Muscle energetics in exercising horses

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Abstract
An optimally functional musculoskeletal system is crucial for athletic performance and even minor perturbations can limit athletic ability. The introduction of the muscle biopsy technique in the 1970s created a window of opportunity to examine the form and function of equine skeletal muscle. Muscle histochemical and biochemical analyses have allowed characterization of the properties of equine muscle fibres and their influence on, and adaptation to, physical exertion. Analyses of exercise responses during standardized treadmill exercise and field studies have illustrated the role of cellular energetics in determining athletic suitability for specific disciplines, mechanisms of fatigue, adaptations to training and the affect of diet on metabolic responses. This article provides a review of the tools available to study muscle energetics in the horse, discusses the muscular metabolic pathways and summarizes the energetics of exercise.

Keywords: muscle biopsy; exercise; glycogen; glucose; lipid; muscle fibre

Introduction
The tremendous range of athletic capacity of equine athletes can be attributed to both years of genetic selection for prowess for a particular form of exercise as well as the remarkable plasticity of muscle, which readily adapts to physical training. The introduction of the muscle biopsy technique and the expanding array of histochemical, biochemical and molecular applications developed over the last three decades have improved our understanding of muscle structure, function, adaptation to training and limitations of performance. In this review, modern methods to assess the relationship between the physical characteristics of skeletal muscle and the biochemical response and adaptation to exercise will be reviewed; the complexity of muscle energetics in equine athletes will also be briefly summarized. Supplemental detailed reviews of muscle biochemistry and physiology can be found elsewhere¹⁻⁵.

Techniques to measure metabolic and contractile properties of muscle
The first part of this paper discusses the techniques available for assessment of substrate metabolism, especially in relation to the contractile properties of the muscle fibres. These techniques allow a quantitative and
qualitative assessment of the effects of exercise, training and dietary manipulation on substrate metabolism at the level of the muscle and the whole body.

**Muscle biopsy technique**
The percutaneous needle muscle biopsy technique was introduced to equine research by Lindholm and Piehl as well as by Snow and Guy. Since that time, it has proved to be an invaluable tool in defining the histological, histochemical and biochemical properties of equine skeletal muscle. Standardization of the site of the muscle biopsy is imperative because equine skeletal muscles have a heterogeneous distribution of muscle fibre types within the muscle. Deeper regions within locomotory muscles have contractile and metabolic characteristics similar to those of postural muscles. In addition, fibre types vary among different muscles in the same horses as well as across horses and breeds. The selection of the specific muscle to biopsy therefore is of critical importance and will depend on its propulsive or postural role. Many studies of equine athletes utilize the gluteus medius muscle or the semitendinosus muscle because of its importance in locomotion and demonstrated metabolic adaptations to exercise and training. Other investigators have also used the triceps brachii, along with the masseter (as a non-exercise muscle control sample site).

When sampling site and depth are consistent and potentially involve several sites, repeatable results are obtained. Muscle biopsy has provided a wealth of information regarding the histochemical, biochemical and metabolic properties of various muscles within the same horse, between horses and breeds, as well as responses to exercise. A detailed review of the technique for performing percutaneous needle muscle biopsy and sample preparation can be obtained elsewhere.

**Contractile properties of muscle fibres**
The contractile machinery provides the fibre’s ability to shorten and lengthen through a highly organized structure. Contractile speed varies according to both the myosin heavy-(MyHC) and light-chain isoforms expressed at the protein level in a given muscle fibre. The contractile strength of a fibre is directly related to the cross-sectional area of that fibre.

**Histochemical studies of contractility**
Fibre typing of equine skeletal muscle first relied upon histochemical analysis, which revealed the acid and alkali stabilities of the myofibrillar adenosine triphosphatase (ATPase) activity in each fibre type. Slow- and fast-twitch fibres can be further subdivided into four types based on the sensitivity of the myosin ATPase enzyme to acid or alkaline preincubation. Classification then included types I, IIA, IIB and intermediated or IIC fibres. The speed of contraction was found to be fastest in type IIB fibres, intermediate in type IIA and slowest in type I fibres. The type IIC fibres, which were mainly found in foals, would indicate fibres’ transformation.

