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Topic of General Interest

Candidate genes for performance in horses, including monocarboxylate transporters[1]

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ABSTRACT.- Regatieri I.C., Curi R.A., Ferraz G.C. & Queiroz-Neto A. 2017. **Candidate genes for performance in horses, including monocarboxylate transporters.** *Pesquisa Veterinária Brasileira 37(1):66-72.* Laboratório de Fisiologia de Exercício Equino e Farmacologia, Departamento de Morfologia e Fisiologia Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho, Via de Acesso Prof. Paulo Donato Castellane s/n, Jaboticabal, SP 14884-900, Brazil. E-mail: iregatieri@hotmail.com

Some horse breeds are highly selected for athletic activities. The athletic potential of each animal can be measured by its performance in sports. High athletic performance depends on the animal capacity to produce energy through aerobic and anaerobic metabolic pathways, among other factors. Transmembrane proteins called monocarboxylate transporters, mainly the isoform 1 (MCT1) and its ancillary protein CD147, can help the organism to adapt to physiological stress caused by physical exercise, transporting lactate and H^+ ions. Horse breeds are selected for different purposes so we might expect differences in the amount of those proteins and in the genotypic frequencies for genes that play a significant role in the performance of the animals. The study of *MCT1* and *CD147* gene polymorphisms, which can affect the formation of the proteins and transport of lactate and H^+, can provide enough information to be used for selection of athletic horses increasingly resistant to intense exercise. Two other candidate genes, the *PDK4* and *DMRT3,* have been associated with athletic potential and indicated as possible markers for performance in horses. The oxidation of fatty acids is highly effective in generating ATP and is controlled by the expression of *PDK4* (*pyruvate dehydrogenase kinase, isozyme 4*) in skeletal muscle during and after exercise. The *doublesex and mab-3 related transcription factor 3* (*DMRT3*) gene encodes an important transcription factor in the setting of spinal cord circuits controlling movement in vertebrates and may be associated with gait performance in horses. This review describes how the monocarboxylate transporters work during physical exercise in athletic horses and the influence of polymorphisms in candidate genes for athletic performance in horses.

INDEX TERMS: Genes, performance, horses, monocarboxylate transporters, fatigue, lactate, polymorphisms.

RESUMO.- [**Genes candidatos para desempenho em equinos, incluindo transportadores de monocarboxilatos.**] Algumas raças de equinos são altamente selecionadas para atividades desportivas. O potencial atlético de cada animal pode ser medido pelo seu desempenho nas competições equestres. Um alto potencial atlético depende, entre outros fatores, da capacidade do animal de produzir energia através dos metabolismos aeróbio e anaeróbio. As proteínas transmembrana chamadas transportadores de monoxarboxilato, principalmente a isoforma 1 (MCT1) e sua proteína auxiliar CD147, podem ajudam o organismo a se adaptar ao estresse fisiológico causado pelo exercício físico, transportando íons lactato e H^+. Algumas raças de equinos são selecionadas para diferentes objetivos, portanto é provável que existam diferenças nas quantidades de transportadores monocarboxilatos e na frequência genotí-

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pica dos seus respectivos genes. O estudo de polimorfismos nos genes das proteínas MCT1 e CD147, afetando a sua formação e o transporte dos íons lactato e H^+, podem fornecer informações suficientes para a seleção de equinos com capacidade de serem altamente treinados e resistentes a intensos exercícios. Dois outros genes candidatos que têm sido relacionados com potencial atlético e utilizados como possíveis marcadores para desempenho em equinos são o *PDK4* e o *DMRT3*. A oxidação de ácidos graxos é altamente efetiva para produção de ATP e é controlada pela expressão do gene *PDK4* (*pyruvate dehydrogenase kinase, isozyme 4*) no musculo esquelético durante e após do exercício físico. O gene *DMRT3* (*doublesex and mab-3 related transcription factor 3*) codifica um importante fator de transcrição no controle dos movimentos em vertebrados e pode ser associado com a marcha em algumas raças de equinos. Esta revisão descreve como agem os transportadores de monocarboxilatos durante o exercício físico em equinos atletas e qual a influência de alguns polimorfismos em genes candidatos para o desempenho atlético em equinos.

TERMOS DE INDEXAÇÃO: Genes, desempenho, equinos, transportadores monocarboxilatos, fadiga, lactato, melhoramento genético, polimorfismos.

INTRODUCTION

The role of horse has changed over the years in social, economic and political aspects. Horses were used as means of transport, traction, to work in the field, and even in wars on the battlefield. While recreation, sports and status were important for a minority aspect of the horse industry in 1900, this comprises the largest role of horses today. Breeders continue to value the superior breeding animal and superior breeding characteristics, since the horses are widely used in sports and leisure activities nowadays.

