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Advances in Equine Nutrition

Volume IV

Edited by

J.D. Pagan

MUSCLE ADAPTATIONS DURING GROWTH AND EARLY TRAINING

STEPHANIE J. VALBERG AND LISA BORGIA
University of Minnesota, St. Paul, Minnesota

Introduction

It is well documented that mammalian muscle, while influenced by heredity, is highly plastic tissue that is capable of changing dramatically in response to growth, exercise, hormonal influences, disease, and dietary deficiencies. The increased demand for performance capacity in young racehorses has prompted investigations to determine if instituting training at a young age can have a major impact on muscle development, thereby promoting long-term benefits to equine performance. The purpose of this review is to highlight the changes that occur in foals and weanlings with growth as well as to review the findings from studies where training has been introduced to foals in the weanling or yearling stage.

Much of our knowledge of skeletal muscle in horses has been gained through the study of muscle biopsies. A muscle biopsy technique for horses was developed in the 1970s (Lindholm and Piehl, 1974; Snow and Guy, 1980). The technique involves insertion of a 6-mm diameter needle into the muscle through a 10-mm skin incision and removal of approximately 400 mg of tissue from a standardized depth. A battery of tinctorial and histochemical stains are applied to specially frozen muscle to identify muscle fiber sizes, shapes, and contractile and metabolic properties, as well as neuromuscular junctions, nerve branches, connective tissue, and blood vessels. Biochemical evaluation of skeletal muscle samples provides quantitative information regarding the activity of various metabolic enzymes and substrate and metabolite concentrations. Muscle biopsy has greatly expanded our understanding of normal muscle structure and function, as well as disease processes.

Contractile Fiber Types

Skeletal muscle comprises approximately 50% of the body's mass. It consists largely of long multinucleated spindle-shaped cells (myofibers) that are highly specialized by virtue of a structured array of muscle-specific contractile proteins that confer the ability to shorten rapidly and efficiently. Myosin, one of the main contractile proteins, possesses enzymatic properties that allow the hydrolysis of adenosine triphosphate (ATPase). The speed of contraction of individual muscle fibers differs depending on the type of myosin and the activity of the myosin ATPase. Contractile properties

of muscle fibers can be differentiated using a histochemical staining technique that is based on the sensitivity of myosin ATPase activity to acid and alkaline pre-incubation. Slow twitch type I fibers stain darkly after acid and lightly after alkaline pre-incubation. In contrast, fast twitch type II myofibers stain lightly with acid and darkly with alkaline pre-incubation (Brooke and Kaiser, 1970). Some fibers do not reverse their staining properties in acid and alkaline media and are classified as type IIC or intermediate myofibers. This likely corresponds to fibers containing either slow and fast twitch myosin or embryonic myosin. Further, type IIA and type IIB myofibers may be differentiated if muscle sections are pre-incubated at pH 4.6 prior to ATPase staining.

Myosin also has structural properties that can be used to differentiate fiber types. Two identical myosin heavy chains (MHC) and two pairs of light chains form each myosin molecule. Different forms of MHC (termed isoforms) are expressed in individual muscle fibers. Immunohistochemical techniques recently have been developed to specifically identify fiber types based on antibodies directed against various MHC isoforms (Gorza, 1990). Embryonic and slow twitch myosin isoforms as well as type 2a, type 2b, and 2x (also called 2d) in skeletal muscle and type 2m fibers in the jaw muscles can be distinguished by this technique. Some hybrid fibers contain a mixture of these isoforms (i.e., type 2a/x). Fibers with type 2x myosin contract faster than fibers with type 2a myosin, which in turn contract faster than type 1 myosin.

Unfortunately, type IIB fibers distinguished by myosin ATPase activity do not correspond to type 2b fibers distinguished by immunohistochemical staining for MHC. Rather, type IIB fibers correspond more closely with type 2x fibers, and type 2b fibers correspond to a very rapidly contracting fiber type found in rodents and camelids (Gorza, 1990; Linnane et al., 1999). In the following sections, information derived from studies using histochemistry is designated by Roman numerals for fiber types (I, IIA, IIB), whereas studies using immunohistochemistry use types 1, 2a, and 2x.

