

# **Advances in Equine Nutrition**

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**J.D. Pagan**



## **SKELETAL MUSCLE FUNCTION AND METABOLISM**

**S.J. VALBERG and J.M. MACLEAY**

*Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, Minnesota, USA*

Skeletal muscle provides the explosive power of the jumper and cutting horse, the stamina of the endurance horse and the fine motor control of the advanced dressage horse. In this paper the pertinent aspects of muscle function and metabolism that confer this remarkable athleticism will be discussed.

### **Structure**

Skeletal muscle consists of bundles of long spindle shaped cells called muscle fibers that attach to bone by tendinous insertions. The blood vessels and nerves that nourish and control muscle function run in thin sheets of connective tissue that surround bundles of muscle fibers. Each nerve branch communicates with one muscle fiber at the motor end. The nerve and all the muscle fibers that it supplies are together termed a motor unit. Each time that a nerve is stimulated all of the muscle fibers under its control will contract.

A muscle's unique ability to contract is conferred by the highly organized parallel, overlapping arrangement of actin and myosin filaments. These repeating contractile units or sarcomeres extend from one end of the cell to another in the form of a myofibril. Each muscle fiber is packed with myofibrils that are arranged in register giving skeletal muscle a striated appearance under the microscope. Muscle contraction occurs when the overlapping actin and myosin filaments slide over each other, serving to shorten the length of the muscle cell from end to end and mechanically pulling the limb in the desired direction. The sliding of the filaments requires chemical energy in the form of ATP.

### **Muscle fiber types**

Different forms of myosin (isoforms) can be found in different muscle fibers and these different isoforms affect the speed with which a muscle cell can contract. In addition, fibers with specific contractile speeds will also possess characteristic metabolic energy producing capacities (Table 1). Type 1 fibers contract slowly, are

ideally suited for endurance and are able to hold a tetanic twitch for long durations without fatigue. Their resistance to fatigue is in part related to their high density of mitochondria which confer a high aerobic or oxidative capacity. In addition to having the highest oxidative capacity, type 1 fibers also have the highest lipid stores, the highest density of capillaries, the lowest glycogen stores and the lowest glycolytic enzyme capacity of the three fiber types. Fast-twitch muscle fibers or type 2 fibers are readily divided in the horse into type 2A and 2B fibers. Type 2B fibers have the fastest contractile speed, the largest cross-sectional area, the highest glycogen stores and glycolytic capacity and the lowest oxidative capacity. As such they are ideally suited to short fast bursts of power. Type 2A fibers are intermediate in contractile speed and metabolic properties between type 1 and type 2B fibers.

**Table 1.** THE PROPERTIES OF MUSCLE FIBER TYPES IN THE UNTRAINED HORSE

	<i>Type 1</i>	<i>Type 2A</i>	<i>Type 2B</i>
Speed of contraction	slow	fast	very fast
Fatigability	low	intermediate	high
Oxidative (aerobic) capacity	high	intermediate	low
Glycolytic (anaerobic) capacity	low	high	high
Glycogen content	low	high	high
Fat content	high	intermediate	low

Most large muscles in the horse contain a mixture of muscle fiber types. The muscle fiber composition, the percentage of type 1, 2A and 2B fibers, and muscle fiber cross-sectional areas vary greatly between muscle groups, among individual horses and between breeds. These proportions are not constant, as training can alter the fiber composition and fiber size in the same muscle over time. Propulsive locomotor muscles such as the gluteus contain a predominance of fast-twitch type 2 muscle fibers, with the highest density of type 1 fibers located deeper within the muscle. In general, Quarter horses and Thoroughbreds have the highest percentage of fast-twitch muscle fibers, 80-90%; Standardbreds an intermediate number, 75%; and donkeys have the lowest percentage of type 2 fibers in locomotor muscles. With growth and training, there is a change in the length and breadth of a fiber, and a change in the proportion of fiber types rather than an increase in the number of muscle fibers. In young horses intensively trained at speed, the proportion of type 2A fibers increases concomitant with a decrease in type 2B fibers.