The techniques to produce athletic horses in Brazil include aspects from stable management and grazing to horse breeding. The desire to improve the genetic quality of the Brazilian horse population has resulted from the increasing number of horses and sporting events in the country. Although breeders of athlete horses want genetically superior animals, able to perform better on competitions, the gap between research and practical application prevents the realization of consistent and continuous selection programs.

Studies involving exercise physiology and horse breeding techniques using candidate genes that can influence athletic performance should be performed to link research and practical applications in horse competitions. The transmembrane protein named monocarboxylate transporter isoform 1 (MCT1) and its ancillary protein CD147 can assist adaptation to physiological stress caused by physical exercises. The *PDK4* gene controls the oxidation of fatty acids, which are highly effective in generating energy in the skeletal muscle. The *DMRT3* gene is involved in movement coordination and associated with gait in some horse breeds. This review describes the role of the *MCT1, CD147, PDK4 and DMRT3* genes in the physiology of skeletal muscle or function of the nervous system, the bioenergetic system in horses and how the monocarboxylate transporters work. Furthermore, it overviews some genetic studies about these candidate genes for performance that can be used to improve the athletic performances of horses.

BIOENERGETICS

The character of a horse is based on genetically controlled characteristics, as well as non-hereditary aspects including environment, nutrition, health, and training. Active horses that practice any sport, when subjected to frequent training, become highly capable of performing more intense activities. The endurance of Arabian horses, for prolonged labor is known while other breeds, such as Quarter Horses, are equipped for short and intense exercises. However, little is known about the mechanisms responsible for maintaining the energetic and acid-base homeostasis during the exercise of these animals.

Arabian horses are widely used in equestrian events due to its ability to resist prolonged periods of work with minimal care and food. They are also used to improve breeds, give them refinement, endurance, and intelligence, as well as jumping, racing and endurance events, in general.

Quarter Horses, known for their great docility and versatility, are used in a variety of equestrian sports like reining, working cow horse, and racing. This breed is able to perform quick starts, sudden stops, has great ability to change direction and tremendous ability to turn on itself.

The muscle contraction for any exercise results from the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by the enzyme myosin ATPase, releasing inorganic phosphate and energy. There are several pathways to produce ATP as creatine phosphates, glycogen reserves, and lipids oxidation, but it is accepted that glucose and glycogen breakdown is the most important of them. During the aerobic metabolism, glycogen reserves are converted to glucose and then to pyruvate that is oxidized to CO_2 and H_2O by molecular oxygen for energy production. This pathway for energy production generates large amount of ATP; however, it takes a long time due to numerous reactions. Animals that exercise short times with great intensity need to produce energy fast due to intense energy requirements. In this case, the predominant pathway to energy production becomes the anaerobic metabolism. ATP is produced when glucose is converted to pyruvate and then to lactate by lactic fermentation (Nelson & Cox 2005). This production of lactate can generate accumulation of lactate and H^+ ions in the muscle fibers and the blood, which are the main cause of cellular acidosis, and fatigue (Fitts 1994, Hogan et al. 1995). According to Allen et al. (2008), fatigue can be understood as any decline in muscle performance. As a defense, cells adapt to the physiological stress caused by exercise through various mechanisms to prevent cellular acidosis and lactate accumulation.

Lactate is an important intermediate metabolite that can move quickly between different cells within a muscle, among different muscles and between muscle and plasma. Up to 50% of the total lactate can be found in red blood cells of horses after intense exercise, and therefore, it is speculated that the red blood cells are responsible for re-

ducing the concentration of blood lactate levels during maximal exercise (Pösö et al. 1995, Väihkönen et al. 1999). The lactate influx into the erythrocytes may be influenced by various factors and becomes greater with lower pH and increased temperature (Väihkönen et al. 1999). Lactate can enter the erythrocytes by three routes (Poole & Halestrap 1993), (1) free diffusion of acids not dissociated, (2) monocarboxylate transporters (MCTs), or (3) protein band 3 (anion exchanger 1, AE1). The AE1 membrane carrier protein exchanges a chloride or bicarbonate for a lactate anion, but in horses, MCTs are the main lactate transporters (Skelton et al. 1995).

MONOCARBOXYLATE TRANSPORTERS (MCTS)

Monocarboxylate transporters (MCTs) are transmembrane proteins that facilitate the transport of lactate, and other substrates such as pyruvate, acetoacetate, and β-hydroxybutyrate, into and out of cells across the plasma membrane (Juel & Halestrap 1999). MCTs are present in erythrocytes and various tissues such as the heart, intestines, liver, testis, brain cells, neurons and cancerous cells (Halestrap 2013).

