Journal of Equine Veterinary Science 43 (2016) 66–71

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

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

ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 9 May 2016
Accepted 9 May 2016
Available online 17 May 2016

Keywords:
Anaerobic metabolism
Fatigue
Lactate
Performance
Western blotting

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 ± 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 ± 0.37 OD) than in Arabians (1.02 ± 0.07 OD). Quarter Horses (3.23 ± 0.39 OD) also showed increased contents of CD147 than Arabians (0.89 ± 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).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.05.004

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.

Table 1
Number of animals, age (mean ± standard deviation), and sex of Arabian and Quarter Horses divided into performance groups.

Group	N	Age (Mean ± Std)	Female	Male
AR LP	20	3–16 (9.05 ± 3.94)	7	13
AR HP	20	8–16 (11.00 ± 2.58)	6	14
QH LI	20	2–22 (9.05 ± 6.23)	15	5
QH HI	20	2–23 (9.65 ± 6.26)	16	4

Abbreviations: AR, Arabian; HI, high Speedy Index group; HP, high-performance group; LI, low Speedy Index group; LP, low-performance group; N, number of animals; QH, Quarter Horses; Std, standard deviation.

2.2. Analysis of the Proteins of RBCs

2.2.1. Blood Samples

Blood samples of each animal were collected in duplicate by venipuncture of the left jugular vein in the neck using vacuum tubes containing 7.5 mg of EDTA. Subsequently, RBCs were separated from plasma by centrifugation at 2,000*g* for 10 minutes. The supernatant was discarded, and the remaining hematocrit was stored at −80°C until RBC membrane isolation.

2.2.2. Isolation of RBCs Membrane

Red blood cell membranes were isolated according to the methodology recommended by Koho et al [19]. Briefly, samples containing 1 mL of hematocrit were washed by 35 mL of Na-Phosphate buffer (5 mM, pH 8.0) in a refrigerated centrifuge (4°C, 22,000*g*, 15 minutes) 4 to 5 times to ensure complete hemolysis of RBCs and remove residual hemoglobin. Red blood cell membrane pellets were suspended in 100 μL of fresh Na-Phosphate buffer and stored at −80°C.

2.2.3. Western Blotting

The isolation of MCT1 and CD147 proteins was performed using polyacrylamide electrophoresis gel containing sodium dodecyl sulfate (SDS-PAGE 10%), according to the technique described by Laemmli [20], and subsequent Western blotting was carried out according to the protocol described by Kitaoka et al [15].

After quantification of total protein using the Pierce BCA Protein Assay Kit (Thermo Fischer Scientific Inc), 40 ng of protein from each sample with Laemmli buffer was run via SDS-PAGE at 125V, 1 hour 30 minutes. Separated proteins were transferred to a nitrocellulose membrane by electrophoresis on ice with 20% methanol (100V, 1 hour). The membrane was blocked with 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween and incubated overnight with primary antibody anti-MCT1 or anti-CD147 (SIGMA), washed and incubated with secondary antibody anti-rabbit peroxidase conjugate (DAKO). The anti-MCT1 and anti-CD147 antibodies are reactive to the human species, but were previously validated for horses [21].

After incubation with antibodies, the intensity of the bands containing MCT1 and CD147 proteins was visualized by chemiluminescence using the capture imaging system (ChemiDoc MP System–Bio-Rad) using the substrate Immun-Star HRP, Chemiluminescent kit, Bio-Rad.

A sample of a known horse with high expression of CD147 [19] was chosen as standard to be used as control for quantification of bands in all analyses. The value of this control sample was normalized to 1.0, and the values of the subsequent samples were expressed relative to the control sample value. The bands were quantified using an optical density arbitrary unit with the software Image Lab (version 4.0 Bio-Rad).

2.3. Statistics of Protein Expression Analysis

The values were described as mean ± standard error. Data were analyzed by analysis of variance using the PROC MIXED procedure of SAS software (Institute, Cary, NC). 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. P values $< .01$ were considered statistically significant [22].

3. Results

Monocarboxylate transporter isoform 1 protein was found in the RBC membranes of all studied horses, with a molecular mass of approximately 52 kDa in Arabian and 49 kDa in Quarter Horses (Fig. 1). The CD147 protein was also detected in all animals, at approximately 52 kDa in Arabian and 48 kDa in Quarter Horses (Fig. 1). A high and positive correlation between the total amounts of MCT1 and CD147 in RBCs was found ($r = 0.95$, $P < .0001$).

The effect of age was significant ($P < .01$) only for MCT1. Positive and weak correlations between MCT1 and age ($r = 0.32$, $P < .01$) and between CD147 and age ($r = 0.37$, $P < .001$) were estimated. The effect of sex was not significant for any breeds or performance groups. However, the observed values of protein amounts for both breeds grouped within sex showed that mares had more MCT1 and CD147 than male horses (Table 2).

