even some hays can do the same. On the other hand the stress of fasting could have a hyperinsulinaemic effect in some individuals. Nevertheless it is preferable to standardise the sampling protocol by fasting for 6 hours. In occasional cases where the fasting procedure causes obvious distress then poor quality hay (perhaps soaked to reduce soluble carbohydrates) can be offered. IR is likely when fasted serum insulin is >20mIU/L (or >30 mIU/L on hay) using data derived at The Liphook Equine Hospital Laboratory (**NB low fasted insulin values do not rule out IR – see below**). Evidence suggests that different methodology and analysers in different laboratories may produce significantly different results so it is vital that laboratories accurately determine their own acceptable reference ranges.

ii. Resting hyperglycaemia

Although a key component of human metabolic syndrome, hyperglycaemia is uncommonly encountered in insulin resistant equids due to the effects of compensatory hyperinsulinaemia.

iii. Other formulaeic proxies or “insulin sensitivity indices”

Some calculated proxies may overcome some of the possible problems and sources of confusion when using simply serum insulin or plasma glucose alone and have generally been shown to correlate more closely with gold standard methodology than the raw insulin and glucose data alone. However, such proxies are mathematically derived from the “raw” insulin and glucose data so often may not give any more information than is self evident from the insulin and glucose values. Numerous formulaeic estimates have been proposed initially from human studies and more recently in equine studies. Proxies are generally calculated from a single blood sample taken from a starved individual. These are attractive in terms of ease of use and reasonable reliability but very few studies have looked at these in horses thus far. Resting insulin and resting glucose could be regarded as the simplest proxies for IR as discussed above but more refinement is achieved in the examples in the table 5.

Proxy for	Test name	Acronym	Formula
IR	fasting insulin	-	-
	homeostasis model assessment for IR	HOMA-IR	$[\text{fasting ins} \times \text{fasting gluc}] \div 22.5$
Insulin sensitivity	quantitative insulin sensitivity check index	QUICKI	$1 \div [\log \text{fasting ins} + \log \text{fasting gluc}]$
	fasting glucose to insulin ratio	FGIR	$\text{fasting gluc} \div \text{fasting ins}$
	reciprocal inverse square of insulin	RISQI	$1 \div \text{insulin}^{-0.5}$
Pancreatic β cell function	homeostasis model assessment of percentage β cell function	HOMA-B%	$[20 \times \text{fasting ins}] \div [\text{fasting gluc} - 3.5]$
	modified insulin to glucose ratio	MIRG	$[800 - 0.3 \times (\text{ins} - 50)^2] \div [\text{gluc} - 30]$
	fasting insulin to glucose ratio (insulinogenic index)	I:G ratio	$\text{fasting ins} \div \text{fasting gluc}$

Table 5. Various “proxies”, their acronyms and formulae for their calculation.

b. Dynamic testing for insulin resistance

Although high resting insulin (and abnormal calculated proxies) strongly suggests IR, unfortunately a low or normal resting insulin does not rule out the presence of IR for 2 main reasons. Firstly, in some cases of longstanding IR then the pancreas may fail to sustain increased secretion and subsequently serum insulin levels may become normal or low (=type 2 diabetes mellitus). These cases should be obvious when cross checking plasma glucose (glucose will be high) and are probably uncommon. Secondly, and more commonly, not all cases of IR will demonstrate high insulin levels at rest. Many cases require a stimulation test which may demonstrate excessive endogenous insulin secretion in response to a glucose challenge (oral or iv) and/or delayed return to normoglycaemia following glucose challenge (=glucose intolerance).

Although the best validated dynamic test available to use in practice is the combined iv insulin-glucose tolerance test (see below), this is often unattractive in practice situations due to the requirement for hospitalization. Therefore, at the Liphook Equine Hospital we have been investigating the usefulness of a simple and straightforward oral glucose challenge test that is easy to use at the home stables:

i. In-feed oral glucose challenge test

This test may be used in suspected IR cases that are found to have normal fasted insulin concentrations or can also be used as a first-line for investigation of suspected insulin resistance. The protocol is:

- Fast horse/pony overnight (12 hours)
- Ask owner to give a non-glycaemic feed (eg chaff) containing 1 g/kg bodyweight glucose or dextrose powder (wet the feed to facilitate mixing and ingestion)
- Take a blood sample to measure serum insulin 2 hours after the feed is consumed.

