INVESTIGATING MYOPATHIES

Exertional rhabdomyolysis has long been recognised to be associated with changes in workload and nutrition. Horses may exhibit sporadic exertional rhabdomyolsyis as a result of extreme over-exertion resulting in muscle damage or chronic exertional rhabdomyolysis in which they recurrently suffer from repeated bouts of muscle damage as a result of an underlying myopathy. Only recently have researchers started to define different myopathies, with different aetiologies that often lead to a common presentation. Broadly, horses with chronic exertional rhabdomyolysis may be classified as having recurrent exertional rhabdomyolysis (RER), polysaccharide storage myopathy (PSSM) or idiopathic chronic exertional rhabdomyolysis. RER is caused by a defective calcium channel, PSSM is caused by defective glycogen metabolism and research is ongoing into the underlying disease processes responsible for those cases currently considered “idiopathic”; it is likely there will be numerous causes.

SIGNALMENT AND HISTORY

Signalment and history may give an indication of diagnosis. PSSM is known to affect Quarter Horses and related breeds, Warmbloods, Hannovarians, Connemaras, Cobs, Welsh Ponies and most Draft Breeds and has also been identified in Thoroughbred crosses, Arab crosses and polo ponies. RER is a disease of Thoroughbreds and breeds with Thoroughbred ancestry. Both conditions are inherited as autosomal dominant traits.

CLINICAL SIGNS

Clinical signs and demonstration of increased muscle enzymes provide an indication that a myopathy may be present. Clinical signs may also give a suggestion of the underlying cause but the typical presentation of muscular stiffness post-exercise is non-specific. Horses with PSSM may also exhibit progressive poor performance, a shivers-like gait, muscle wasting, weakness or back pain.

MUSCLE ENZYMES

Creatine kinase (CK) is responsible for energy production in muscle and is therefore present in all skeletal muscle as well as the myocardium and brain. Exchange does not occur between the CSF and plasma and the central nervous system can therefore be discounted as a source of plasma CK. CK is released in response to myolysis with a 3-5 fold increase in plasma concentration approximated to indicate myolysis of around 20g of muscle. However, some muscle cells appear to release CK without being lysed and the potential significance of increased CK concentrations appears to vary from patient to patient and breed to breed. There is therefore much conjecture over the level of increase that is considered clinically relevant and likely to indicate the presence of a myopathy. In the horse, assessment of different CK isoenzymes is not informative. Peaks in CK occur within 4-6 hours of skeletal muscle damage. Plasma half-life is very short and once myolysis ceases CK concentration should return to normal levels within days.

Aspartate aminotransferase (AST) is found in skeletal and cardiac muscle but also within the liver. Increases therefore need to be interpreted alongside increases in specific muscle or liver enzymes. Following myolysis AST takes 24h to reach peak plasma concentrations. Plasma half-life is 7-10 days so AST concentrations can remain increased for weeks after a single episode of rhabdomyolysis.

The differing rates of increase and decrease in CK and AST concentrations can be very useful in diagnosis of myopathies as it enables approximation of when muscle damage has occurred. If increases are identified then serial measurement of CK is helpful in determining whether there is ongoing myolysis and when it is safe to resume work.

Lactate dehydrogenase (LDH) may be used to identify muscle damage but is present in all tissues and is therefore non-specific. In addition to skeletal muscle, cardiac muscle and hepatic tissue are the primary sources. LDH may exist as one of 5 isoenzymes (which comprise different proportions of its 2 subunits, H or M) that are present in different quantities in different tissues. Measurement of subunits may assist in identifying the source of LDH; however, measurement of individual isoenzymes has not been validated in horses and offers little information that cannot be obtained from more routine biochemistry analytes. Cardiac troponin is a more reliable indicator of cardiac muscle damage than LDH isoenzymes.

URINALYSIS

In acute rhabdomyolysis, pigmenturia may be visible grossly as a result of the excretion of myoglobin. In commercial laboratories there is no reliable means of differentiating whether pigmenturia is due to the presence of myoglobin or haemoglobin. Urine dipsticks are an insensitive indicator for myoglobin. If myoglobin is identified then plasma urea and creatinine should be measured to ensure there is no evidence of renal damage.