Metabolic Fiber Types

Type 1 fibers generally have higher concentrations of triglycerides and myoglobin and are better suited to derive their energy by oxidative phosphorylation via the electron transport system following the oxidation of fatty acids and glucose via the Krebs cycle. The oxidative capacity of type 1 fibers can be demonstrated by dark histochemical staining for the activity of oxidative enzymes such as succinate dehydrogenase (SDH) and reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADHTR) (Dubowitz et al., 1973).

In general, type II fibers are suited to derive energy for contraction by anaerobic glyco(geno)lysis. Fast twitch fibers, particularly type IIx and IIb fibers, tend to have higher concentrations of glycogen as well as higher activities of enzymes associated with glycogenolysis and glycolysis such as phosphofructokinase (PFK) and lactate dehydrogenase (LDH) activity. The oxidative staining of type II fibers can be variable with some fibers containing higher oxidative staining than others. In general type 2a

fibers have higher oxidative staining than type 2b fibers. However, this is not always the case and may vary with age and training (Valberg et al., 1988). A further means to subtype fibers is by both their ATPase and oxidative staining for SDH or NADH. This fiber typing distinguishes slow twitch, fast twitch oxidative, and fast twitch glycolytic (or non-oxidative) fibers.

Measures of Metabolic Capacity

Aerobic pathways such as the citric acid cycle, ß oxidation of free fatty acids, and the electron transport chain are located within mitochondria and provide the bulk of ATP for the cell as long as oxygen is plentiful. The activity of key enzymes in the pathways can be used as an indicator of oxidative capacity. Citric acid cycle enzymes such as citrate synthase (CS) and succinate dehydrogenase (SDH) are often measured in snap frozen muscle biopsies as oxidative markers. Often used as a marker for the capacity for ß oxidation of free fatty acids is 3-hydroxy-acyl-CoA dehydrogenase (HAD). Anaerobic pathways such as glycolysis, creatine phosphate, and the purine nucleotide cycle are found within the cell cytoplasm. Markers for the capacity for anaerobic glycolysis that are commonly measured in frozen muscle include PFK and LDH enzyme activities.

The main fuels for aerobic muscular contraction are fatty acids and glucose, which are supplied by intramuscular (lipid droplets, ß glycogen particles) and extramuscular (liver and adipose tissue) depots during exercise. The rate-limiting factor in the supply of plasma free fatty acid (FFA) to muscle appears to be the rate of FFA release from adipose tissues (Bennard et al., 2005). The rate-limiting factor in the extramuscular supply of glucose to working muscle is glucose uptake by the myofibers under the influence of insulin. Muscle triglyceride levels can be estimated by Oil red O staining in muscle. Glycogen stores in muscle can be measured biochemically in snap frozen samples and estimated from periodic acid Schiff's stains of frozen sections.

Variation in Muscle Fiber Type Composition

The speed and force developed by a muscle during contraction differs qualitatively and quantitatively depending on its fast and slow twitch fiber type composition. Most muscles in horses contain a mixture of types 1, 2a, and 2x fibers (or I, IIA, and IIB, depending on the technique used for fiber typing). Locomotor muscles have a high proportion of type 2a and 2x fibers relative to type 1 fibers, whereas postural muscle have a higher proportion of type 1 fibers than most locomotor muscles. The proportion of muscle fiber types within a given muscle will also vary along its length and depth.

Generally, deeper muscles or portions of muscles have a higher percentage of type 1 muscle fibers. Due to this variation, when comparing the fiber type composition of different individuals, a standardized site within a muscle must be used. Fiber type composition of muscles on the left and right side of the body will be identical if

samples are taken from the same site and depth. The gluteus muscle is often chosen for study in horses because it is a major propulsive muscle active in locomotion, is easily accessible, and shows adaptations to growth and training. Some studies have also evaluated the semimembranosus or tendinosus muscle.

