The Veterinary Journal 209 (2016) 5–13

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

Skeletal muscle adaptations and muscle genomics of performance horses

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ARTICLE INFO

Article history:
Accepted 29 November 2015

Keywords:
Equus caballus
Skeletal muscle mass
Fibre type composition
Muscle metabolism
Equine genomics

ABSTRACT

Skeletal muscles in horses are characterised by specific adaptations, which are the result of the natural evolution of the horse as a grazing animal, centuries of selective breeding and the adaptability of this tissue in response to training. These adaptations include an increased muscle mass relative to body weight, a great locomotor efficiency based upon an admirable muscle-tendon architectural design and an adaptable fibre-type composition with intrinsic shortening velocities greater than would be predicted from an animal of comparable body size. Furthermore, equine skeletal muscles have a high mitochondrial volume that permits a higher whole animal aerobic capacity, as well as large intramuscular stores of energy substrates (glycogen in particular). Finally, high buffer and lactate transport capacities preserve muscles against fatigue during anaerobic exercise. Many of these adaptations can improve with training. The publication of the equine genome sequence in 2009 has provided a major advance towards an improved understanding of equine muscle physiology. Equine muscle genomics studies have revealed a number of genes associated with elite physical performance and have also identified changes in structural and metabolic genes following exercise and training. Genes involved in muscle growth, muscle contraction and specific metabolic pathways have been found to be functionally relevant for the early performance evaluation of elite athletic horses. The candidate genes discussed in this review are important for a healthy individual to improve performance. However, muscle performance limiting conditions are widespread in horses and many of these conditions are also genetically influenced.

Introduction

The extraordinary speed, endurance and strength of modern equine athletes is a result of the horse's natural evolutionary history as a grazing animal, centuries of selective breeding that has resulted in the wide variety of horse breeds, and the remarkable plasticity of almost all body systems to respond to training. The superior athletic capacity of the horse has been attributed to a number of anatomical and physiological adaptations of body systems involved in exercise. Some of these adaptations occur in the tissue responsible for movement generation and affect skeletal muscle mass, muscle architectural design, fibre-type composition, muscle contraction and muscle energetics.

The cellular and molecular mechanisms underlying both the design and the adaptability of equine muscles have been the object of intensive investigation during the past 40 years. Most of this research has centred on the use of percutaneous needle biopsy technique and has resulted in a greater understanding of the response of this tissue to exercise and training (Rivero and Piercy, 2014). Furthermore, the complete sequencing of the equine genome (Wade et al., 2009) is a major advance that will impact the understanding of equine muscle physiology. The rapid technological advances in equine genomics has enabled gene expression profiling to explore muscle responses to exercise and training in equine athletes, to identify muscle-related candidate genes useful in early performance evaluation and to detect genomic markers of inherited muscle diseases.

This review focuses specifically on skeletal muscle characteristics that contribute to the horse as a supreme athlete, including the adaptive response of this tissue to training and their implications for performance. The objective is to relate, with an integrative perspective, earlier studies with new results from the emerging field of equine muscle genomics.

Skeletal muscle mass

Since the larger the muscle, the larger its potential power output, skeletal muscle mass is important for equine performance (Kearns

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http://dx.doi.org/10.1016/j.tvjl.2015.11.019
1090-0233/

et al., 2002). Thoroughbred racehorses have a very high skeletal muscle mass comprising over 53–57% of total bodyweight, compared to that of non-athletic horse breeds (42%) (Gunn, 1987). The hindlimb muscles of the Quarter-Horse (bred for acceleration) are also of a significantly greater mass than those of the Arab horses (bred for endurance), having greater isometric force potential (Crook et al., 2008).

Equine muscles usually respond to training by increasing muscle mass, especially in the hindquarters (Rivero et al., 1996a). This adaptation has been associated with an increase in abundance (per fibre) of most protein constituents (hypertrophy) rather than an increase in the number (hyperplasia) of muscle fibres. But a hyperplasic growth of muscle fibres after training cannot be ruled out in some breeds (in particular, Thoroughbreds and Standardbreds), that usually respond with a prominent increase in muscle mass associated with minimal or no changes in muscle fibre sizes (Rivero et al., 1996a, 2002). Horses in training that are fed high-protein diets have increased rates of muscle protein synthesis that improve skeletal muscle mass (Graham-Thiers and Kronfeld, 2005; Urschel et al., 2011), and thus muscle strength. The relationship between muscle mass and muscle strength is explained by the physiological cross-sectional area (PCSA) of a muscle, defined as the sum of the cross-sectional area of their constituent muscle fibres, being a function of muscle mass, and the muscle's maximum isometric force potential being a product of PCSA and the maximum isometric stress of vertebrate skeletal muscle (Crook et al., 2008). Hence, larger muscles will have an increased capacity for powerful contractions.

