Review

Skeletal muscle adaptations and muscle genomics of performance horses

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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
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 hyperplastic 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 drive’s system based on functional specialisation of thoracic and pelvic architectures, suggesting a functional similarity. However, 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.

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 (≤2000m), whereas horses with a T/T genotype have greater stamina and are suited for long-distance races (>2000m). Thoroughbreds that are heterozygote at this locus (C/T) are best suited to middle distance races. An insertion polymorphism located in the promoter 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.
Fibre type composition

Myosin heavy chain (MyHC) is considered the best marker for fibre typing in mammalian muscles (Schiaffino and Reggiani, 2011). Three MyHC isoforms (termed types 1, 2A and 2X) are functionally expressed in adult equine skeletal muscles (Rivero et al., 1999). A minute amount (<1%) of the fastest MyHC-2B isoform is also expressed, despite a MyHC-2B pseudogene present in the equine genome (Chikuni et al., 2004). This suggests that an ancestral MyHC-2B gene has lost its function in horses during the course of their natural history. This functional absence has been attributed to the large body size of the horse (Li et al., 2011), but the relationship between functional presence of MyHC-2B and body size is not absolute, since this isoform is widely expressed in the skeletal muscle of large mammals, such as llama (Graziotti et al., 2001) and pig (Quiroz-Rothe and Rivero, 2004).

Immunohistochemical and in situ hybridisation analyses of equine skeletal muscle sections show the existence of a spectrum of fibre types with pure and hybrid myosin content according to the scheme 1 ↔ 1 + 2A ↔ 2A ↔ 2A+2X ↔ 2X (Rivero et al., 1996b; Eizema et al., 2003). As discussed below, the relative proportions of these fibre types change as a function of exercise-training. Fibres typed as 2B based on ATPase staining in fact are 2X fibres based on myosin content (Rivero et al., 1996a). Multivariate analysis of contractile, metabolic and morphological properties of individual equine muscle fibres results in optimal discrimination (100%) of myofibres according to the MyHC they express (Quiroz-Rothe and Rivero, 2001). This indicates that the MyHC isoform expressed in a fibre is closely related with phenotypic differences in contractile and non-contractile features observed among fibre types.

Clearly, a large number of proteins are differentially expressed in the various fibre types, and it has been proposed that the simple classification based on myosin composition is only convenient for communication until more complete profiles of gene expression at the transcript and protein level become available (Schiaffino and Reggiani, 2011). Microarray analysis of whole muscle samples has not much contributed so far to help in better delineating fibre types in skeletal muscles. However, as well as influencing muscle hypertrophy, a significant association with muscle fibre type proportions has been found to be a functional consequence of myostatin gene variation. Skeletal muscle fibre typing was performed for a set of Quarter-Horses with different myostatin genotypes: horses with the ‘sprint’ variant (C-allele or SINE Insertion) had 9% more type 2X fibres than horses with the alternate allele (Petersen et al., 2013). It is unclear whether the phenotype is affected directly by the SNP or the SINE, however, fibre type analysis in horses that had the SINE insertion but not the C-allele indicated that the promotor region insertion polymorphism is the most likely protagonist to influence muscle fibre type variation (Petersen et al., 2014). Functional analysis of the gene will shed light on the mechanism by which genetic variation leads to such dramatic phenotypic variation.

Fibre-type composition varies extensively between and within equine muscles, depending largely on function. In general, forelimb muscles have a greater percentage of type 1 and type 2A fibres and a lower percentage of 2X fibres than the hindlimb muscles (Kawai et al., 2009). This difference reflects again the functional specialisation of thoracic and pelvic limbs of the horse (Payne et al., 2005) and substantiates the general concept that muscle architecture and fibre-type composition are strongly correlated in mammals (Graziotti et al., 2012). Equine locomotor muscles usually contain high percentages of types 1 and 2A fibres in deep regions and a predominance of 2X fibres in superficial portions (Lopez-Rivero et al., 1992). This intramuscular fibre-type regionalisation clearly reflects a division of labor between muscle subvolumes and has practical implications on the biopsy sampling procedure of equine muscles.