### **Muscle fiber recruitment**

When a muscle contracts during exercise, it does so in response to a predetermined recruitment of particular muscle fibers. This orderly recruitment of muscle fibers

leads to smooth, coordinated movement. Each motor contains fibers of the same type. As exercise begins, a select number of motor units are recruited to provide the power to advance the limb. At slow exercise intensities, type 1 muscle fibers and a small number of type 2A muscle fibers are stimulated. The force produced by any muscle is proportional to the cross-sectional area that is active. As the speed or duration of exercise increases, more muscle fibers will be recruited and this occurs in the order of their contractile speed. Only at near-maximal exercise intensities or after several hours of submaximal exercise are type 2B fibers recruited.

### **Excitation - contraction - coupling**

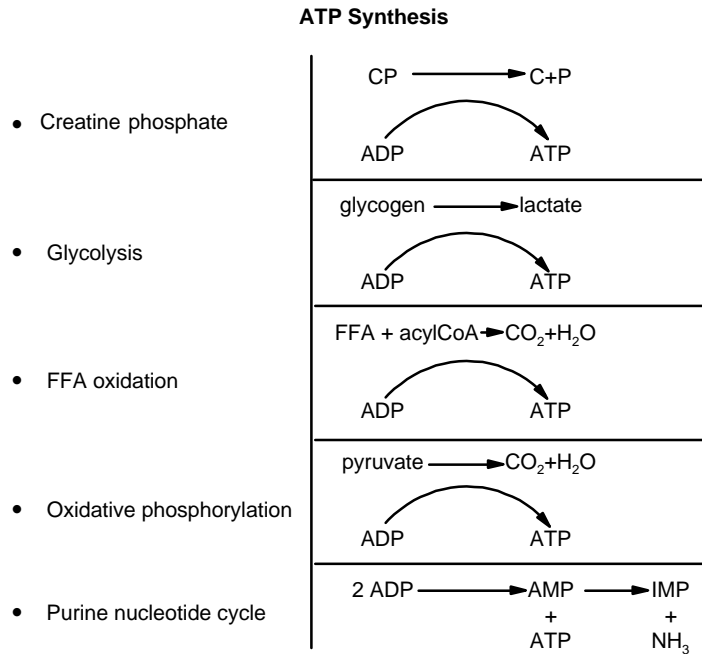
When a nerve is stimulated, a wave of electrical depolarization occurs that quickly reaches the neuromuscular junction. In response, the nerve terminal releases acetylcholine which binds to the motor-end plate of the muscle fiber and initiates electrical depolarization of the muscle cell membrane. Electrical depolarization of the muscle cell membrane triggers the release of calcium that is sequestered in intracellular membranous storage sites (sarcoplasmic reticulum) into the myoplasm via the calcium release channel. Increased concentrations of intracellular calcium allows the interaction of actin and myosin filaments which then slide over each other in a ratchet like fashion to produce a contraction (cross-bridge cycling). Tension is generated as the shortening filaments tug both ends of the myofiber toward the middle. Muscle must relax after each contraction by actively pumping calcium back into the storage sites preventing actin-myosin interaction. In addition, ion pumps in the cell membrane actively repolarize the muscle cell membranes. All of the processes necessary for relaxation are active, meaning that they require energy in order for them to occur.

### **Metabolic responses to exercise**

#### **ENERGY PATHWAYS**

The basic unit of energy in the cell is adenosine triphosphate or ATP. This molecule stores energy in the form of a high energy phosphate bond. When cleaved, the products include adenosine diphosphate (ADP), inorganic phosphate (Pi), and energy for use in cellular functions. During muscle contractions, ATP is utilized for actin-myosin cross-bridge cycling, and cell membrane and sarcoplasmic reticulum ion pumps. The processes of glycogenolysis, glycolysis, the Krebs cycle, oxidative phosphorylation by the electron transport chain, oxidation of free fatty acids (FFA), and purine nucleotide deamination all serve to supply the muscle cell with ATP (figure 1). A number of interdependent factors appear to influence the metabolic pathways used for energy production during exercise in the horse. These include the speed and duration of exercise; the muscle fiber composition; the properties of the muscle fibers

recruited, including capillarization, oxidative and glycolytic capacities; and the availability of oxygen and different energy substrates.