Performance of this test on 20 horses at Liphook Equine Hospital with no evidence or suspicion of IR has indicated a mean serum insulin measured 2 hours after the glucose-feed of 32 mIU/L and a normal reference interval of up to 85 mIU/L. Thus values >85 mIU/L are indicative of insulin resistance.

ii. the combined insulin-glucose tolerance test (CGIT)

The combined insulin-glucose tolerance test (CGIT) which monitors the glycaemic response to a combination of exogenous glucose and insulin (Eiler *et al* 2005). The protocol for the CGIT is outlined in table 6.

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| <ul style="list-style-type: none">• overnight fast• plain and oxalate fluoride tube collected for basal glucose and insulin• 150 mg/kg 40-50% glucose solution iv (150ml 50% per 500kg) followed by 0.1 iu/kg soluble insulin (0.5 ml 100iu/mL per 500kg) iv• collect further blood samples for plasma glucose at 1min, 5min, 15min, then q 10 mins up to 45 mins, then q 15 mins up to 2½ hrs• also test the 45 min sample for serum insulin |
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Table 6. Protocol for the Combined Glucose Insulin Tolerance Test

The peak serum glucose occurs around 1 to 5 mins and reaches 2 to 2½ x baseline. The serum glucose normally remains greater than baseline for between 30 and 45 minutes, followed by a negative phase for a further 1 to 2 hours where the serum glucose is below the original baseline. Insulin resistant horses are expected to have a higher peak and a longer positive (>45 mins) and shorter negative phase (and perhaps no negative phase at all) (Figure 1). A 45 minute insulin concentration > 100 mIU/L also implies IR.

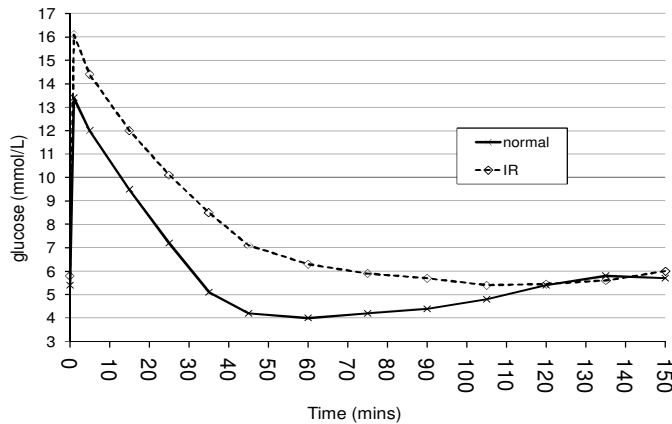


Figure 1. Examples of curves obtained from a normal horse and a horse with insulin resistance using the combined glucose insulin tolerance test

3. Estimates of dyslipidaemia

There is currently insufficient evidence to include estimates of dyslipidaemia in a definition of EMS although further studies may change or clarify this position. The original definition of EMS proposed by Treiber *et al* (2006) included serum triglyceride >0.64 mmol/L as one of the defining criteria although as previously mentioned these criteria may well not apply to other populations. This has been confirmed by some but not all subsequent studies. Although further studies have found plasma levels of non-esterified fatty acids, VLDL and HDL-cholesterol to be higher in obese, insulin resistant horses, the relationship with laminitis was not examined.

4. Blood pressure

Blood pressure measurement is not a popular technique in equine practice although can be achieved with cheap and readily available cuffed manometers. Hypertension was found to be present in laminitis-prone ponies during the summer in one study when compared with non-laminitic controls.

5. Other adipocytokines

Various cytokines released from adipose stores are involved in IR and are worthy of further investigation as possible future tests. These include leptin, adiponectin, retinol binding protein and several others. These are currently under evaluation at the Liphook Equine Hospital to investigate their potential usefulness.

6. Other tests

A few other parameters such as increased serum uric acid have been shown to be related to laminitis susceptibility but are limited in their availability and perhaps require further investigation.

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Carter RA, Treiber KH, Geor RJ, Douglass L, Harris PA (2009): Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. *Equine Vet Journal* 41: 171-178.

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