FRACTIONAL ELECTROLYTE EXCRETIONS

Whilst they offer no assistance in diagnosing myopathies, measurement of electrolye levels may be useful in ensuring that deficiency is not contributing to the instability of myocytes. However, measurements of plasma electrolyte concentrations are of little value as they correspond poorly to whole body status. Simultaneous measurement of blood and urine concentrations and calculation of fractional excretion values gives a better indication of deficiency.

EXERCISE TESTING

Clinical signs and demonstration of increased muscle enzymes provide an indication that a myopathy may be present. Clinical signs may also give a suggestion of the underlying cause but the typical presentation of muscular stiffness post-exercise is non-specific. Horses with PSSM may also exhibit progressive poor performance, a shivers-like gait, muscle wasting, weakness or back pain.

PSSM GENOTYPING

Identification of the genetic mutation (GYS-1) responsible for some PSSM cases has enabled diagnosis of this condition by DNA extraction from a single blood sample. A variety of breeds, including Quarter Horse, Appaloosa, Warmblood, Connemara-cross, Cob, Polo Pony and Thoroughbred-crosses have been demonstrated to carry the mutation in the UK and blood testing should be considered in any horse that is not pure Thoroughbred. In Throroughbreds and in horses testing negative for the GYS-1 mutation a muscle biopsy should be perfomed.

MUSCLE BIOPSY

Muscle biopsy is required to achieve a definitive diagnosis in the majority of horses with exertional rhabdomyolysis. It is a straightforward procedure that is associated with minimal risks (to the patient!) other than incision breakdown and protracted wound healing. There are risks to the person performing the procedure who has to stand behind the horse; however, if stocks or an alternative barrier are used, the horse is well sedated and adequate local analgesia is used then the procedure is safe and straightforward.

Muscle biopsies can either be collected from the semimembranosus muscles which have a predominance of type II muscle fibres or the sacrocaudalis dorsalis medialis muscle which are mostly type I fibres. In exertional rhabdomyolysis cases it is type II fibres that are affected and the semimembranosus muscle is therefore the most suitable site for biopsy. Samples may require multiple stains and results may therefore take longer than conventional histopathology.

Performing a Muscle Biopsy
• Organise same day or overnight courier service prior to sampling and check with the laboratory prior to sending.
• Sedate the horse and aseptically prepare a 20 cm deep x 10 cm wide site
• Inject around 10 ml local anaesthetic subcutaneously. Avoid injection into the muscle layer.
• Wet several sterile gauzes with chilled sterile saline and squeeze them out thoroughly so they remain damp,but not wet.
• Make a 5 cm incision dorsoventrally in the skin and subcutaneous tissue, exposing the underlying muscle belly.
• Keep the muscle exposed with Gelpi or similar retractors
• Make 2 parallel incisions 3cm long between the muscle fibres, about 1cm apart.
• While holding the incised muscle proximally, incise the proximal region, undermine the strip at a depth of around 1 cm and finally incise distally. Avoid holding the muscle anywhere other than the proximal and distal extremities.
• Carefully wrap the sample in the damp gauze
• Close the incision in 3 layers: muscle, subcutis and skin
• Divide the sample into 2 pieces along its length holding it at the ends only. Pin one piece at either end onto card or a wooden tongue depressor and place it in 10% formalin in a screw top container. Ensure there is 20 x the volume of formalin to muscle.
• Remove the remaining sample from the gauze and place on the inside surface of a screwtop plastic container (on its own). DO NOT INCLUDE THE WET GAUZE.
• Place both samples in an insulated container containing ice packs but not in direct apposition to the icepacks. Place cotton wool between the muscle and ice block to prevent the muscle from freezing.
• Seal and post the box by courier or hand deliver.

FURTHER READING:

- Ledwith, A and McGowan, C. (2004) Muscle biopsy: a routine diagnostic procedure. Equine Veterinary Education 16, 62-67.
- Stanley, R. *et al*. (2009) A glycogen synthase 1 mutation associated with equine polysaccharide storage myopathy and exertional rhabdomyolysis occurs in a variety of UK breeds. Equine Veterinary Journal 41, 597-601.
- PSSM genotyping: http://www.laboklin.co.uk/laboklin/GeneticDiseases.jsp?catID=HorsesGD