Significant individual variations can be detected in the fibre type composition of equine muscles. For example, the proportion of type 1 fibres in the gluteus medius muscle was found to vary from 10 to 85% in a large cohort of horses of the same breed (Rivero and Barrey, 2001). Muscle biopsies from equine athletes also show marked variations in fibre-type composition according to performance profile, with a fast fibre predominance in sprinters (Barrey et al., 1999) and a tendency for slow type 1 and fast type 2A abundances in elite endurance horses (Rivero et al., 1993). There is now abundant evidence of the influence of genetic factors on fibre-type composition of equine muscles (López-Rivero et al., 1989; Rivero et al., 1996c; Rivero and Barrey, 2001; Petersen et al., 2013). Thus, selective breeding for centuries for specific performance profiles has been highly efficient for this muscular trait, particularly in some athletic breeds (Fig. 1). While a preliminary understanding of the contribution of the myostatin gene to fibre type composition is known, the complex nature of the molecular basis of genetic variation of equine muscle fibre-type composition remains largely to be established. The rapid progress in the sequencing of individual horse genomes should lead to the identification of polymorphisms or variants of specific genes implicated in defining the fibre phenotype of each equine athlete.

Fig. 1. Gluteus medius muscle biopsies removed from equivalent sampling depths from two different breeds of athletic horses; sections are stained with myofibrillar adenosine triphosphatase after preincubation at pH 4.45. Note the high proportion of fast fibres (stained weakly or moderately) in the Thoroughbred (bred for speed) and the high percentage of slow fibres (stained darkly) in the Arab horse (bred for endurance). Bar scale, 150 μm.
The most common adaptive response to training includes a fibre-type transformation in the direction 2X → 2AX → 2A (Serrano et al., 2000; Rivero et al., 2007). This functional plasticity involves a differential expression of MyHC isoforms and other contractile and metabolic genes, thus allowing fine tuning of muscle performance (McGivney et al., 2010). The MyHC-2X transcript is rapidly (within 12–24 h) down-regulated in horse muscle after exercise training (McGivney et al., 2010). Hybrid fibres coexpressing MyHCs 2A and 2X at the protein level but a single expression at the transcript level are frequent phenotypes in horse muscles under fibre-type transformation (Eizema et al., 2005; Rivero et al., 2007). However, this adaptation is reversible, since a return to sedentary activity levels down-regulates MyHC-2X at the protein level but a single expression at the transcript level (12–24 h) down-regulated in horse muscle after exercise training (Rivero et al., 2007).

As a greater number of fast-twitch motor units can be recruited at high-intensity exercise following training and type 2A fibres can sustain high power outputs for longer than 2X, the immediate functional implication of this adaptation is an overall improvement of both muscle shortening velocity, relevant for both speed and strength, and aerobic capacity, which increase endurance.