There are 14 MCTs isoforms identified in mammalian cells (Price et al. 1998), which have not yet specific and determined function and differ from one another by specific tissue and substrate. The monocarboxylate transporter isoform 1 (MCT1), encoded by the same gene name (*MCT1*), is the most widely distributed, found in most tissues, including oxidative and glycolytic muscles, besides erythrocyte membranes (Halestrap & Meredith 2004, Koho et al. 2006). The MCT1 is often studied in athletic horses because it is fundamental to the transport of lactate and H^+ ions from the plasma into the erythrocytes, thus maintaining the acid/base homeostasis and slowing the systemic acidosis and muscle fatigue caused by cellular decreasing of pH after intense exercises. The MCT1 gene in human, also called SLC16A1, was mapped to chromosome 1p13.2--p12 (Garcia et al. 1994, Jackson et al. 1995, Carpenter et al. 1996). In horses, the MCT1 has 5 exons and was mapped to chromosome 5 (UCSC Genome Browser 2002, sequence NM_001246296 - chr5:55,589,142-55,598,920).

Some MCTs need an accessory protein to its proper location and operation in the plasma membrane. The ancillary protein of the MCT1 is an integral glycoprotein of the plasma membrane belonging to the immunoglobulin superfamily, designated Cluster of Differentiation 147 - CD147 (Kirk et al. 2000, Gallagher et al. 2007), encoded by the same gene name (*CD147*). This ancillary protein, also known as OX-47, basigin (BSG), CE9, HT7, neurothelin, M6 or EMMPRIN in other species (Fossum et al. 1991, Seulberger et al. 1992, Schuster et al. 1996) is present in many adult tissues, including the membranes of erythrocytes and muscle (Koho et al. 2006). The CD147 protein is needed to maintain the catalytic activity of MCT1 and its translocation to the plasma membrane (Wilson et al. 2005), where they form a complex consisting of two molecules of MCT1 and two of CD147 (Wilson et al. 2002, Halestrap & Meredith 2004). Kaname et al. (1993) mapped the human CD147 gene on chromosome 19p13.3. In horses, the CD147 gene has 5 exons, with unknown location on chromosomes (UCSC Genome Browser 2002, sequence EF564280 - chrUn:48,184,877-48,186,120).

LACTATE TRANSPORT IN MCT1

The ionic form of lactic acid crosses the plasma membrane by diffusion, but at physiological pH, most of the lactic acid is in the dissociated form and crosses the membrane through the monocarboxylate transporter. This transport is carried out by a hydrogen proton at 1:1 ratio and does not consume energy (Merezhinskaya & Fishbein 2009, Halestrap 2013). The lactate transport in MCT1 involves an orderly sequential mechanism. First, a proton H^+ combines with the MCT1 and opens a binding site for the lactate anion. Subsequently, the ions are transferred via amino acids residues through the MCT1 and released across the membrane (Halestrap 2013).

The direction of the lactate transport through MCTs (inflow or outflow) may change and depends predominantly on the concentration gradient of the anion and proton through the membrane, besides the pH gradient (Halestrap 2012). Based on different rates of lactate influx into the erythrocytes, Väihkönen & Pösö (1998) divided equines into two groups: one with high lactate transport activity (HTA) and another with low lactate transport activity (LTA) by doing so, the lactate transport is called bimodal. This bimodal distribution cannot be caused by MCT1 but by the variable expression of its ancillary protein CD147 (Kirk et al. 2000). Koho et al. (2006) corroborated this result by reporting that Standardbred horses divided into HTA and LTA groups had the same amount of MCT1 but observed a correlation between the amount of CD147 and the lactate transport activity ($P<0.001$) in the membrane of red blood cells.

Evidences of lactate transport in MCT1 were also reported in cancerous cells where the center of hypoxia is glycolytic and produces the lactic acid, which is captured by the peripheral cells expressing MCT1, to be used in oxidative phosphorylation. When the MCT1 is inhibited, the aerobic cells use glucose instead of lactate to produce energy. Thereby, the cancer cells die due to lack of glucose and intracellular acidification, inhibiting tumor growth (Semenza 2008, Chiche et al. 2011, Le Floch et al. 2011, Parks et al. 2011).