**Immunohistochemical studies of myosin heavy chains**
The development of monoclonal antibodies for specific MyHC isoforms provided a more accurate means to discern specific fibre types in equine skeletal muscle. Immunohistochemical staining identified several MyHC isoforms in equine muscle that are encoded for by separate genes. These included three MyHC isoforms that define five fibre types: pure I, IIA and IIX fibres and the hybrids (i.e. coexistence of two MyHC isoforms) I + IIA and IIA + IIX fibres (Fig. 1). In situ hybridization with RNA probes specific for each MyHC isoform shows that the majority of fibres express identical mRNA and protein isoform, whereas hybrid fibres present a mismatch between coexpression at the protein rather than the mRNA level. Type IIB fibres identified previously by the myofibrillar ATPase histochemical technique were shown to represent type IIX fibres, whereas the true IIB MyHC isoform commonly found in rodents was not identified in equine muscle. Further, no cDNAs encoding the IIB gene have been identified in horses. Thus, although requiring a more complex analysis, immunohistochemistry provides more specific and accurate information regarding MyHC isoform(s) in equine muscle than traditional histochemical analysis.

**Metabolic properties of muscle fibres**
The metabolic properties of equine muscle can be evaluated through histochemical and tinctorial stains, as well as by biochemical and molecular assays.

**Histochemical assessment of enzyme activities**
Tinctorial and histochemical stains of enzyme activities reveal distinctive metabolic capacities in various fibre types. Modified Gomori Trichrome stains may also demonstrate the oxidative capacity of skeletal muscle fibres. Amylase–periodic acid Schiff (PAS) readily demonstrate the quantitative capacity of skeletal muscle fibres. Amylase–periodic acid Schiff’s stains may also be useful in examining oxidative capacity as the number of capillaries surrounding each muscle fibre can be assessed with this stain. However, oxidative capacity and capillaries are not univocally correlated. The glycolytic capacity of muscle fibres can be evaluated...
using stains for phosphorylase, phosphofructokinase (PFK) or glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity. The phosphorylase stain has the disadvantage of being dependent on \textit{in situ} glycogen, whereas stains coupled to PFK enzyme activity are hampered by the lability of PFK. The GPDH stain was developed to assess the capacity of cytosolic GPDH to reduce nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) to NADH. However, it is unclear whether the GPDH assayed truly represents glycolytic capacity or whether it also or alternatively measures oxidative capacity via flavine adenine dinucleotide (FAD)-dependent GPDH in mitochondria\textsuperscript{44}. Nevertheless, the activity of this enzyme is highly correlated with that of other enzymes directly involved in glycolysis (discussed in Quiroz-Rothe and Rivero\textsuperscript{45}). The oxidative capacity of the fast-twitch fibres has, in earlier studies, been evaluated using either SDH or NADH stains, and fibres were then classified as FT (low oxidative) or FTH (high oxidative) in addition to ST fibres\textsuperscript{4,46}.

**Histological stains for energy substrates**

The major sources of energy for muscle contraction are intramuscular glycogen and triglycerides, as well as blood-borne glucose and fatty acids. Periodic acid Schiff's stains readily demonstrate the presence of glycogen in myofibres and Oil red O stains highlight the presence of lipid droplets\textsuperscript{47}. Sequential staining of muscle sections for glycogen content and contractile properties have been used to assess the pattern of recruitment of muscle fibres\textsuperscript{19,46–53}.

**Immunofluorescent and immunohistochemical stains**

Detailed identification of structures within myofibres can be accomplished using monoclonal antibodies coupled to fluorescent tags. The location of insulin-sensitive glucose transporters GLUT-4 within intracellular storage pools and in their active position within the sarcolemma in equine muscle has been characterized by this method\textsuperscript{54,55}. Furthermore, localization of isoforms of the Ca\textsuperscript{2+}-ATPase (SERCA) within equine skeletal muscle has been accomplished by SERCA immunohistochemistry\textsuperscript{45,56}.

**Biochemical assays**

A more quantitative means to assess metabolic capacity of skeletal muscle is to measure the activity of enzymes or substrates in whole muscle homogenates or on pools of fibres or single fibres of identified type. Frequently used markers of oxidative capacity include assays of citrate synthase (CS) or SDH activity within the Krebs cycle or 3-OH-acyl-CoA dehydrogenase (HAD) in free fatty acid oxidation. Glycolytic capacity is often assessed by determining lactate dehydrogenase (LDH) activity or PFK activity. The LDH usually indicates the capacity for lactate production. The activity of hexokinase (HK) is used to evaluate the capacity for phosphorylation of glucose. Assessment of the concentrations of triglycerides, glycogen, glucose-6-phosphate, pyruvate, lactate and adenosine nucleotides provides further information regarding the metabolic state of muscle at the time of biopsy. Metabolite analyses on whole muscle must be evaluated.
with caution as this represents only a mean value for the metabolic responses in different fibre types57.