**Muscle contraction**

The contractile machinery of skeletal muscle is organised in highly ordered supramolecular structures termed sarcomeres. These consists of thick (myosin) filaments and thin (actin) filaments, which represent the contractile machinery, and a cytoskeletal scaffold, composed of transverse structures, the Z-disk and M-band, and longitudinal filaments composed of giant proteins titin and nebulin, running in parallel with thin and thick filaments (Fig. 2). The basic structure of the sarcomere is very similar in different types of muscles; contractile and cytoskeletal proteins exist as multiple isoforms (Fig. 2), being responsible for the wide variations in both active and passive mechanical properties in different fibre types (Schiavino and Reggiani, 2011).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain/Breed</th>
<th>Body mass (kg)</th>
<th>Vmax Type 1 (Lo/s)</th>
<th>Vmax Type 2A (Lo/s)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>C57BL/6J</td>
<td>25</td>
<td>1.39 ± 0.09 a</td>
<td>3.37 ± 0.19 d</td>
<td>Marx et al. (2006)</td>
</tr>
<tr>
<td>Rat</td>
<td>Sprague-Dawley</td>
<td>450</td>
<td>1.36 ± 0.11 a</td>
<td>3.00 ± 0.21 b</td>
<td>Marx et al. (2006)</td>
</tr>
<tr>
<td>Dog</td>
<td>Beagle</td>
<td>10</td>
<td>1.07 ± 0.09 b</td>
<td>3.02 ± 0.11 b</td>
<td>Marx et al. (2006)</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>80</td>
<td>0.70 ± 0.06 c</td>
<td>1.91 ± 0.13 d</td>
<td>Marx et al. (2006)</td>
</tr>
<tr>
<td>Horse</td>
<td>Quarter Horse</td>
<td>400</td>
<td>0.74 ± 0.13 b</td>
<td>2.53 ± 0.14 b</td>
<td>Marx et al. (2006)</td>
</tr>
<tr>
<td>Cattle</td>
<td>Meat production</td>
<td>450</td>
<td>0.35 ± 0.02</td>
<td>1.10 ± 0.21</td>
<td>Toniolo et al. (2005)</td>
</tr>
<tr>
<td>Rhinoceros</td>
<td>White</td>
<td>2500</td>
<td>0.59 ± 0.12</td>
<td>1.98 ± 0.19 c</td>
<td>Marx et al. (2006)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Superscripts denote significant statistical differences (P < 0.05, ANOVA) between values, i.e., a similar superscript beside two species values indicates that there is no significant differences between species; cattle samples are excluded from this analysis.
(McGivney et al., 2010). Besides sarcomeric myosin, transcripts of all major components of the thin filaments (actin, tropomyosin and the troponin complex) are highly abundant in equine muscles. Thus, the α-skeletal isoform of actin is the third most highly expressed gene in equine muscle and has been implicated as a candidate athletic performance gene in Thoroughbreds (Gu et al., 2009). Interestingly, the gene encoding the splicing variant of troponin T, expressed in fast fibres, is significantly up-regulated in response to exercise training, suggesting enhanced contractibility in these fibres (McGivney et al., 2010).

Titin, a giant structural protein that spans a half sarcomere from the Z-disk to the M-band (McGivney et al., 2010). Titin exists in different isoforms with longer variants expressed in slow fibres and shorter isoforms in fast fibres (Prado et al., 2005). Considered as a molecular spring, titin is a major determinant of resting passive tension of myofibres, and the expression of longer isoforms implies a lower level of passive tension or higher muscle fibre extensibility. Thus, resting passive tension is higher in fast than in slow fibres (Mutungi et al., 2003).

The α-actinin-3 gene (ACTN3), which forms links between thin filaments in the Z-disk, is also over-expressed in equine skeletal muscles (McGivney et al., 2010), particularly in those with abundant type 2X fibres (Mata et al., 2012). For these fibres, ACTN3 plays a far-ranging beneficial effect in generating forceful contractions at high speed (Schroder et al., 2011). The equine ACTN3 gene has recently been sequenced (Mata et al., 2012) and compared between different horse breeds separated by their athletic tasks (Thomas et al., 2014). A SNP of this gene has been considered to be functionally relevant. Both allele and genotype frequency analyses of this polymorphism showed significant differences between breeds (Thomas et al., 2014). The absence of the homozygous AA genotype in Thoroughbreds and the prevalence of the GG genotype among strength thoroughbreds argues that the A-allele could be detrimental to sprint performance and that the G-allele could be detrimental for strength performance. Like other structural genes, the equine ACTN3 gene is significantly down-regulated after training (McGivney et al., 2010), probably related to a parallel 2X-to-2A fibre-type transformation, since the ACTar4N3 protein is more abundant in type 2X than in type 2A fibres (Vincent et al., 2007).