DISTRIBUTION OF MONOCARBOXYLATE TRANSPORTERS

The distribution of monocarboxylate transporters in red blood cells and muscles of horses can be affected by several factors as the horse breed, age, and training. Väihkönen et al. (2002) investigated how the lactate transport activity differs from foals to racehorses and divided Standardbred horses in HTA and LTA. These authors reported a bimodal distribution for all age groups and horses born in the LTA or HTA group remained in their respective groups over time. The effect of age was significant only in the HTA group, where the highest lactate transport activity occurred in foals, decreased from 1 to 3 years and tended to grow again in older horses. Koho et al. (2006) studied how age and training affected the amount of MCT1 and CD147

in the erythrocytes and *gluteus medius* muscle of Standardbred. The racehorses had higher CD147/MCT1 ratio in erythrocytes than moderately trained horses, showing a tendency to increase with training and age. The amount of CD147 was equal in muscles while MCT1 was variable and not dependent on age and training. Mykkänen et al. (2010) also found no correlation between age and the amount of MCT1 and CD147 in the red blood cells of Thoroughbred, Finnhorses, and Standardbred. However, they observed a correlation between MCT1 and CD147 in the three breeds studied. Regatieri et al. (2014) assessed the amount of CD147 in Arabian horses with low and high levels of athletic ability, considering the training of the horses. The amount of CD147 did not change between the horses of the untrained group and those of the 160-km endurance group. The performance results were similar to those reported by Feringer Junior et al. (2014), which divided the Brazilian Sport Horse breed into animals of high and low performance according to their jumping height ability in equestrian events. The authors did not observe a significant difference between the amounts of MCT1 and CD147 in the two performance groups.

Studies also demonstrated that CD147 and MCT1 are distributed differently in muscle fiber membranes. Revold et al. (2010) showed that in Norwegian-Swedish Coldblood racehorses, MCT1 and CD147 were present in high levels in muscle fiber membranes in the following order: I > IIA > IIAX > IIX. As high-intensity exercises change the type of fiber to be recruited (D'Angelis et al. 2005, Yamano et al. 2006), scientific studies show that the quantities of MCT1 and CD147 can change with training. Kitaoka et al. (2010) noted that the MCT1 level in the *gluteus medius* muscle of Thoroughbred increased after 18 weeks of high-intensity training (90-110% VO_2max) and the increase was maintained when the animals continued to be trained at moderate intensity (70% VO_2max). However, values for MCT1 returned to baseline when the animals were stabled without exercising.

Countless factors may be responsible for regulating the expression of MCTs (Halestrap 2013) both at the transcriptional and post-transcriptional levels. The individual variation in the activity of the MCTs can be inheritable, and a recessive allele would be responsible for low lactate transport activity (Väihkönen et al. 2002). Kitaoka et al. (2013) evaluated the MCT1 in muscle of trained and not trained Thoroughbreds and observed that mRNA expression and MCT1 levels in the *gluteus medius* muscle increased during 6 hours after an incremental test session, but no difference was observed after 24 hours, compared to the baseline. Moreover, the authors reported that mRNA expression did not differ between trained and untrained animals, but the amount of protein was higher in the trained horses. Thus, it can be concluded that high-intensity training increases the amount of monocarboxylate transporter, but it does not affect the expression of mRNA, which can be considered an adaptation of lactate transport during exercise.

GENETIC STUDY OF MONOCARBOXYLATES

Due to the various segments in which horses are used in the Brazilian agribusiness complex, the goal and the criteria to be included in genetic breeding programs should be clearly defined. For athletic horses, the study of monocarboxylates combined with genetic breeding of the species can bring significant improvements in the performance of these animals since the MCTs are closely related to performance and muscle fatigue during competitions.

The search for mutations in candidate genes linked to horse performance can provide precise information in favor of the improvements in the performance of equines. In humans, several single nucleotide polymorphisms (SNPs) were found in MCTs proteins (Lean & Lee 2009, Merezhinskaya et al. 2000, Cupeiro et al. 2010). In horses, studies searching for polymorphisms were also conducted. Reeben et al. (2006) tried to find differences between equines classified into high and low lactate transport capacity groups, analyzing sequences in *MCT1* and *CD147* genes of Standardbred horses. They found polymorphisms in *MCT1* (Lys457Gln – AY457175.1:c1573A>C) and *CD147* (Met125Val – EF564280.1:c389A>G), but there was no association between the polymorphism and lactate transport activity. Mykkänen et al. (2011) found no association between Standardbred and Finnhorses healthy and with myopathy that presented the same polymorphisms described by Reeben et al. (2006).

