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Original Research

Quantification of MCT1 and CD147 in Red Blood Cells of Arabian and Quarter Horses

Inaê Cristina Regatieri [a,*], Maria Luiza Mendes Almeida [a], Antônio Raphael Teixeira Neto [b], Rogério Abdallah Curi [c], Guilherme Camargo Ferraz [a], Antonio Queiroz-Neto [a]

[a] Department of Animal Morphology and Physiology, Faculdade de Ciências Agrárias e Veterinárias, UNESP—Univ Estadual Paulista, São Paulo, Brazil
[b] Hospital Veterinário, Faculdade de Agronomia e Medicina Veterinária, Campus Universitário Darcy Ribeiro, Asa Norte, UnB—Universidade de Brasília, Brasilia, Distrito Federal, Brazil
[c] Department of Animal Breeding and Nutrition, Faculdade de Medicina Veterinária e Zootecnia, UNESP—Univ Estadual Paulista, Botucatu, São Paulo, Brazil

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ABSTRACT

During anaerobic glycolysis for energy production, lactate and H^+ ions accumulate in muscle fibers and pass into the blood stream. The monocarboxylate transporter isoform 1 (MCT1) and its ancillary protein cluster of differentiation 147 (CD147) transport H^+ and lactate ions from the plasma into red blood cells (RBCs), thereby maintaining acid/base homeostasis and retarding systemic acidosis and fatigue. The aim of this study was to compare the levels of MCT1 and CD147 protein in the RBC membranes of Arabian and Quarter Horses with different levels of athletic ability. Blood samples were collected from 40 Arabian and 40 Quarter Horses, both males and females, ranging from 3 to 16 years and 2 to 23 years, respectively. The horses were divided into two groups: 20 animals of low performance and 20 animals of high performance for each breed. The amount of MCT1 and CD147 in the plasma membranes of their RBCs was determined by Western blotting analysis with arbitrary optical density (OD) units, using a human-specific anti-MCT1 and anti-CD147 antibodies that were previously validated for horses. The means $\pm$ standard errors were analyzed by repeated measures analysis of variance using the PROC MIXED procedure of SAS software. The effect of age was included as covariate and sex as a class effect in the model. The correlations were analyzed by Pearson correlation test at $P < .05$. Monocarboxylate transporter isoform 1 with a molecular mass of approximately 52 and 49 kDa was found in the RBC membranes of all the Arabian and Quarter Horses, respectively. Cluster of differentiation 147 also was observed in all Arabian and Quarter Horses at approximately 52 and 48 kDa, respectively. A positive correlation was observed between the total amount of MCT1 and CD147 ($r = 0.932$, $P < .001$) in the RBC. The amount of MCT1 was significantly ($P < .0001$) higher in Quarter Horses (3.03 $\pm$ 0.37 OD) than in Arabians (1.02 $\pm$ 0.07 OD). Quarter Horses (3.23 $\pm$ 0.39 OD) also showed increased contents of CD147 than Arabians (0.89 $\pm$ 0.06 OD). However, there was no statistical difference in the amount of the protein between the low- and high-performance groups in either breed. Results indicate that the levels of MCT1 and CD147 are different between Arabian and Quarter Horses and the most probable explanation is that different pathways are used for the production of energy for each breed.

* Corresponding author at: Inaê Cristina Regatieri, Laboratory of Equine Exercise Physiology and Pharmacology, Department of Animal Morphology and Physiology, Faculdade de Ciências Agrárias e Veterinárias, UNESP—Univ Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, São Paulo, Brazil.
E-mail address: iregatieri@hotmail.com (I.C. Regatieri).

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1. Introduction

Arabians and Quarter Horses are known for their exceptional sports skills. Quarter Horses are adapted to short and intense exercises such as short races and western competitions, whereas Arabian horses usually perform better on long distance endurance rides. The ability of each animal to exercise can be inferred based on biological variables such as blood lactate concentration and heart rate. With those variables, we can determine the intensity and training type suitable for each animal [1,2]. High performance, in certain sports, depends mainly on the efficiency of the animal to use the energy produced through aerobic and∖or anaerobic pathways [3]. During high-intensity exercise, the requirement of ATP is higher than what is produced by aerobic pathway, and thus, the deficit is supplied by anaerobic glycolysis. The anaerobic pathway of energy produces ions lactate and H^+, which can lead to an accumulation of ions H^+ in muscular fiber and a decrease in blood pH, accelerating fatigue process [4–6].