**Muscle energetics**

Available evidence suggests that equine skeletal muscle displays intrinsic metabolic adaptations that cover a number of aspects of myofibre structure and function, such as a structural basis for these metabolic features, substrate and by-product transport across sarcolema and coordinated integration of metabolic pathways to regenerate ATP in response to exercise (Fig. 3). It is evident that all these aspects are fibre type-dependent and they can be enhanced by training (see Votion et al., 2008, for relevant discussion). The strong interdependence that exists between oxidative and glycolytic capacities in equine muscle fibres (Quiroz-Rothe and Rivero, 2001) clearly represents an inverse coupling between their intrinsic capacities for synthesising ATP from aerobic and anaerobic pathways. Thus, while fibre types represent a range of capacities and are not strictly dichotomous, the fibres with the highest oxidative capacity tend to have the lowest glycolytic capacity, and vice versa.

As energy metabolism is inversely related to body mass (Kleiber’s law), the metabolic activity of skeletal muscles is higher in smaller than in larger species (Weibel and Hoppeler, 2005). Several studies have shown a significant inverse relationship between the total volume occupied by mitochondria in muscle fibres (Vmt) and body mass in a variety of terrestrial and marine mammals with near 3000-fold difference in body size (Watson et al., 2007; Velten et al., 2013). However, this relationship is not absolute, since some species such as the horse have Vmt higher (about 7% of fibre area; Hoppeler et al., 1987) than smaller species like human (2–5%; Fuglevand et al., 1999) and mammals of similar body size like cattle (about 3%; Kayar et al.,

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**Fig. 3.** Schematic diagram showing some metabolic differences between fast and slow muscle fibres. Pathways prevalent in fast or slow muscle fibres are shown as green or red arrows, respectively. DHAP, dihydroxyacetone phosphate; GLUT4, glucose transporter 4; F-6-P, fructose-6-phosphate; FAT/CD36, fatty acid traslocase; FFA, free fatty acids; F-1, 6-P, fructose-1, 6-bisphosphate; G-3-P, glyceraldehyde-3-phosphate; G-6-P, glucose-6-phosphate; GPDH1, glycerol phosphate dehydrogenase (cytosolic); GPDH2, glycerol phosphate dehydrogenase (mitochondrial); HK, hexokinase; LDH, lactate dehydrogenase; MCT1, monocarboxylic acid transporter 1; MCT4, monocarboxylic acid transporter 4; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; TG, triglycerides.
Similarly, activities of key aerobic muscle enzymes, such as citrate synthase and β-hydroxyacyl-coenzyme A dehydrogenase, are of similar range in horses and other athletic species with smaller body size such as dogs and humans (Table 2). These overall results indicate that, among terrestrial mammals, horses’ locomotor muscles have V_{mt} and oxidative capacity greater than could be predicted from the size of this species in accordance to the Kleiber’s law. The intrinsically high muscle oxidative capacity of the horse is commensurate with its exceptional maximal oxygen consumption rate (VO_{2max}) that is more than twice as high in racehorses (Young et al., 2002) compared to elite human athletes (Saltin and Astrand, 1967) and 2.7-fold greater in horses than cattle (Kayar et al., 1989). A significantly proportional relationship between VO_{2max} and muscle V_{mt} has been documented in horses (Tyler et al., 1998).