**Gene transcription**
A rapidly developing application for equine muscle biopsies is the use of real-time RT-PCR which provides a means to evaluate gene transcription within muscle under varying metabolic stimuli. It was recently used to assess GLUT-4 gene transcription and glycogen branching enzyme gene transcription in equine muscle58,59.

**Structure and function of muscle fibre types**
Co-localization of contractile and metabolic staining within individual fibres shows that, in untrained horses, muscle fibre types often have characteristic metabolic properties. In general, type I fibres have the highest oxidative capacity and lipid stores as well as the lowest glycolytic capacity and glycogen stores. In contrast, type IIX fibres have the lowest oxidative capacity and lipid stores and the highest glycolytic capacity and glycogen stores in the untrained state. Type IIA fibres are intermediate in these capacities. Some overlap exists, however, among muscle fibre types resulting in a continuum rather than exclusive metabolic and contractile properties (see Table 1)44,45,60,61.

At rest, type I motor units are primarily recruited for posture. Their smaller size, high lipid stores, high oxidative capacity and high capillary density make them ideally suited to resist fatigue through oxidative metabolism. At exercise, motor units are recruited in the rank order I → IIA → IIX depending on the intensity and duration of exercise. The large cross-sectional area of type IIX fibres, as well as their high glycosogen stores and glycolytic capacity, makes them ideally suited for high-intensity maximal aerobic and anaerobic exercises. However, high-force type IIX fibres are less resistant to fatigue than type I fibres (Table 1).

**Assessment of whole-body substrate utilization and energy partitioning during exercise**
Although the measurement of tissue samples from muscle biopsy described above provides useful information about metabolic and contractile profiles of muscle fibres, these local and systemic measures are 'static' measures that do not allow quantitative assessment of the actual rates of substrate use62. Metabolic studies in humans and more recently in horses utilize stable isotope tracer methodology in combination with indirect calorimetry for calculation of whole-body substrate oxidation rates63-66. Using a constant rate infusion of a stable isotopically labelled tracer of glucose, rates of glucose production (mainly hepatic glucose production) and utilization (mainly muscle glucose uptake) can be estimated during submaximal exercise62,63. Thus, in contrast with measurement of blood glucose concentrations, this technique allows one to monitor the dynamics of glucose turnover during exercise or after feeding62. Furthermore, indirect calorimetry can be used to calculate whole-body oxidation rates, derived from measurement of the horse’s oxygen consumption, carbon dioxide production and respiratory exchange ratio (RER). The main assumptions are that measure of gas exchange at the level of the lung (measured by RER) accurately reflects the actual metabolic gas exchange at the cellular level (RQ) and that protein contribution is negligible. From concurrent calculations of whole-body rates of carbohydrate oxidation and the rate of plasma glucose disappearance, it is also possible to estimate the contribution by blood glucose and muscle glycogen (plus lactate) to total carbohydrate utilization64. The main assumption is that rate of glucose disappearance is equal to the actual oxidation rate of glucose derived from the blood, although it is possible that some glucose is used for glycogen resynthesis during low-to-moderate-intensity exercise64. The main limitation is that this state-of-the-art technique can only measure substrate utilization during submaximal exercise. A further disadvantage is the cost associated with the isotope and the instrumentation required for measurement of isotopic enrichment62. Detail review of these techniques can be found elsewhere62.

**Muscular metabolic pathways**

The second part of this paper discusses the muscular metabolic pathways. The ATP hydrolysis supports the cross-bridge cycle as well as the activity of ion pumps.

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### Table 1 Fibre-type features of equine skeletal muscle

<table>
<thead>
<tr>
<th>Variables</th>
<th>Properties of muscle fibre types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific ATP activity</td>
<td>From low to high: I &lt; IIA &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Speed for shortening</td>
<td>From slow to fast: I &lt; IIA &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Resistance to fatigue</td>
<td>From high to low: I &gt; IIA &gt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Power output</td>
<td>From low to high: I &lt; IIA &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Metabolic properties</td>
<td>From low to high: I &lt; IIA &lt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>From low to high: I &lt; IIA &lt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Oxidative capacity</td>
<td>From high to low: I ≥ IIA &gt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Glycolytic capacity</td>
<td>From low to high: I &lt; IIA &lt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Morphologic characteristics</td>
<td>From small to large: I ≤ IIA &lt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Fibre size (cross-sectional area)</td>
<td>From high to low: I ≥ IIA &gt; IIX &lt; IIX &lt; IIX</td>
</tr>
<tr>
<td>Capillary bed</td>
<td>From high to low: I ≥ IIA &gt; IIX &lt; IIX &lt; IIX</td>
</tr>
</tbody>
</table>
and channels participating in excitation–contraction coupling, in particular, the sarcoplasmic reticulum calcium ATPase pump. Since the local ATP reserves can only sustain contraction for a few seconds and ATP needs to be produced at the site of its utilization, oxidative phosphorylation from glucose and lipid substrates is the main pathway for ATP synthesis under aerobic conditions.