Myostatin gene

Recent advances in the understanding of the genomic infrastructure for the horse have enabled the identification of polymorphisms associated with racing performance phenotypes. In particular, variation at the *myostatin* gene locus has been found to be strongly associated with a horse's best racing distance among elite flat racing Thoroughbred horses (Hill et al., 2010b). Research has shown that for a particular single nucleotide polymorphism (SNP) within the gene, horses with a C/C genotype are suited to fast, short-distance races (≤1600 m), whereas horses with a T/T genotype have greater stamina and are suited for long-distance races (>2000 m). Thoroughbreds that are heterozygote at this locus (C/T) are best suited to middle distance races. An insertion polymorphism located in the promotor region of the gene has also been shown to be associated with the distance trait (Hill et al., 2010c) and the insertion and SNP are completely concordant. In Quarter Horses the C-allele is at very high frequency; a result of recent intense selection for speed in the breed. Selection for speed / short bursts of power exercise has been the most strongly selected trait among horses during the specialisation of horse breeds (Petersen et al., 2013).

Myostatin is involved in the inhibition of muscle growth through negative regulation of both myoblast proliferation and differentiation; hence, myostatin acts to limit skeletal muscle mass by regulating both the number and growth of muscle fibres. Recent results suggest that regulation of *myostatin* gene expression influences skeletal muscle mass and therefore racing performance in Thoroughbred horses in training (Tozaki et al., 2011). After six months of training, animals with the genotype associated with suitability for short-distance racing (C/C) had the highest bodyweight to withers height ratio, while those with a genotype associated with suitability for long-distance racing (T/T) had the lowest. Thus, compared to the T/T genotype, the C/C genotype seems to promote a significantly higher training-induced increment in skeletal muscle mass, either by functionally suppressing myostatin or by decreasing its expression level. The extent of the effect of training on myostatin expression was realized following transcriptional analysis – among 58 genes detected to have decreased expression following training, myostatin showed the greatest decrease (McGivney et al., 2010).

Muscle architecture

Much of the superior locomotor capacity of the horse can be explained in terms of its admirable muscle-tendon architecture: the arrangement of muscle fibres within the muscle, relative to the axis of muscle force generation. The total force exerted across a muscle is the sum of active force generated by the contractile machinery and passive force provided by fascia and elastic structures of the muscle-tendon complex. Most equine natural gaits typically consist of stretching–shortening cycles, in which lengthening (eccentric) and shortening (concentric) actions of the muscle-tendon complex in the limbs are repeated during each cycle (Butcher et al., 2009). During these bouncing gaits, elastic energy is stored in tendons and intramuscular connective tissues during the eccentric (loading) phase of the cycle, and this energy is then reused and added to the active energy produced by the contractile apparatus of muscle fibres during the concentric (uploading) phase. As a consequence of its natural evolution and more recent selective breeding, the horse has maximised the effective utilisation of this elastic energy recovery mechanism; such adaptation may be explained by the consumption of relatively less metabolic energy than would be expected based on the substantial demands of an animal of comparable body size and running speed (Taylor et al., 1982). When compared with other species of similar body size, the intercept in the linear equation of the relationship between oxygen consumption and speed, which is considered as a main component of the energetic cost of locomotion, was lowest for the horse (Taylor et al., 1982). In a racehorse galloping at high speed, thoracic limb protraction is largely a passive action likened to a catapult mechanism in which, during limb loading, energy is stored in elastic structures, such as the internal tendon and *lacertus fibrosus* of the *biceps brachii* muscle, which is released rapidly when the toe leaves the ground (Wilson et al., 2003). It is estimated that without this effective mechanism, muscles involved in this action would need to be about 100 times larger.

Equine cursorial locomotion is often described as a 'rear-wheel-drive' system based on functional specialisation of thoracic and pelvic limbs (Payne et al., 2005). The forelimbs support a greater proportion of the body mass, whilst the hindlimbs provide the power required for displacement (Witte et al., 2006). In both the pelvic and thoracic limbs of the horse there is a proximal-to-distal reduction in muscle volume and fascicle length (Payne et al., 2005). Hence in general, proximal limb muscles are highly specialised for doing active work, while distal limb muscles are specialised for economically generating force. Distal thoracic and pelvic limb muscles have similar architectures, suggesting a functional similarity. However, compared to proximal thoracic limb muscles, proximal pelvic limb muscles are larger and have shorter fascicles, suggesting that pelvic limb muscles have likely sacrificed the ability to exert force over a wide range of motion (due to their short fascicles) for the ability to produce large amounts of force (due to their large muscle mass) (Payne et al., 2005).

Recent studies have compared the architecture of hindlimb muscles in two breeds situated at either end of equine athletic performance, the Quarter-Horse and the Arab (Crook et al., 2008). Compared to those of the Arab, Quarter-Horse hind limb muscles are of greater mass, but have similar fascicle lengths and pennation angles. This implies greater isometric force potential. Thus, Quarter-Horse hind limb muscles are suited for rapid acceleration, whilst Arab hind limb muscles are optimised to function at maximum economy.