Transmembrane proteins called monocarboxylate transporters can help the body adapt to the physiological stress caused by physical exercise. Among 14 different isoforms [7], monocarboxylate transporter isoform 1 (MCT1) is the most frequently found isoform in mammalians and is located in muscle and erythrocyte membranes [8]. The MCT1 transporter needs an ancillary protein, specifically the cluster of differentiation 147 (CD147) glycoprotein, to enable proper position and operation at the plasma membrane [9,10]. These proteins transport the ions H^+ and lactate from plasma into red blood cells (RBCs), thereby maintaining acid/base homeostasis and retarding systemic acidosis and fatigue.

Studies have been performed to determine differences in amount and expression of monocarboxylate transporters among different ages, genders, breeds, and physical training [11–13]. Koho et al [8] studied the effect of age and training on MCT1 and CD147 amount in RBC and gluteus medius muscle of Standardbred horses. Animals between 2 and 14 years old were divided into race fit and moderately trained. Monocarboxylate transporter isoform 1 content in muscle did not change significantly; however, race fit horses had higher amount of CD147/MCT1 ratio in RBC, showing an increasing trend associated with age and training. Kitaoka et al [14] showed that MCT1 content in gluteus medius muscle of Thoroughbreds at 24 months of age was significantly higher compared with 2 months of age (86% increase), suggesting an increase in lactate oxidation capacity during animals growth.

Kitaoka et al [15] reported that detraining also can change the amount of MCT1 in Thoroughbred horses. The authors showed that the amount of MCT1 had an increase of 31% in gluteus medius muscle after 18 weeks of high-intensity training (90%–110% VO_2max). The increase was maintained in the moderate-intensity detraining group (70% VO_2max) and returned to the pretraining level in the stall rest detraining group. Kitaoka et al [16] evaluated changes in MCT1 expression in the gluteus medius muscle in a single incremental test exercise in trained and untrained Thoroughbreds. The authors observed a temporary increase in the expression of MCT1 mRNA and in the MCT1 contents after 6 hours the test for both trained and untrained horses was performed. However, the protein contents were significantly greater for trained horses at all points during the observation period, which may represent a mechanical adaptation to physiological changes caused by exercises.

Mykkänen et al [12] studied the levels of MCT1 and CD147 in Thoroughbreds, Standardbreds, and Finnhorses in different sexes. The authors noticed that Thoroughbred mares had higher levels of CD147 than male horses. When considering all breeds together, there were no differences among sexes. Väihkönen and Pösö [17] also reported that lactate transport activity in RBC of Standardbred mares was higher than in stallions.

The changes on MCTs levels are dependent on various factors such as breed and physical training, Therefore, the aim of this study was to quantify the MCT1 and CD147 protein contents in the RBCs of Arabian and Quarter Horses. Animals were divided into groups that exhibited high and low performance, and correlations between the proteins, the breeds, and group performance were investigated. The findings of this study are important in the search for potential methods to improve the performance of athletic horses if we find out how the monocarboxylate transporters work in different breeds and different athletic skills.

2. Material and Methods

2.1. Animals

Arabian horses, of both genders, were selected according to their performance and separated in two groups: 20 animals of low performance, which were pasture grazed and not in training at the time, but could have had some training during their lifetime, and 20 animals of high performance, which were athletic horses that participated in endurance competitions of 160 km and had won at least once in an official competition of the Fédération Equestre Internationale.

Two hundred ninety-six animals composed the Quarter Horses base population. Quarter Horses, born between 1982 and 2011, registered with the Brazilian Quarter Horse Association (ABQM), were also divided into two groups, differentiated by the ABQM Speed Index (IV) in horse races. This measure is a merit registration on race and was created to allow animal performance comparisons under different conditions such as racetrack, track type, country, climate, and distance [18]. Based on the distribution curve from the IVs of the base population animals ($n = 296$), phenotypes were adjusted for the systematic effects of environment (fixed effects), gender, interaction between race track, and distance (228, 275, 301, 320, 365, 402, and 502 meters), between the year of the race (1988–2013), and the age of the animal at race (2, 3, and 4 years). Later, 20 horses of the highest performance index and 20 horses of lowest performance index were selected. The age, sex, and number of animals considered in each performance group are described in Table 1.

All horses were in good health, and the procedures involving the animals were performed in accordance with the Brazilian standards of animal welfare issued by the Ethics Committee on Animal Use.