Under most physiological conditions, glucose entrance across plasma membranes into the muscle cell is the rate-limiting step in glucose utilization. Glucose transport occurs primarily by facilitated diffusion that uses a family of structurally related proteins (GLUT-1 to GLUT-12) as glucose carriers. For instance, GLUT-4 is the major isoform in the skeletal muscle. Whereas GLUT-1 and GLUT-5 isoforms are mainly associated with the cell surface and are not insulin stimulated, the translocation of the GLUT-4 protein from an intracellular (non-active) pool to the plasma membrane (active site) is largely regulated by insulin- and contraction-dependent processes.

Glycolysis begins with glucose-6-phosphate obtained either from blood glucose and phosphorylation by HK or from mobilized stored intracellular glycogen and proceeds through a series of steps to produce pyruvate (Fig. 2). The rate-limiting step in glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-diphosphate by the enzyme PFK, whose activity is regulated by the ATP/ADP (ADP:adenosine diphosphate) ratio. When this ratio decreases the activity of PFK increases, thus resulting in a greater production of pyruvate. Under anaerobic conditions, pyruvate in the cytoplasm is converted to lactate by LDH. Anaerobic glycolysis is efficient in terms of kinetics but not in terms of ATP synthesis yield per glucose. With aerobic metabolism, pyruvate is transported into the mitochondrion and undergoes oxidative decarboxylation to form acetyl-coenzyme A (acetyl-CoA). Oxidative metabolism is highly efficient in terms of energy yield but not in terms of kinetics.

Fatty acids from circulating very-low-density lipoproteins or from stored muscle triglycerides are a prime substrate for aerobic metabolism. Sarcoplasmic short- and medium-chain fatty acids (fewer than ten atoms of carbon) can freely enter the mitochondrial matrix, where they form acyl-coenzyme A (acyl-CoA). In contrast, long-chain fatty acids are esterified first as acyl-CoA and then as acylcarnitine by carnitine palmitoyltransferase I and II (CPT I and CPT II) before they cross the mitochondrial membranes. β-Oxidation starts with acyl-CoA oxidation catalysed by the HAD, culminating in the formation of acetyl-CoA (which enters the Krebs cycle—also named the tricarboxylic acid cycle or citric acid cycle) and the production of shortened acyl-CoA (two C fragments are removed by β-oxidation sequence), with concurrent reduction of one FAD and one NAD⁺.

Metabolites resulting from aerobic glycolysis and fatty acid β-oxidation enter the Krebs cycle as acetyl-CoA. In this mitochondrial process, several oxidation steps are involved that result in the formation of oxaloacetate, which may be used to repeat the Krebs cycle. The oxidants utilized are NAD⁺ and FAD. For each acetyl-CoA that undergoes the whole process, three NADH + H⁺, one FADH₂ and one GTP are generated. The reduced coenzymes will be reoxidized by the electron transport chain (see further) to provide ATP. Fibres that contain a lot of mitochondria have higher oxidative capacity than fibres poorly furnished with that organelle.

Pathways of oxidative energy conservation

The reducing power of various substrates is converted into phosphate potential through the process of respiratory chain and ATP synthase: oxidoreduction energy of reduction substrates converges into two reduced coenzymes, NADH + H⁺ and FADH₂, which deliver their electrons to the electron transport chain. During the electron transport from coenzymes to oxygen, a proton electrochemical gradient is built by proton pumps. The ATP synthesis relies on the consumption of this proton gradient. The respiratory chain is made up of four complexes (complex I–IV) named as follows: I, NADH–ubiquinone oxidoreductase; II, succinate–ubiquinone oxidoreductase; III, ubiquinol–cytochrome c oxidoreductase and IV, cytochrome c oxidase.

Energetics of exercise

This last part reviews the energetics of exercise. The relative contribution of aerobic and anaerobic pathways for energy production, as well as the source of energy, depends on the type, intensity and duration of the exercise. Training and nutrition are also important determinants of the pathway used for energy production.