When standardized techniques are used to assess muscle fiber composition, individual horses have been shown to have a wide variation in muscle fiber type composition. This phenomenon has been attributed to effects of genetic background, breed, sex, age, and state of training. Heritability is believed to have a strong influence on MHC isoforms in muscle and is estimated to be 13% (Rivero et al., 1996; Barrey et al., 1999). Breed differences have been extensively studied in horses (Snow and Valberg, 1994). In general, Quarter Horses and Thoroughbreds have the highest percentage of type II fibers in the gluteus medius, about 80-90%; Standardbreds and Andalusians have an intermediate number, about 75%; and donkeys have the lowest percentage of type II fibers in locomotor muscles. There are, however, wide variations between individuals of the same breed.

Stallions have a higher proportion of type IIA and lower proportion of type IIB fibers on average in their locomotor muscles than mares (Rivero et al., 1993a; Roneus, 1993). No differences in type I fibers or oxidative enzyme activities have been identified between age-matched Standardbred mares and stallions (Roneus, 1993). Andalusian mares have a higher percentage of type I fibers than stallions (Rivero et al., 1993a), a finding which has been inconsistently reported in Thoroughbreds (Snow and Guy, 1980; Roneus et al., 1991).

With growth and training, there is a change in the length and breadth of all fibers, and a change in the proportion of fiber types rather than an increase in the number of muscle fibers.

Effect of Growth on Metabolic and Contractile Properties

In the embryo, primitive muscle cells migrate to their position in the limb where their eventual fiber type is influenced by innate developmental directives, temporal and positional factors, neural enervation, and activation of specific signal transduction pathways. Fetal muscle cells initially express perinatal MHC, which is subsequently replaced by one of the fast or slow twitch MHC isoforms. Further development of subtypes of fast twitch fibers (2a, 2ax, or 2x) occurs in concert with the emergence of thyroid function (Emerson and Hauschka, 2004). Positional factors dictate that those portions of muscles that are primarily postural have a higher percentage of slow twitch type 1 muscle fibers. Eventual neural enervation dictates that all fibers supplied by the same nerve branch have the same muscle fiber type. These factors combine to produce a mosaic of fiber types with fiber type predominance programmed into certain muscles or portions of muscles. Muscle remains plastic well into adulthood, however, and contractile fiber types may be altered by growth and training.

The change in metabolic and contractile properties of the gluteus medius muscle with growth and development have been studied in Standardbreds, Quarter Horses,

Andalusians, Arabians, and Thoroughbreds (Essen-Gustavsson et al., 1983; Thornton and Taylor, 1983; Kline and Bechtel, 1990; Fowden et al., 1991; Roneus et al., 1991; Galisteo et al., 1992; Rivero et al., 1993a; Roneus, 1993; Yamano et al., 2005). A confounding factor in some of these studies is the degree of standardization of the biopsy site. Ideally, the same relative position within the muscle would be sampled as the animal grows. In studies where standardization was not accomplished, attributions of variation in both metabolic and contractile properties with growth may in fact reflect the sample depth-age relationship. In particular, if biopsy sample depth was not increased as foals aged, samples from older foals would underestimate any real increase in oxidative fiber types or oxidative capacity.

PERINATAL PERIOD

In utero, maternal glucose is the primary source of energy for equine muscle. During the third trimester of pregnancy, the equine fetus shows a clear insulin response to blood glucose, which serves to drive glucose into muscle and increase glycogen stores (Fowden et al., 1984; Fowden et al., 1991). Newborn foals continue their dependence on glucose as fuel via mare's milk, which is relatively high in sugar (59% DM) and low in lipid (13% DM) relative to other domestic animals (Rossdale, 1967). Muscle glycogen concentrations in newborn foals are relatively high (119-220 μmol/g wet weight) and similar to those found in yearling and two-year-old horses (Fowden et al., 1991; Valberg et al., 2001; de la Corte et al., 2002).