**Figure 1.** Skeletal muscle metabolic pathways used to supply ATP for muscle contractions.

### Energy Sources

Creatine phosphate serves as a small store of immediate energy within the muscle fibers. Initially, cleavage of the phosphate from ATP or the creatine phosphate molecule supplies energy until ATP production from glucose or glycogen begins. Glucose is stored in the body in the form of glycogen, a long, branched polymer of glucose molecules. The largest deposits of glycogen are in the liver and muscle cells. Up to 8% of the liver's weight and up to 1-2% of the muscle's weight may be in the form of glycogen. Sympathetic stimulation of the nervous system, as occurs in exercise, causes an increase in the circulating levels of epinephrine and glucagon. These hormones activate phosphorylase enzymes that breakdown glycogen to phosphorylated glucose molecules. The muscle or liver cells shift from glycogen formation to glycogenolysis to supply free glucose. When this process occurs in the liver, free glucose is released into the blood stream. Muscle cells obtain glucose from the blood and from glycogen stored within the muscle cell. Fat serves as another energy-rich fuel source. Free fatty acids released from adipose tissue or from the liver can be taken up by the muscle cells and burned aerobically. Small intracellular triglyceride stores are also present within type 1 and 2A muscle fibers.

### **Metabolic pathways**

Anaerobic pathways such as glycolysis, creatine phosphate and the purine nucleotide cycle are found within the cell cytoplasm. This pathway, which converts glucose to pyruvate and then lactate, provides 2 molecules of ATP for each molecule of glucose metabolized. Aerobic pathways such as the Krebs cycle, oxidation of FFA and the electron transport chain are located within the cell's mitochondria and provide the bulk of ATP for the cell so long as oxygen is plentiful. Specific transport mechanisms are present in the outer mitochondrial membrane to move FFA and pyruvate into the mitochondria for further metabolism. The efficiency of mitochondrial pathways is demonstrated by the ability to generate 38 molecules of ATP from oxidation of one molecule of glucose or the generation of up to 146 molecules of ATP from oxidation of a FFA.

### **AEROBIC EXERCISE**

At submaximal exercise speeds, oxygen is readily available and slow-twitch fibers as well as fast-twitch fibers with a high oxidative capacity are recruited. Intramuscular supplies of ATP and creatine phosphate (CP) are quickly utilized and energy must be derived from glycolytic or oxidative pathways. A drop in the ATP: ADP + Pi ratio activates glycolysis, and ATP for contraction will then be provided by the metabolism of glycogen to pyruvate. Pyruvate is converted to acetyl-CoA in the mitochondria and is completely oxidized by the Krebs cycle and the electron transport chain. The amount of blood-borne substrates (glucose and FFA) available for oxidation increases within 15 minutes of submaximal exercise in conjunction with increasing concentration of cortisol and low insulin concentrations. High rates of oxidative phosphorylation result in inhibition of phosphofructokinase and a slowing of glucose oxidation in favor of beta-oxidation of FFAs. As such, the rate of intramuscular glycogen utilization steadily declines over time as the oxidation of FFA increases. Oxidative metabolism is highly efficient. It provides much more ATP per molecule of substrate (glucose or FFA) than glycolysis without altering intracellular pH. By using FFA, intramuscular glycogen stores are spared. Fatigue during prolonged submaximal exercise occurs when a combination of the following occurs: intramuscular glycogen concentrations become depleted, muscle temperatures become markedly elevated, electrolyte concentrations are altered or neuromuscular fatigue occurs (Table 2). Very little lactic acid accumulates at fatigue in horses performing submaximal exercise.