**Muscle responses to exercises**

All the fibres of a single motor neuron (i.e. that respond to its action potential) belong, in general, to the same fibre type and are dispersed throughout the muscle mass (for details, see Rivero and Piercy). Glycogen in muscle is utilized during most types of exercise and glycogen utilization increases with increasing work intensity, being most pronounced during the first work bouts during interval work. During low-intensity submaximal exercise, muscle triglycerides, blood glucose and free fatty acids (released from adipose tissue and/or liver stores) are the main sources of energy for type I and IIA fibres. With prolonged low-intensity exercise, the uptake of free fatty acids by muscle increases substantially, gradually becoming the major source of energy. During extremely prolonged low-intensity exercise
FIG. 2 Interconnection between the contractile apparatus and pathways of energy metabolism. Action potential causes the sarcoplasmic reticulum to release large quantities of calcium ions (Ca\(^{2+}\)) that enable muscle contraction. When muscle stimulation ceases, the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) pumps back Ca\(^{2+}\) into the sarcoplasmic reticulum. There are several pathways for adenosine triphosphate (ATP) synthesis, which may simplistically be subdivided into anaerobic and aerobic pathways. The anaerobic pathway takes place in the cytosol and includes the coupling of phosphocreatine (PC) to adenosine diphosphate (ADP) by the creatine kinase (CK), deamination of adenosine nucleotides (with formation of adenosine monophosphate (AMP) and inosine monophosphate (IMP)) and glycolysis. Activity of the PFK is considered as an indicator of the capacity for anaerobic metabolism. Assay of GPDH is considered as a measure of the glycolytic capacity of the muscle cell. Blood glucose across the sarcolemma may be increased in response to exercise owing to glucose transporter type 4 (GLUT-4), which is moved to the cell surface from intracellular sites. The activity of hexokinase (HK) is used to evaluate the capacity for phosphorylation of glucose, which is the first step of glycolysis from blood glucose. The aerobic pathway involves several mitochondrial processes: free fatty acid \(\beta\)-oxidation, the Krebs cycle and the electron transport chain. Activity of the 3-OH-acyl-CoA dehydrogenase (HAD) is considered as a measure of lipid oxidation capacity, activity of the citrate synthase (CS) as an indicator of Krebs cycle activity and activity of the succinate dehydrogenase (SDH) as a measure of oxidative capacity of the muscle cell. The pyruvate resulting from the cytosolic glycolysis may enter the Krebs cycle or, in the absence of oxygen, be converted to lactate by the lactate dehydrogenase (LDH). Activity of LDH is considered as a measure of the capacity for anaerobic metabolism. Reduced forms of nicotinamide adenine dinucleotide (NADH + H\(^{+}\)) and of flavine adenine dinucleotide (FADH\(_{2}\)) that are produced during glycolysis and the Krebs cycle are used by the electron transport chain to regenerate ATP.

**Diagram Description**
- **Glycogenolysis**:
  - Muscle glycogen → Glucose-1-P → ATP → Glucose-6-P → ADP → ATP
- **Creatine Kinase**:
  - Creatine + ADP → ATP + Creatine
- **Glycolysis**:
  - Glucose-6-P → Fructose-6-P → ADP → ATP → Glucose-1,6-di-P → Pyruvate → LDH (Lactate)
- **Muscle Contraction**:
  - ATP → ADP → ATP
- **Electron Transport Chain**:
  - NADH + H\(^{+}\) → FADH\(_{2}\) → Oxaloacetate → Citrate → Malate → Fumarate → Succinate → Succinyl-CoA → SDH (Succinate Dehydrogenase)
further motor units are recruited, including IIAX, and finally the type IIX motor units\textsuperscript{80,72}. Catabolism of protein and amino acid metabolism may also contribute to provision of energy during this type of tough endurance exercise\textsuperscript{72,73}.