The intensity of the bands of MCT1 and CD147 proteins was significantly ($P < .0001$) higher in Quarter Horses than in Arabians (Tables 3 and 4). There was no statistical difference between the amounts of MCT1 and CD147 proteins in the athletic performance groups of both breeds.

4. Discussion

The values for the molecular weight of the CD147 protein corroborate, in part, with the results observed by Koho et al [19], who used a human-specific antibody to identify MCT1 and CD147 in the RBCs of Standardbred horses. The authors found more intense bands with a molecular mass of 76–80 kDa for MCT1 and 52 kDa for CD147. A similar result was found in a later study by Koho et al [21] using horse-specific antibodies. The authors detected bands for MCT1 and CD147

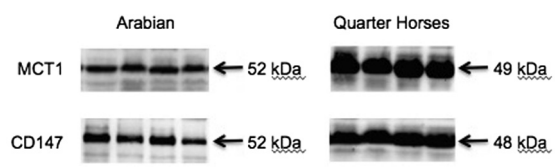

Fig. 1. Bands of MCT1 and CD147 proteins in red blood cells membrane of Arabian and Quarter Horses. CD147, cluster of differentiation 147; MCT1, monocarboxylate transporter isoform 1.

Table 2
Number of animals, mean, and standard error of MCT1 and CD147 detected in Western blotting of red blood cell membranes of Arabian and Quarter Horses, in arbitrary units of optical density, within sex.

Protein/Sex	N	Mean	Standard Error	*P* Value
CD147				
Female	44	2.72	0.30	<.01
Male	36	1.25	0.33	
MCT1				
Female	44	2.70	0.28	<.001
Male	36	1.20	0.31	

Abbreviations: CD147, cluster of differentiation 147; MCT1, monocarboxylate transporter isoform 1; N, number of animals.

Table 4
Amount of CD147 detected in Western blotting of red blood cell membranes of Arabian and Quarter Horses, in arbitrary units of optical density.

CD147	N	Mean	Standard Error	Minimum	Maximum	*P* Value
AR	40	0.88	0.06	0.17	1.77	<.0001
QH	40	3.23	0.38	0.24	9.45	
AR LP	20	0.94	0.09	0.31	1.77	.3808
AR HP	20	0.83	0.09	0.17	1.70	
QH LI	20	2.92	0.60	0.24	9.30	.4059
QH HI	20	3.55	0.46	0.55	9.45	

Abbreviations: AR, Arabian; CD147, cluster of differentiation 147; HI, high Speedy Index group; HP, high-performance group; LI, low Speedy Index group; LP, low-performance group; QH, Quarter Horses.

at 50 kDa in the RBCs of Standardbred, Finnhorse, Warmblood, and Icelandic horses. Mykkänen et al [12] also found CD147 bands at 50 kDa using horse-specific antibodies. Bands were observed for MCT1 at 47–50 kDa.

The correlation between the amounts of MCT1 and CD147 in our study was similar to the studies of Koho et al ([19], $r = 0.82$, $P < .001$) and Feringer Junior et al ([23], $r = 0.67$; $P < .0001$). Koho et al [8] found positive correlation between CD147 in muscle and in RBCs ($r = 0.62$, $P < .05$) and between the amounts of CD147 in muscle and lactate transport activity in RBC ($r = 0.64$, $P < .05$). Koho et al [19] also reported positive correlation between the amount of MCT1 in the cremaster muscle and in the RBC membrane of horses ($r = 0.74$, $P < .05$). Our results showed positive and moderate correlation between the monocarboxylate transporters and age. Despite the fact that effect of sex in our study does not influence the amount of proteins within breed and performance group, when the observed values of MCT1 and CD147 within sex were analyzed1horses showed higher levels of protein. The same pattern was observed for Thoroughbreds [12].

Significant differences between levels of MCT1 and CD147 in Arabian and Quarter Horses were observed. Mykkänen et al [12] reported a higher proportion of CD147 in Thoroughbred (88%), followed by Finnhorses (85%) and Standardbred (82%). Koho et al [21] analyzed the expression of MCT1 and CD147 in RBC and gluteus muscle of Standardbred, Finnhorse, Warmblood, and Icelandic horses divided into two groups: high and negligible expression of MCT1 and CD147. This study showed evidence of an inter-individual variation of MCT1 and CD147 in horses. Studies in humans and other species like rats showed that content of MCT1 in muscle vary based on whether the measurement was made before, during, or after the exercise [24–34].