Few studies have addressed the metabolic characteristics of skeletal muscle of newborn foals. Kline (1990) found that Quarter Horses and Standardbreds followed from 1 to 100 days of age showed a significant increase in glycolytic enzymes (250% increase in LDH) and a decline in oxidative enzymes (37% decrease in HAD) in muscle. This corresponded to a tendency for fast twitch glycolytic fibers to increase and fast twitch oxidative fibers to decrease in these foals. The depth of the biopsy was not adjusted for growth in this study. We performed a study evaluating the activity of three enzymes involved in the ß oxidation of fat in gluteal muscle of Thoroughbred foals using a technique to standardize the depth of sampling. The activity of the enzyme crotonase decreased from 7.2 ± 1.6 μmol/g/min at 48 h of age to 4.4 ± 0.4 μmol/g/min at 1 month of age, whereas the activity of HAD and thiolase remained unchanged at 5.7 ± 2.2 to 4.1± 0.4 μmol/g/min and 1.1 ± 0.13 to 1.1 ± 0.3 μmol/g/min.

Mature fast and slow twitch MHC isoforms are expressed in newborn foal muscle. Fast twitch fibers in foals may coexpress perinatal MHC up to 10 weeks of age (Dingboom et al., 2002). In addition, many slow twitch fibers in foal muscle coexpress cardiac MHC isoforms up to 22 weeks of age.

BIRTH TO 1 YEAR OF AGE

The metabolic changes that occur in gluteal muscle with growth are not consistent across the studies available. Standardbred weanlings followed from 6 months to 1

year of age showed no change in CS or HAD activity and a decline in glycolytic capacity (LDH) (Essen-Gustavsson et al., 1983). In contrast, a study of Arabian and Thoroughbred crossbred foals showed a 23% decline in oxidative enzyme activity (SDH) and oxidative staining intensity of fast twitch fibers from 2 weeks to 8 months of age (Thornton et al., 1983). A 25% increase in glycolytic capacity (PFK activity) was also recorded. It is unclear what the depth of sampling was in these foals. This decline in oxidative fast twitch fibers was also documented in Arabian and Andalusian horses from birth to 1 year of age in a study where depth was not adjusted (Galisteo et al., 1992). A semi-quantitative study of SDH activity indicated an increase in activity in all fiber types with growth in Thoroughbred foals (Eto et al., 2003). We evaluated the activity of three enzymes involved in ß oxidation of fat in Thoroughbred foals using a biopsy technique that accounted for changes in muscle size with growth. The activity of the enzyme crotonase increased from 4.4 ± 0.4 µmol/g/min to 6.9 ± 0.9 µmol/g/min at 1 year of age, HAD increased from 4.1± 0.4 to 6.0 ± 1.2 µmol/g/min, and thiolase remained unchanged at 1.2 ± 0.1µmol/g/min. Thus, it would appear that there might be a variable change in oxidative capacity during the first year of life and an increase in glycolytic capacity.

From 6 months to 1 year of age, Standardbred foals showed no change in the percentage of type 1 fibers, an increase in type IIA fibers and a decrease in type IIB fibers (Essen-Gustavsson et al., 1983). In a study of Arabian and Andalusian foals, the percentage of type I and IIA fibers increased, and type IIB decreased from 10 days to 1 year of age (Galisteo et al., 1992, Rivero et al., 1993a). Thoroughbred foal gluteal muscles showed no change in the percentage of type 1 fibers from birth to 1 year of age, an increase in the percentage of type 2a fibers, and a decrease in 2x fibers (Eto et al., 2003). In warmblood foals, the percentage of type 1 and type 2a fibers increased and the percentage of hybrid type 2a/2x and type 2x fibers decreased from birth to 1 year of age (Dingboom et al., 1999; Dingboom et al., 2002). Thus, there is a small increase in type 1 fibers and a consistent increase in fast twitch type 2a (IIA) fibers and a decrease in type 2x (IIB) in foals of a variety of breeds as they reach 1 year of age. Between 1 year and 2 years of age, these changes in fiber type proportions continue and appear to level off at about 3 1/2 years of age in those breeds that have been studied (Roneus et al., 1991).

