INDICATORS OF FITNESS AND PERFORMANCE

ERYTHROCYTES

Oxygen carriage to skeletal muscle is an important factor in overall fitness, and in horses there is a clear association between total red cell volume and performance. As horses enter training, red cell parameters are expected to increase for a couple of months before reaching a plateau and they may therefore be used as an indicator of how well the animal is responding to training. The same trend should be observed each season, with a slight year-on year increase in total red cell volumes through the first few years of competition.

Fit thoroughbreds would be expected to have red cell numbers, packed cell volumes (PCVs) and haemoglobin concentrations toward the top end of, or exceeding, published reference ranges for the general horse population. Factors that affect hydration status and degree of splenic contraction (such as excitement, stress, time since exercise and time since feeding) all influence the distribution of red cells within the circulatory system, resulting in individual horses demonstrating significant intra-day variation and potentially confounding monitoring.

As a result of excitement, PCV may increase by up to 10L/L and remain elevated for up to two hours. One means of eliminating these variables is to measure maximal haematocrit levels within a minute of the cessation of hard exercise. This approach is not widely utilised as it poses logistical difficulties and exercise will serve to confound the measurement of other haematological and biochemical variables that may be of interest. If samples are to be collected at rest, it is best to standardise collection; the optimal time being early morning before feeding, exercise or turn-out on a day following a rest day or a following a day of light work.

Declining PCVs in performance horses are a concern as they may be an indication of subclinical or clinical disease. It is hypothesised that subclinical or clinical disease may result in reduced sympathetic tone and hence an increase in splenic sequestration of erythrocytes. Primary anaemia is rarely identified in performance horses and investigations of apparent reductions in red cell volumes should initially focus on other conditions that commonly affect performance horses, such as infectious respiratory disease and equine gastric ulcer syndrome.

LEUCOCYTES

Measurement of absolute and relative leucocyte numbers is of no value in the assessment of fitness *per se*; however, they are important in the detection of clinical and subclinical disease that will have a bearing on performance. In young performance horses, assessment of the leucogram a few days before competition is a popular and useful tool that is used to detect animals that may have faced infectious challenge and may therefore be likely to underperform.

The relative concentrations of leucocytes within the circulating and peripheral pools will be influenced by factors such as stress and excitement and the conditions of sampling must therefore be considered when interpreting results. Sample collection should be avoided within six hours after exercise or stressful incidents. If this is not possible, the results should be interpreted cautiously. The typical glucocorticoid-mediated response that accompanies the stress of exercise and competition (as well as Cushing's disease and exogenous glucocorticoid administration) results in neutrophilia, lymphopenia and eosinopenia and an overall increase in leucocyte numbers. However, this effect may be balanced during high-intensity exercise or short-term excitement (such as during venepuncture) by increased blood volume and lymphocyte release secondary to splenic contraction.

INDICATORS OF FITNESS AND PERFORMANCE

In performance horses, relative neutropaenia or a 'reversed differential' (i.e. neutrophils present in similar or lower numbers than lymphocytes) is commonly observed in animals that are performing poorly or showing signs of lethargy. Whilst this presentation is well-recognised it is not well explained. It is generally assumed to be a result of viral infection or an equine post-viral fatigue syndrome and may be associated with overtraining. Horses with poor performance and relative neutropaenia often have elevations in muscle enzymes that might be due to viral myositis or to post-viral fatigue syndrome. Horses presenting in such a manner may take many months to recover and it is generally recommended that they are rested until the leucocyte differential returns to normal. Fatigue and related symptoms may persist for months or even years.

MUSCLE ENZYMES

Increases in creatine kinase (CK) and aspartate aminotransferase (AST) may indicate various myopathies such as recurrent exertional rhabdomyolysis, polysaccharide storage myopathy or viral myositis. Increases may also be observed in response to initiation of training or overtraining which may or may not indicate underlying myopathy. Confusion surrounds what constitutes normal and abnormal values for AST and CK and the degree of increase that should be expected after exercise. Exertion is expected to result in an increase in circulating AST and CK but post-exercise values may still remain within published reference ranges if the baseline is low. Sequential samples from the same horse may be more helpful than relying on published reference ranges, which are inevitably wide. Measurements performed before and after exercise (4 and 24 hours) are also a useful means of determining whether myopathy is present; a significant increase post exercise is an indication of underlying pathology (see later chapter on investigating myopathies).

Though not likely to be relevant to performance horses muscle enzymes may also be increased in horses that are catabolic.

LIVER ENZYMES

Gamma-glutamyl transferase (GGT) has been investigated as a measure of performance in addition to being a marker of hepatic disease. In response to training, GGT appears to increase in a linear manner and has been associated with poor performance; it is unclear whether this is due merely to overtraining or to subclinical liver disease. High GGT may be observed on routine blood samples in the absence of clinical signs and in cases that have recovered from hepatic disease. An increase in GGT is not totally specific for hepatic disease or overtraining therefore and more research needs to be performed into its significance in the performance horse.

ELECTROLYTES

Abnormal electrolyte levels have been associated with poor performance and with exertional rhabdomyolysis. As most performance horses receive a plethora of supplements, deficiencies are uncommon. Furthermore, circulating electrolyte levels provide a very poor assessment of whole-body status and detection of excesses or deficiencies should be performed by calculating renal clearances from simultaneously collected urine and blood samples rather than by simply measuring blood levels.