In horses performing high-intensity maximal exercise, type IIX motor units are rapidly recruited in addition to type I, IIA and IIAX fibres, anaerobic glycolysis is the main source of energy and large amounts of lactate are produced leading to acidosis\textsuperscript{71,74,75}. Muscle can temporarily generate ATP using breakdown of phosphocreatine and through the muscle adenylate kinase pathway\textsuperscript{57,74-77}. Cleavage of phosphocreatine enables transfer of the phosphate group to ADP in order to reconstitute ATP. This source of energy is rapidly depleted, since the amount of phosphocreatine stored is very small. With the adenylate kinase pathway, one ATP and one adenosine monophosphate (AMP) are generated using two ADP molecules. The AMP molecule can then be deaminated to ionosine monophosphate (IMP) within the purine nucleotide cycle, with concurrent production of ammonia. High lactate, ammonia and IMP levels and low ATP levels are seen after intense exercise in the muscle of Standardbreds and especially in type II fibres\textsuperscript{57,78,79}. To sustain muscle contraction beyond the first few minutes of exercise, horses rely heavily on glycogen, which is the obligate substrate to sustain anaerobic ATP production\textsuperscript{80}. The importance of muscle glycogen reserves as an anaerobic energetic fuel to prevent fatigue during high-speed exercise has been highlighted in studies that manipulated muscle glycogen stores after strenuous exercise\textsuperscript{80,81}. For instance, substantial depletion of muscle glycogen stores in horses before exercise is associated with decreased anaerobic capacity, during a subsequent high-speed exercise test\textsuperscript{80,81}. Replenishment of muscle glycogen stores by glucose infusion after glycogen-depleting exercise restores anaerobic capacity, evident as restoration of the maximum accumulated oxygen deficit and run time to fatigue during high-speed exercise\textsuperscript{81}. Similarly, a 41\% decrease in muscle glycogen concentration impaired the capacity for work in horses dragging a sled, suggesting a reduction in anaerobic capacity\textsuperscript{82}. During intense exercise, excessive production of lactic acid occurs in association with fatigue and is believed to alter metabolism of the muscle cells\textsuperscript{83} and induce sarcoplasmic reticulum dysfunction\textsuperscript{84,85}. For example, low pH inhibits the enzyme PFK, thus decreasing efficiency of the anaerobic glycolysis. Furthermore, the glycolytic enzymes are in close proximiy to the sarcoplasmic reticulum Ca transport system\textsuperscript{86}. Thus, impaired glycolytic flux and selective depletion of muscle glycogen associated with sarcoplasmic reticulum may impair excitation-contraction coupling and calcium flux, and thus may contribute to fatigue\textsuperscript{87,88}. On the other hand, products of AMP deamination stimulate glycolytic enzymes. Fatigue is also thought to be related to the accumulation of Pi, ADP, AMP and further degradation products\textsuperscript{77,89,90} concomitant with depletion of glycogen stores\textsuperscript{80,81} observed in the type I and IIA oxidative fibres\textsuperscript{51,77}. Depletion of sources of energy in these fibres will induce progressive recruitment of type IIX fibres and further lactate production. Thus, the metabolic response to such exercise is highly influenced by the muscle fibre composition and by the oxidative and glycolytic capacities of the muscle fibres. Those properties are most probably responsible for the different resistance to fatigue observed between horses\textsuperscript{91}. Capillary supply of muscles, and especially of type IIB fibres, is of importance for aerobic capacity and exercise tolerance in Standardbred trotters\textsuperscript{92}.

In prolonged low-to-moderate-intensity (submaximal) exercise, lipids are the predominant source of energy. However, glycogen depletion (observed in type I and IIA oxidative fibres\textsuperscript{49,93}) also likely contributes to fatigue\textsuperscript{71} because acetyl-CoA produced by free fatty acid oxidation needs oxaloacetate (produced from pyruvate) to enter the Krebs cycle and proceed to sufficient ATP synthesis through oxidative phosphorylation (see Fig. 2). Evidence of the role of muscle glycogen as a major energy substrate during submaximal exercise was clearly demonstrated by combined use of isotopic tracer and indirect calorimetry methods. Intramuscular carbohydrates (primarily glycogen) account for c. 50\% of the total energy expenditure during submaximal exercise performed at 30\% of maximum oxygen uptake during the early phase of the exercise (0–30 min). However, as the exercise duration increases, glycogen utilization counts for only 20\% of the total energy expenditure, with a concomitant increase in lipid utilization at 60–90 min of exercise\textsuperscript{53}. Conversely, with increased work intensity (from 30 to 60\% of maximum O\textsubscript{2} uptake), there is an increase in the rates of both muscle glucose uptake and muscle glycogen breakdown, with concomitant decrease in lipid oxidation (Fig. 3). Indeed, the increase in energy expenditure associated with increased workload was met almost entirely by an increase in muscle glycogen utilization, which was c. 60\% of the total energy expenditure\textsuperscript{53} (see Fig. 3). Administration of carbohydrate before or during exercise, either in the form of glucose (2 g kg\textsuperscript{-1}, PO or 34.9 \textmu mol kg min\textsuperscript{-1} IV) or a grain meal (corn, 51.4 kJ of digestible energy kg\textsuperscript{-1} of diet), enhanced glucose use and decreased lipid oxidation during subsequent submaximal exercise, without altering the contribution of muscle glycogen utilization to total energy expenditure\textsuperscript{53,65} (see Fig. 4). These studies clearly demonstrate the influence of nutrition on substrate utilization and energy partitioning during subsequent exercise. However, it is worth noting...
that feeding of forage 2–3 h before exercise has a minimal effect on substrate utilization during exercise (Fig. 4).