Thus, glycolytic metabolism appears to be of prime importance in young foals with a gradual increase in the ratio of type IIA:IIB with development over the first year of life.

Adaptations to Training

BIRTH TO 1 YEAR OF AGE

A study of five Standardbred colts was conducted where weanlings between 7 and 8 months of age were started into training and continued until 17-18 months of age

(Essen-Gustavsson et al., 1983). Training consisted of walk for 5 min and trot for 1800 m with the distance gradually increasing over the next 4 months to 3600 meters. This exercise was performed 4-5 days/week. For the next 6 months, yearlings were trained with a sulky at a trot over 3000 meters 4-5 days/week at increasing tempo. During this period, 1 day of speed training (500 m/min or faster) over 1600 m was added. An age- and sex-matched control group remained untrained. The increase in type IIA:IIB ratio that occurred with growth was similar between trained and untrained groups. The activity of the oxidative enzyme CS increased significantly (18%) in the trained group but not the untrained group and glycolytic enzyme activities declined (LDH 33%) similarly with age in both trained and untrained weanlings.

Thoroughbreds. A training study of Thoroughbred foals began at 2 months of age and continued to 1 year of age with foals weaned at 5 months of age. An age-matched control group was untrained. Foals began treadmill exercise with 15 s of cantering interspersed with 4 min of trot and gradually increased their speed, reaching 3.3 m/s of trot and 11 m/s of cantering (Eto et al., 2003). There was no difference in fiber type composition between the control and training group; however, SDH activity was higher in type 2x fibers of trained foals than controls. Muscle fiber sizes were larger in the trained vs. untrained foals.

Warmbloods. A comparison of Dutch warmbloods that were either stall-rested, turned out on pasture, or chased in the paddock for repeated sprints from birth to 5 months of age showed little effect of exercise on contractile fiber types (Dingboom et al., 1999; Dingboom et al., 2002).

Training studies of two- to four-year-old Standardbred, Andalusian, and Thoroughbred horses show an increase in the type IIA:IIB or 2a:2x ratio and an increase in oxidative capacity (CS: 31% increase Andalusians, 50% increase Standardbreds; SDH: 37% increase Thoroughbreds) (Roneus et al., 1992; Serrano et al., 2000; Yamano et al., 2005). When training is imposed on young growing weanlings, it does not appear to have a major impact on muscle development over and above the changes which occur naturally with growth. Enhanced oxidative capacity of type 2b fibers may occur with training but this change is not permanent, as studies have shown that after 3 months of detraining, oxidative enzyme activity will revert to pre-training levels (Serrano et al., 2000).

Conclusion

In summary, the metabolic and contractile adaptations in skeletal muscle that are present at birth provide the means by which young foals stand within minutes of being born and develop the quick burst of speed and rapid glycolytic metabolism necessary to evade predators. Equine muscle contains high muscle glycogen content

and glycolytic capacity from birth, and mare's milk provides a rich source of sugar for energy metabolism. During the first years of a horse's life, there is a shift in fiber type proportions in favor of type 2a fibers at the expense of type 2b fibers. Depending on the breed, there may be a gradual increase in the oxidative capacity of type 2 fibers in the first year of life, which progressively evolves over the next 2 years providing enhanced staying power and a slightly slower speed of muscle contraction (decreased type 2a:2x ratio). Initiating training at less than a year of age does not appear to hasten the changes that occur naturally with growth and its impact appears to be less in young growing horses than is seen when training is begun at 18 months to 3 years of age.

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