Koho et al. (2012) examined the expression of MCT1 and CD147 proteins in Standardbred, Finnhorse, Warmblood and Icelandic horses while searching for polymorphisms. The authors found two SNPs in the *MCT1* sequence: Val432Ile:1498G>A and Lys457Gln:1573A>C, that did not explain the variation in the expression of the gene. In the CD147 gene they discovered the SNPs Met125Val:389A>G and Ile51Val:168A>G, and two SNPs (888G>C e 990C>T) in the 3′-untranslated region gene, which is known to regulate the stability of mRNA. The authors concluded that the SNPs at nucleotides 389 and 990 do have an effect on the expression of the MCT1–CD147 transport complex because heterozygotes Standardbreds for those SNPs showed low amount of CD147. Also, that was the first time that the Ile51Val polymorphism was reported. They found this polymorphism in one Warmblood with low expression of MCT1 and CD147 in comparison to other Warmbloods. Wherefore, the authors suggested that this SNP might affect the formation of the MCT1-CD147 complex and therefore, the activity of lactate transport across the RBC membranes.

CANDIDATE GENES FOR PERFORMANCE

There was a significant increase in publications in various areas related to horses (Almeida & Silva 2010) and some of them show low heritability of athletic performance traits, which result in the difficulty of identifying selective criteria for athletic traits (Koenen et al. 1995, Wallin et al. 2003, Bokor et al. 2005). In Brazil, studies (Mota et al. 1999, Villela et al. 2002, Mota & Corrêa 2004) estimated genetic parameters for Quarter Horse racing performance traits and showed low heritability for final placement, racing time and Speed Index, indicating difficulties to select animals based solely on their individual performance, as determined by this parameter. The Speed Index (IV) is a Register of Merit in racing given by the Brazilian Association of Quar-

ter Horse Breeders (ABQM) and was created to allow animal performance comparisons under different conditions as racetrack, track, country, climate and distance (Evans 1989). According to last years results, each racetrack has a table with the speed index, compiled from the average of the three fastest wins each year for three consecutive years, for each distance. The average of nine wins will represent the IV = 100. Every second fraction is equal to an IV point, and this point varies with the distance of each race.

Although it is likely that many genes influence athletic performance in horses at the moment, few genetic variants are related to performance traits. Two candidate genes, *PDK4* and *DMRT3,* have been studied for athletic potential as possible markers for performance in horses. The expression of the *pyruvate dehydrogenase kinase, isozyme 4 - PDK4* (Gu et al. 2009, Hill et al. 2010), located on equine chromosome 4 (ECA4) helps to control metabolism and glucose conversion to acetyl-CoA for production of ATP (Andrews et al. 1998). This regulation of glucose conversion is tightly controlled by conversion of pyruvate to acetyl-CoA in the mitochondrion by catalytic function of the pyruvate dehydrogenase complex (PDC), which is controlled by pyruvate dehydrogenase kinase (PDK). The PDK blocks the formation of PDC, resulting in the beta-oxidation of fatty acids to acetyl-CoA as a substrate for oxidative phosphorylation. The oxidation of fatty acids is highly effective in generating ATP and is controlled by the expression of *PDK4* in skeletal muscle during and after exercise (Pilegaard & Neufer 2004, Eivers et al. 2009). As the *PDK4* is involved with physical exercise through energy production, polymorphisms in this gene have been successfully associated with performance traits in horses. In Thoroughbred racehorses, the SNP g.38973231A>G was strongly associated with racing performance (Hill et al. 2010). In this study, individuals of AA and AG genotype performed better than the GG genotype.

The *doublesex and mab-3 related transcription factor 3 gene* (*DMRT3*) located on equine chromosome 23, is related with the setting of spinal cord circuits controlling movement in vertebrates. It is involved in a neural network for the coordination of the locomotor system that controls limb movement. A nonsense mutation (DMRT3_Ser301STOP) at SNP g.22999655C>A of the *DMRT3* gene was significantly associated with gait performance in horses, especially performance of the harness racing horses, pace and tölt of Icelandic horses (Andersson et al. 2012). In a validation study with several breeds, the frequency of allele A was close to 100% in gaited breeds, with high frequencies of homozygous AA; and 0% in non-gaited breeds. This fact suggests that the A allele of *DMRT3* may negatively affect the performance of velocity breeds (Andersson et al. 2012). Promerová et al. (2014) tested horses of 141 breeds and found that the g.22999655C>A SNP is highly prevalent (frequency>0.90) or fixed among breeds used for harness racing or selected for performing 4-beat gaits (walk, trot, canter, and rack) at high speeds.

Given the role of each of the described genes in the physiology of skeletal muscle or functioning of the nervous system, they are candidate genes for athletic performance. To link this knowledge to animal breeding, studies should be conducted to determine and compare the genotypes in different horse breeds with diverse skills and performance, including polymorphism association. These studies further contribute to the knowledge of the metabolism, muscle physiology and genetics of the chosen/studied horse breeds.

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