Because glycogen is a limiting fuel for contracting muscle for both high- and low-to-moderate-intensity exercise, the capacity to maximize muscle glycogen replenishment after exercise is an important factor for optimizing performance in horses competing on successive days or on several occasions during the same day. However, complete resynthesis of muscle glycogen pool requires 48–72 h after exercise, after conventional or high-carbohydrate diet. Intravenous glucose administration hastened glycogen stores replenishment after strenuous exercise but, in the equine muscle glycogen depleted by exercise, starch-rich meals failed to enhance GLUT-4 gene expression, GLUT-4 protein content and muscle concentrations within the first 24 h after exercise. A 30% increase in muscle glycogen concentrations in horses fed starch-rich meals was observed only 72 h after exercise compared with horses fed conventional diet. Mechanisms underlying the slow glycogen replenishment after exercise in horses are not well known, but may include limited ability of the small intestine to digest starch, slower activity of the muscle glycogen synthase enzyme and lower recruitment of GLUT-4 at the plasma membrane compared with values reported in humans and rodents. For instance, glycogen synthase activity increased twofold after

Fig. 3 Contribution of energy from different substrate sources with work intensity and time. With increasing work intensity there is an increase in muscle glycogen utilization, with a concomitant reduction in the rate of lipid oxidation in equine skeletal muscle. Contribution of energy from different substrate sources during the 20- to 30-min (at 30% of maximum O2 uptake) and 35- to 45-min (at 60% of maximum O2 uptake) periods of exercise in six horses. Modified from Geor et al. and used with permission from the Journal of Applied Physiology.

Fig. 4 Relative caloric contributions from oxidation of muscle glycogen, lipid and blood glucose during exercise. Administration of carbohydrates (either in the form of oral glucose or starch-rich meals) before exercise increases plasma glucose utilization and decreases lipid utilization, without altering the use of muscle glycogen during the 30- to 60-min period of a submaximal exercise (at 50–55% maximum O2 uptake). Panel A. Relative caloric contributions from oxidation of muscle glycogen, lipid and blood glucose in six horses during exercise after oral administration of water or glucose (2 g kg⁻¹). ** Values significantly different for both glucose and fat utilization in glucose compared with water trial, P < 0.05. Modified from Geor et al. and used with permission from the Journal of Applied Physiology. Panel B. Relative caloric contributions from oxidation of muscle glycogen, lipid and blood glucose during exercise after withholding feed, feeding hay or feeding grain 90 min before exercise. * Values significantly different for fat utilization in grain compared with hay trial, P < 0.05. Significant increase in the caloric contribution from oxidation of glucose in the grain trial compared with the fast and feeding trials was noticed during the 5- to 30-min period of exercise. Modified from Jose-Cunilleras et al. and used with permission from the Journal of Applied Physiology.
glycogen-depleting exercise in horses, whereas a five- to ten-fold increase was reported in humans under similar condition. Furthermore, in contrast with other species, GLUT-4 protein content is unchanged or only mildly increased immediately after exercise and IV glucose infusion after exercise enhanced muscle glycogen synthesis but attenuated the increase in GLUT-4 protein content. Because of the slow rate of glycogen synthesis (up to 72 h), the interval between exercise bouts may be inadequate for complete or partial restoration of muscle glycogen stores, which may contribute to a decline in performance during subsequent exercise in the horse. The effect of pre-exercise and, more recently, post-exercise feeding on muscle glycogen substrate and exercise performance have been well studied, but are beyond the scope of this review.

Contractile and metabolic profile in relation to performance
Specific athletic abilities are greatly influenced by genetic factors, and significant variations in muscle fibre composition are observed among breeds and types of horses known to have a predisposition for specific disciplines. For example, Arabian horses, known to have high endurance capacities, have in their locomotory muscles a greater percentage of type I fibres than Thoroughbreds horses, known to be sprinters. In addition, muscle fibre proportion correlates with performances. Among endurance horses, the better performers have higher percentages of type I and IIA fibres, larger type I and IIA fibres, higher activities of oxidative enzymes, higher lipid oxidation capacity and lower percentages of type II fibres. On the contrary, performances requiring short duration, high-intensity exercise are correlated with high percentages of type II fibres. Standardbred trotters with higher racing performance have a higher type IIA:IIB ratio and more high-oxidative type IIB fibres than horses with a lower racing performance.

The proportion of slow- to fast-contracting fibres is thought to be somewhat inherited and to be influenced by age and gender; however, it may be modified by training programmes (see below).