CONCLUSION

Attempting to determine fitness from resting blood samples remains fraught with difficulty and there is little evidence to suggest that it may be done reliably. Assessments of lactate and erythrocyte levels at and immediately after exercise are probably the most helpful but require veterinary attendance at exercise. The main value of clinical pathology lies in detecting underlying conditions, such as muscular or infectious disease, that may have a considerable bearing on performance, especially at the top level.

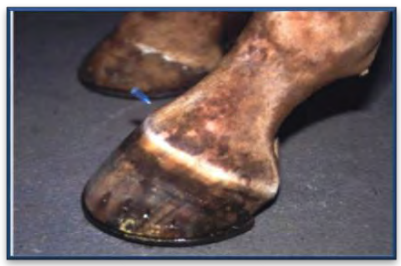
SYNOVIAL FLUID ANALYSIS

In a clinical setting examination of synovial fluid has traditionally been limited to cytology, measurement of protein concentrations and bacterial culture. When there is inflammation within or adjacent to the joint (e.g. following synoviocentesis, intra-articular medication, damage to periarticular tissue) the leucocyte count, neutrophil percentage and protein concentration are all expected to increase. Following bacterial inoculation, increases in synovial fluid leucocyte count are seen within 8 hours of inoculation and reach significant levels within 12 to 24 hours. If bacteria are present within the joint then the increases will generally be more marked and the neutrophil percentage will be higher. The total protein concentration and leucocyte count may vary depending on the duration of sepsis and the virulence of the bacteria. Synovial fluid evaluation is of no benefit in the investigation of chronic osteoarthritis.

	Total leucocyte count (x 10^9/L)	Neutrophil percentage	Protein concentration (g/L)
Normal	<5	< 10	< 20
Inflammatory "flare"	8 - 40	50 - 90	20 - 40
Sepsis	> 20	> 90	20 - 80

LEH laboratory has developed a PCR that detects preserved sections of bacterial DNA and therefore provides a useful adjunct to conventional synovial fluid cytology and culture. PCR has the advantage that it will detect the presence bacteria that are dead or not amenable to culture, detects bacteria that are present in low numbers and crucially provides results within hours. Provided the sample is collected without the potential for any contamination (which should be standard practice for synoviocentesis) a positive result provides evidence that bacteria are present within the affected joint/sheath/bursa and lavage should be performed without delay. This offers a major advantage over waiting for the results of culture and additional important information if the results of cytology are equivocal.

Antibodies to Borrelia can also be detected in synovial fluid which may increase suspicions of Lyme disease as a cause of polyarthritis. However, the test is unvalidated and the clinical significance of the results is uncertain.



INVESTIGATING EXERTIONAL MYOPATHIES

Exertional rhabdomyolysis has long been recognised to be associated with changes in workload and nutrition. Horses may exhibit sporadic exertional rhabdomyolysis as a result of extreme over-exertion resulting in muscle damage or chronic exertional rhabdomyolysis in which they recurrently suffer from 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 and is autosomal dominantly inherited. A heritable defect in the glycogen synthase gene (GSY1) is responsible for a substantial proportion of PSSM cases (termed PSSM type 1) and this mutation been found in a wide range of European and American horse breeds and crosses thereof. A reasonable number of cases with histologically confirmed PSSM are negative for the GSY1 mutation (termed PSSM type 2), indicating that the pathogenesis of PSSM is rather more complex, and aetiologies more diverse, than first thought when the GSY1 mutation was discovered! Research is ongoing into the underlying disease processes responsible for those cases currently considered non-PSSM, non-RER, "idiopathic"; it is likely there will be numerous causes.

SIGNALMENT AND HISTORY

Signalment and history may give an indication of diagnosis. PSSM should be considered as a possible aetiology in every breed of horse presenting with a recurrent myopathy except pure Thoroughbreds; it 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.

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. 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 around 4-6 hours following acute skeletal muscle damage and therefore it is possible to test too early after onset of signs, leading to very modest changes in CK where higher values were expected. 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 and remain high even after the muscle has recovered. Nevertheless AST is better than CK for identifying low grade recurrent bouts of myolysis as AST tends to persist at high levels whereas CK may rapidly normalise.

INVESTIGATING EXERTIONAL MYOPATHIES

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.

EXERCISE TESTING

Given the dynamics of muscle enzyme increases described above, where there is a suspicion of myopathy it can often be useful to measure CK at its expected peak 4-6 hours after an exercise bout. Samples collected pre-exercise and 4-6 hours after exercise (depending on severity of myopathy) often demonstrate a significant increase in the post exercise sample – confirming exertional myopathy. Clearly when a marked myopathy is expected then the exercise test should not be too strenuous. In contrast when the clinical presentation is nothing more than poor performance then a more strenuous exercise test is more appropriate.

URINALYSIS

In acute rhabdomyolysis, pigmenturia may be visible grossly as a result of the excretion of myoglobin. In commercial laboratories there are 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 ensures that deficiency is not contributing to the instability of myocytes. However, measurements of plasma electrolyte concentrations 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 – separate section explaining clearance ratios.

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 and crosses have been identified with the mutation.

Blood testing for the GSY-1 mutation 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 performed.

INVESTIGATING EXERTIONAL MYOPATHIES

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 (power/exertion muscles) or the sacrocaudalis dorsalis medialis (tail-head) muscle which are mostly type I fibres (postural muscles). In exertional rhabdomyolysis cases it is type II fibres that are affected and the semimembranosus muscle is therefore the most suitable site for biopsy. Non-exertional myopathies such as motor neurone disease or atypical myopathy are best diagnosed from type I muscle. 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