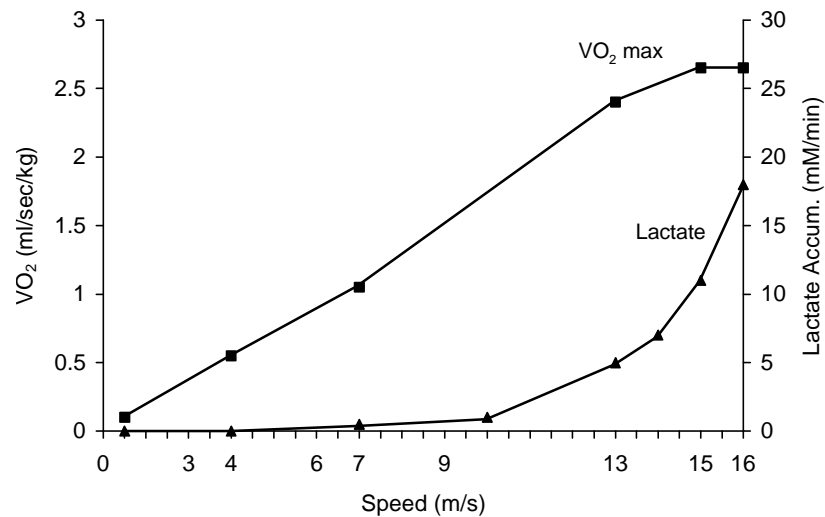
**Table 2.** FACTORS THAT CONTRIBUTE TO FATIGUE DURING AEROBIC AND ANAEROBIC EXERCISE INTENSITIES.

<i>Aerobic Exercise</i>	<i>Anaerobic Exercise</i>
<ul style="list-style-type: none"> <li>• glycogen depletion</li> <li>• hyperthermia</li> <li>• electrolyte depletion</li> <li>• myalgia and motivation</li> </ul>	<ul style="list-style-type: none"> <li>• lactic acidosis</li> <li>-inhibition of PFK</li> <li>-decreased muscle tension</li> <li>• depletion of CP and ATP</li> </ul>

PFK= phosphofructokinase  
 CP= creatine phosphate  
 ATP= adenosine triphosphate

### ANAEROBIC EXERCISE

With any form of exercise a small amount of anaerobic metabolism occurs, but at submaximal speeds the majority of energy is produced by aerobic metabolism. As the speed of exercise increases so does the energy demand placed on the muscle. More muscle fibers are recruited including type 2B fibers and more oxygen is consumed by the horse until it reaches a speed where the delivery of oxygen or the ability to utilize oxidative processes becomes limiting. At the point of maximum oxygen consumption (VO<sub>2</sub>max) any further energy must be generated by anaerobic glycolysis or deamination of ATP (figure 2). By converting pyruvate to lactate, NADH is oxidized to NAD thereby further facilitating glycolysis. With their rich supply of glycolytic enzymes and limited capacity for oxidative phosphorylation, type 2B fibers are uniquely suited for anaerobic glycolysis. With type 2B fiber recruitment at speeds at and beyond the point of maximal oxygen uptake, an exponential rise in blood lactate accumulation occurs (figure 2). The advantages of anaerobic glycolysis are that oxygen is not required and that it provides a rapid supply of ATP. Depletion of glycogen stores does not limit maximal exercise because anaerobic glycolysis is inhibited by a lactic acidosis before total muscle glycogen is depleted. Low intramuscular pH caused by lactate and hydrogen ion accumulation inhibits phosphofructokinase enzyme, which is the rate limiting step in glycolysis (Table 2). Muscle pH can fall as low as 6.4 following maximal exercise at which point both glycolysis and excitation contraction coupling are inhibited. The ability to buffer or remove hydrogen ions becomes very important for muscle function during maximal exercise. Intracellular buffering of hydrogen ions occurs by proteins and dipeptides such as carnosine. Diffusion and active transport of lactate into the general circulation where bicarbonate acts as a major blood buffer are also important for intramuscular pH. The amount of lactic acid in the circulation following exercise is in part directly related to the percentage of low oxidative type 2B fibers in the muscle and the duration of high intensity exercise.



**Figure 2.** The oxygen consumption and rate of lactate accumulation in a Thoroughbred horse. (Data courtesy of Dr. JH Jones).