Table 3
Amount of MCT1 detected in Western blotting of red blood cell membranes of Arabian and Quarter Horses, in arbitrary units of optical density.

MCT1	N	Mean	Standard Error	Minimum	Maximum	*P* Value
AR	40	1.04	0.08	0.24	2.66	<.0001
QH	40	2.99	0.35	0.16	9.19	
AR LP	20	1.14	0.07	0.54	1.67	.2363
AR HP	20	0.95	0.14	0.24	2.66	
QH LI	20	2.60	0.49	0.27	8.81	.2627
QH HI	20	3.38	0.48	0.16	9.19	

Abbreviations: AR, Arabian; HI, high Speedy Index group; HP, high-performance group; LI, low Speedy Index group; LP, low-performance group; MCT1, monocarboxylate transporter isoform 1; QH, Quarter Horses.

Furthermore, other factors, such as intensity of the exercise, can regulate the changes in MCT [35,36]. Kitaoka et al [16] observed that incremental exercise increased the amount of MCT1 in the gluteus medius muscle of Thoroughbreds, but after 24 hours, there was no difference detected compared with the baseline. In this case, the increase in the amount of MCT1 could be attributed to acute exercise.

In our study, we cannot conclude that the largest amounts of proteins found in Quarter Horses were due to exercise because some of the animals were not engaged in exercise when the samples were collected. Thus, it is possible that the significant effect on the amount of monocarboxylate transporters in Quarter Horses is mainly attributed to glycolytic fibers (IIA and IIX) connected to the metabolism of energy production that allow this breed to perform better during short and intense exercises. Arabian horses, athletes in endurance competitions, predominantly use aerobic metabolism for energy production because the exercise practiced by them requires resistance over a prolonged time. This kind of exercise recruits mainly muscle fiber type I, which are oxidative fibers of aerobic metabolism and have large concentrations of mitochondria and mainly use fatty acids and carbohydrates to generate ATP through canonical oxidative phosphorylation. In contrast, it is well known that Quarter Horses excel at running short distances with great speed. This kind of physical effort recruits type IIA and IIX fibers because this exercise demands energy for muscle contraction in greater quantity, requiring rapid production. Thus, metabolism predominantly favors anaerobic glycolysis, producing ATP from glucose and/or glycogen. This pathway of energy production causes accumulation of the ions lactate and H^+ in muscular fiber and blood that, combined with other factors, such as $H_2PO_4^-$ [37], can accelerate the fatigue process. In this way, Quarter Horses have adapted with increased expression of monocarboxylate transporters to transport lactate from the muscle for metabolism in the liver, resulting in reduced fatigue.

According to numerous studies, horses can be divided into two groups: animals of high and animals of low lactate transport activity [8,9,12,17,38]. This bimodal distribution of lactate transport correlates directly with the amount of CD147 protein. In other words, animals that belong to the high transport lactate group have a high amount of CD147 protein. In our study, Quarter Horses showed significantly increased amounts of MCT1 and CD147 protein over Arabians, but differences were not observed in the levels of monocarboxylate transporters between the high- and low-

performance groups of both breeds. Koho et al [19] observed increased amounts of CD147 protein in Standardbred horses with high lactate transport activity over animals with low transport activity. However, the authors did not find statistical differences in MCT1 protein levels between the two groups. Mykkänen et al [12] separated Finnhorse, Standardbred, and Thoroughbred horses of high and low lactate transport activity by the intensity of the bands of MCT1 and CD147. They classified horses of high lactate transport activity as the horses that showed higher amount of CD147. The amount of MCT1 followed the bimodal distribution of CD147 protein, but was statistically significant only in the Thoroughbred breed. The authors did not find correlation between the amount of proteins and performance in Thoroughbreds, as well as in our study, where similar MCT1 and CD147 protein contents were found in the performance groups of Arabian and Quarter Horses. The results for performance in our study are in agreement with that obtained by Feringer Junior et al [23], which divided the Brazilian Sport Horse breed into animals of high and low performance according to jump height in equestrian events. The authors did not observe a significant difference in the amount of MCT1 and CD147 between the two performance groups.

The similarity between different performance groups for Arabian and Quarter Horses may occur as a result of selection for specific kinds of exercises, which has been applied for years for those breeds. For example, Quarter Horses have been selected for their sprinting ability over short distances since the formation of the breed, which can account for the similar protein expression levels of the performance group. The efficiency of the exercise is dependent on cardiovascular and metabolic adaptations and various biomechanical and kinematic variables such as heart rate and stride angle. Trained animals tend to use less oxygen consumption for a given running speed (Running Economy), and then, less monocarboxylate transporters are used to maintain the acid/base homeostasis. For Quarter Horses, we believe that there is a change over time as they become more efficient in exercising. Those animals use less energy from the anaerobic pathway, a phenomenon that could be known as “Glycolytic economy,” and therefore, less MCT1 and CD147 are needed to maintain the homeostasis. It is important to mention that all the Quarter Horses of this study are racing horses and, thus, are submitted to the same level of training and exercises.