Muscle responses to training
It can be predicted that a range of fibre type distributions are required for success at elite levels within each discipline. Linked to plasticity of muscle energetics, specific training has the ability significantly to change the fibre-type composition, metabolic properties, fibre size and/or capillarization within skeletal muscle.

For example, endurance-trained horses show some enlargement of type I and IIA fibres (hypertrophy) and an increased number of capillaries surrounding type I fibres in their locomotory muscles. However, these changes are not consistently observed amongst different breeds. In Standardbred trotters, a period of intensive training will rapidly increase the oxidative capacity and the capillary density in muscle. Long-term draught training programmes in Andalusian horses modify MyHC composition, with an increase in high-oxidative fibres and a decrease in fast-glycolytic fibres. In Standardbred trotters, there is an increase in the type IIA:IIB ratio and in oxidative capacity in skeletal muscle after a draught-loaded interval-training programme. Regular training in Standardbred trotters from 18 months of age until 3–4 years of age resulted in significant changes in muscle composition, with a shift among type II fibres towards fast oxidative IIA fibres and an increase in oxidative capacity. Well-trained racing Standardbred trotters have a high IIA:IIB fibre ratio and a high oxidative capacity. Changes in muscle fibre oxidative capacity were also observed in 2- to 3-year-old trained Thoroughbreds. Additional high-intensity training programmes have the potential to increase anaerobic capacity, which might be of particular interest in ensuring the final acclimatization necessary to compete satisfactorily in a race. However, high-intensive training might increase the risk of lameness and might contribute to overtraining.

Muscle concentration of glycogen stores may also be influenced by training, since it has been shown that low-intensity (in Haflinger ponies) or high-intensity training programmes increase muscle glycogen stores. The GLUT-4 gene expression increases in the hours following glycogen-depleting exercise, which might contribute to refurnishing the glycogen stores used during exertion. Glucose transport capacity from blood to muscle is increased in trained muscle owing to an increase of GLUT-4. Furthermore, training favours development of fibres, capillary network which results in better muscle perfusion and greater blood nutrient availability.

Thus, it appears that training increases the overall capacity of the muscle to respond to specific demands. Fibre-type transitions would occur in a ranked and sequential mode, such as type IIX → IIAAX → IIA → I, according to the energetic requirement enforced by training (i.e. type IIA fast oxidative fibres for sprinters and type I slow oxidative fibres for endurance performers).

Despite apparent healthiness and maintenance of training, some Standardbred horses show diminished performances. These may be red cell hypervolaemic horses which, compared with normovolaemic horses, have a higher oxidative capacity in type II fibres, a lower capillary supply and a lower percentage of the type IIX isoform. In these poor performers, it is assumed that a change in MyHC isoforms from IIX to I occurs. This might explain why these horses were...
unable to complete a sprint (which requires fully recruitment of type IIX fibres).

It is important to realize that not only training but also dietary compositions may influence glycogen storage and metabolic response to exercise. Age and gender are also important factors to consider, as these influence fibre-type composition and, in both Standardbreds and Thoroughbreds, stallions have a higher type IIA:IIB ratio compared with mares.

**Perspectives**

Metabolic adaptations to every type of physical exercise are the result of very wide-ranging responses of muscle cells (among others) to intracellular signals that are provided by metabolic pathway activities, and are the consequences of cellular stress (in its widest meaning). The simplest description of these so-called retrograde responses could be: changes in metabolic by-product concentrations and up-or-down regulation of transcription of DNA coding for specific enzymes (proteins) that control the flux of metabolic pathways, i.e. the sources of these by-products. If modulation of transcription is followed by changes in translation into proteins, then up-or-down regulation of enzyme concentrations (activities) occurs that can modify the rate at which the by-products are processed. A new steady state is therefore achieved.

While genomes are rather constant entities, their protein complements, the so-called proteomes, differ from cell to cell. Furthermore, depending on the cell life cycle and environmental factors, protein expression patterns are constantly adapted in terms of splicing isoforms, expression levels or post-translational modifications following their biogenesis. Accordingly, these complex and dynamic protein networks cannot be fully characterized by gene expression analysis alone, making proteomics (i.e. the study of proteomes) the most unavoidable field in protein science. Proteomics encompasses a two-step analysis of proteomes including (1) a large-scale protein profiling followed by (2) protein identification by mass spectrometry and bioinformatic.

Protein expression proteomics (also called comparative proteomics), which describe proteome variations induced under various conditions, could be the ‘golden way’ of studying cellular adaptations to exercise at the energy metabolism level, as well as at the structural protein level sustaining mechanical work.

**References**

Muscle energetics in exercising horses


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