Equine locomotor muscles also have higher activities of glycolytic enzymes, such as lactate dehydrogenase, than both other athletic species of smaller body size and non-athletic species of similar body size (Table 2). These overall data support a parallel metabolic adaptation of equine muscles for both sustained aerobic metabolism and an enhanced intrinsic ability to produce ATP through glycolysis during high-intensity exercises. Muscle metabolic responses to exercise and training

Equine muscles contain high glycogen stores (300 to 650 mol/g dry weight) (Votion, 2014), particularly in fast fibres. Glycogen is a limiting fuel of muscle contraction for both submaximal and maximal exercise (Votion et al., 2008). In prolonged low-to-moderate intensity exercise (<85% VO_{2max}), lipids are an important source of energy, but glycogen also contributes substantially (up to 60%), especially at increased work intensities (Geor et al., 2000). Thus, intramuscular glycogen depletion has been identified as a major cause of fatigue during such exercise, because free fatty acid oxidation cannot produce sufficient ATP in the absence of pyruvate. To sustain muscle contraction beyond a few seconds of high-intensity exercises (>85% VO_{2max}), horses also rely heavily on glycogen, which is the obligate substrate for glycolysis (Lacombe et al., 2001). Muscle glycogen replenishment after exercise is an intrinsically slow process in the horse that can take up to 72 h (Brojer et al., 2006). Certain strategies such as intravenous glucose infusion (Lacombe et al., 2001), oral acetate administration (Waller et al., 2009a) and rehydration with hypotonic electrolyte solutions (Waller et al., 2009b) have been shown to be useful to enhance muscle glycogen replenishment following exercise, but post-exercise ingestion of starch-rich meals fails in this regard (Jose-Cunilleras et al., 2006).

While there are many other contributors to skeletal muscle fatigue (Allen et al., 2008) in horses performing high-intensity exercise, the local acidosis associated with a rise in intracellular lactate has been identified as a major cause of fatigue (Rivero and Piercy, 2014). The means for the prevention of muscle acidosis requires buffers and transport of protons and lactate anions out of the cell. The buffering capacity of equine muscle is higher than in other species, probably due to a high carnosine content (Sewell et al., 1992), but this mechanism seems to be insufficient to prevent acidosis. The sodium / proton exchange also plays a minor role, so monocarboxylate transporters (MCT) are responsible for most proton efflux. Two MCT isoforms, MCT1 and MCT4, are expressed in equine muscles (Koho et al., 2006). MCT1 expression is related to the oxidative capacity of muscle fibres and decreases in the order 1 > 2X > 2A > 2X (Mykkänen et al., 2010). The fibre type distribution of MCT4 is not known in horses, but in humans its expression is related to the glycolytic capacity of muscle fibres, decreasing in the order 2X > 2A > 1 (Fishbein et al., 2002). In terms of muscle metabolism, the differential cellular location and relative abundance of MCT isoforms contribute to the cell-to-cell lactate shuttle. Thus, lactate formed in glycolytic fibres could be released via MCT4 and transported into oxidative fibres via MCT1, being oxidised by subsarcolemmal mitochondria as a substrate for ATP resynthesis (Fig. 3). Acute incremental exercise results in transient increases of MCT1 and MCT4 transcript expression and protein content (Kitaoka et al., 2013).

An improvement in aerobic capacity is the most common and earliest adaptation to training in equine muscle. This adaptation is phenotypically expressed by significant increments of both the activities of key enzymes involved in the oxidative phosphorylation (Serrano et al., 2000) and mitochondrial volume density of up to 131% (Tyler et al., 1998). In addition to an increase in mitochondrial biogenesis, training induces an increase in mitochondrial performance. Thus, mitochondrial respiratory capacity (ATP production by oxidative phosphorylation) increases 1.7-fold as a function of training and body condition score in the horse (Votion et al., 2012). Overall, these adaptations imply parallel increments in both maximum oxygen uptake (VO_{2max}) and run time to fatigue (Tyler et al., 1998). Training also improves capillary supply of muscle fibres, glucose availability to muscle, insulin sensitivity, GLUT-4 expression and glycogen synthase activity, owing to increased muscle glycogen stores (reviewed in Rivero and Piercy, 2014). The increased aerobic capacity of skeletal muscle following training occurs concurrently with a significant reduction in the net rate of muscle glycogenolysis during prolonged submaximal exercise (Geor et al., 1999), delaying the onset of fatigue by saving muscle glycogen and increasing fat oxidation as a source of energy. However, some studies report that following training, high-intensity exercise results in nearly identical reduction in muscle glycogen (Hinchcliff et al., 2002).