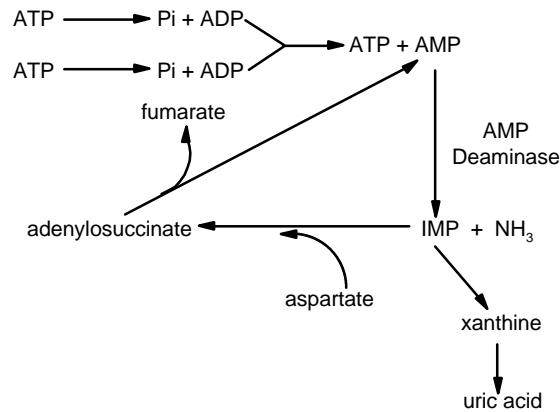
At top speeds, as the intramuscular stores of ATP and energy demands outstrip its innate ability to produce ATP, the cell turns to its last venue of energy production. Surplus ADP in the cell is converted to ATP and AMP in a process called the myokinase reaction (figure 3). At low intramuscular pH, AMP deaminase enzymes are activated and this deaminates AMP into IMP,  $\text{NH}_3^+$  and  $\text{P}_i$  to catalyze the production of ATP from ADP. Short term, this produces ATP for muscle contraction from the excess of intramuscular ADP. However, ammonia diffuses from the cell and the total nucleotide pool is depleted. Plasma ammonia levels begin to increase at a threshold beyond the onset of lactic acid accumulation and correlates strongly with a depletion of ATP within the muscle cells. Before intracellular stores of ADP and ATP are replenished IMP must be reaminated. The reamination of IMP into ADP and ATP takes at least 30-60 minutes. Ultimately, fatigue appears to be related to depleted ATP stores within muscle fibers (Table 2). The levels of lactate and ATP in individual fibers during maximal exercise may be more important for the onset of fatigue than the measured concentrations in whole muscle samples. Depleted fibers might develop a sustained painful contracture similar to rigor, and impair the horse's ability to maintain maximal speed.

## The effect of training

### ENDURANCE TRAINING

Endurance training in horses changes the contractile and metabolic profile of skeletal muscles. After a short period of training, the volume density of mitochondria, and



**Purine Nucleotide Cycle****Figure 3.** The purine nucleotide cycle.

thus the oxidative enzyme capacity, all increase. Over a 6 month period the ratio of type 2A:2B fibers increases, the cross-sectional area of type 2B muscle fibers decreases, and the capillarization of all fiber types increases. These adaptations favor the delivery of oxygen and blood-borne substrates, the early activation of oxidative metabolism and the utilization of FFA in muscle fibers. By sparing muscle glycogen, endurance is enhanced, and fatigue is delayed.

**SPEED TRAINING**

With training at high intensities, the speed at which a horse begins to accumulate lactate should gradually increase, delaying the onset of lactate accumulation and ATP depletion. This is accomplished by an increase in the oxidative capacity (by increased mitochondrial volume) and capillarization of all muscle fiber types, including type 2B fibers. Training of 2 and 3 year-old Standardbred and Thoroughbred racehorses in Scandinavia had been shown to reduce the percentage of type 2B fibers and increase the percentage of type 2A fibers as well as decrease the cross-sectional area of type 2B fibers. Although an increase in oxidative capacity may be metabolically advantageous, a decrease in the percentage of type 2B fibers and a decrease in their cross-sectional areas may also deleteriously affect their speed and force of contraction. Muscle must have the ability to generate energy via anaerobic glycolysis for race horses to be successful. Obviously a balance is required between skeletal muscle fiber metabolic and contractile properties for optimum speed and endurance. Since the muscle fiber composition and fiber properties vary so greatly between horses, achievement of this balance may be different for each horse.

## **References**

- Snow, D.H. and Valberg S.J.: Muscle anatomy, physiology and adaptations to exercise and training. In Rose, R.J. and Hodgson, D.H. (eds) *The Athletic Horse: principles and practice of equine sports medicine*. Philadelphia, PA, W.B. Saunders Co., 1994.
- Hodgson D.H.: Energy considerations during exercise. In Rose, R.J. (ed) *Vet Clinics of North America, Equine Practice*, vol 3, Philadelphia, PA, W.B. Saunders Co., 1985.