Although controversial in the early literature, there is now sufficient evidence that training that includes regular (3–4 sessions per week) high-intensity (>85% VO_{2max}) exercise improves anaerobic capacity in horse skeletal muscles, but this is not reflected in blood lactate concentrations during maximal, exhaustive effort (Hinchcliff et al., 2002; Rivero et al., 2007). Since lactate is an oxidisable substrate (Gladden, 2004) and training increases the expression of MCT isoforms in horse muscle (Revold et al., 2010; Kitaoka et al., 2011), training would improve the muscle capacity to oxidise in
oxidative fibres lactate produced primarily in glycolytic fibres. Accordingly, the excess of lactate associated with an increased glycolysis would be available for oxidative regeneration of ATP and less lactate would be released into the blood (Fig. 3). Another possibility could be an increased buffering capacity (McGowan et al., 2002), but the mechanism underlying this training adaptation remains largely unknown in horses.

**Muscle metabolic genes**

Recent studies have identified temporally altered metabolic genes in equine skeletal muscle following exercise and training (Eivers et al., 2010; McGivney et al., 2010). In particular, genes responsible for oxidative metabolism and mitochondrial biogenesis are differentially increased, while for the genes responsible for glycolysis, the result is usually unaltered. Molecular genomic tools have also enabled the association of sequence variants of specific metabolic genes with athletic performance. In particular, specific polymorphisms in CKM (creatine kinase, muscle) and COX4I2 (cytochrome c oxidase, subunit 4, isoform 2) are significantly, but weakly, associated with racing performance (Gu et al., 2010). A polymorphism in the PDK4 (pyruvate dehydrogenase kinase, isoenzyme 4) gene is, however, strongly associated with elite racing performance and has been suggested be useful for the selection of racehorses with superior racing ability (Hill et al., 2010a). PDK4 plays a role in decreasing glucose oxidation and increasing fatty acid oxidation during submaximal exercise. While these genes may have small effects on the micro-adaptations contributing to exercise performance, it is most likely that a very large number of genes contribute to the overall phenotypic variation in exercise performance adaptations. The rapid advances in the development of genomic tools to assay genetic variation in the horse, such as SNP Chip genotyping platforms and next-generation sequencing, will enable researchers to further understand the suite of genes contributing to muscle adaptation and the superior physiological athletic performance of the horse.

**Conclusions**

During the past 40 years, a clear picture of skeletal muscle adaptations of the equine athlete has emerged. These adaptations are the sum of their natural history as grazing animals, centuries-long selective breeding for performance and the remarkable malleability of the tissue in response to training. They include an increased muscle mass relative to body weight, a great locomotor efficiency based upon an admirable muscle-tendon architectural design, an adaptable fibre-type composition having muscle fibres with intrinsic shortening velocities greater than would be predicted from an animal of comparable body size, a high muscle mitochondrial volume that permits a higher whole aerobic capacity, large intramuscular stores of energy substrate (glycogen in particular) important for fuelling muscle contraction and high buffer and lactate transport capacities to preserve muscle against fatigue during anaerobic exercises. Furthermore, these adaptations can be improved with training.

Major and rapid progress in this area has been made following the sequencing of the equine genome (Wade et al., 2009). As a consequence, genes involved in muscle growth, muscle fibre type, muscle contraction and specific metabolic pathways have been identified that respond to both exercise and training, and a number of these genes have been found to be functionally relevant for early performance evaluation of elite athletic horses. The candidate genes presented here are important for a healthy individual to improve performance, but muscle performance limiting conditions are widespread in horses and many of these conditions are also genetically influenced.

**Conflict of interest statement**

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