This study was limited in that it was impossible to measure lactate transport activity to separate the animals in low- and high-performance group. Animals therefore had to be separated by performance, with subsequent correlation to the amount of protein. Similar to Mykkänen et al [12], classification of the horses into low and high lactate transport activity was to be via protein band intensity; however, the bands detected in this study were inadequate for this type distinction. Therefore, future studies will be conducted to determine whether polymorphisms in DNA could alter the production of monocarboxylate transporters in animals before they practice any kind of physical training or exercise, allowing us to determine the cause of differences in performance in animals of the same breed.

5. Conclusion

Monocarboxylate transporter isoform 1 and CD147 proteins were found in all Arabian and Quarter Horses. Higher amounts of MCT1 and CD147 were observed in Quarter Horses respect to Arabian Horses probably because of the different pathways used by each breed for energy production. There was no significant difference in the amounts of MCT1 and CD147 between the groups of high and low performance of each race.

Acknowledgments

This research study was funded by FAPESP—Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant number: 2011/11080-0, 2012/24193-0, and 2014/20697-9) and CNPQ-Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant number: 307006/2007-7).

References

[1] Piccione G, Arfuso F, Fazio F, Bazzano M, Giannetto C. Serum lipid modification related to exercise and polynsaturated fatty acid supplementation in jumpers and thoroughbred horses. J Equine Vet Sci 2014;34:1181–7.
[2] Arfuso F, Giannetto C, Giudice E, Fazio F, Piccione G. Dynamic modulation of platelet aggregation, albumin and nonesterified fatty acids during physical exercise in thoroughbred horses. Res Vet Sci 2016;104:86–91.
[3] Piccione G, Messina V, Bazzano M, Giannetto C, Fazio F. Heart rate, net cost of transport, and metabolic power in horse subjected to different physical exercises. J Equine Vet Sci 2013;33:386–9.
[4] Fitts RH. Cellular mechanisms of muscle fatigue. Physiol Rev 1994; 74:49–94.
[5] Hogan MC, Gladden LB, Kurdak SS, Poole DC. Increased [lactate] in working dog muscle reduces tension development independent of pH. Med Sci Sports Exerc 1995;27:371–7.
[6] Bazzano M, Rizzo M, Arfuso F, Giannetto C, Fazio F, Piccione G. Increase in erythrocyte osmotic resistance following polyunsaturated fatty acids (PUFA) supplementation in show jumper horses. Livestock Sci 2015;181:236–41.
[7] Price NT, Jackson VN, Halestrap PA. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem J 1998;329:321–8.
[8] Koho NM, Hyyppä S, Pösö AR. Monocarboxylate transporters (MCT) as lactate carriers in equine muscle and red blood cells. Equine Vet J 2006;36:354–8.
[9] Kirk P, Wilson MC, Heddle C, Brown MH, Barclay AN, Halestrap AP. CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J 2000;19:3896–904.
[10] Gallagher SM, Castorino JJ, Wang D, Philp NJ. Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. Cancer Res 2007;67:4182–9.
[11] Revold T, Mykkänen AK, Karlström K, Ihler CF, Pösö AR, Essén-Gustavsson B. Effects of training on equine muscle fibres and monocarboxylate transporters in young Coldblooded Trotters. Equine Vet J 2010;42:289–95.
[12] Mykkänen AK, Pösö AR, Mcgowan CM, McKane SA. Expression of lactate transporters MCT1, MCT2 and CD147 in the red blood cells of three horse breeds: Finnhorse, Standardbred and Thoroughbred. Equine Vet J 2010;42:161–6.
[13] Kitaoka Y, Mukai K, Aida H, Hiraga A, Masuda H, Takemasa T, et al. Effects of high-intensity training on lipid metabolism in Thoroughbreds. Am J Vet Res 2012;73:1813–8.
[14] Kitaoka Y, Hoshino D, Mukai K, Hiraga A, Takemasa T, Hatta H. Effect of growth on monocarboxylate transporters and indicators of energy metabolism in the gluteus medius muscle of Thoroughbreds. Am J Vet Res 2011;72:1107–11.
[15] Kitaoka Y, Masuda H, Mukai K, Hiraga A, Takemasa T, Hatta H. Effect of training and detraining on monocarboxylate transporter MCT1 and MCT4 in Thoroughbred horses. Exp Physiol 2011;96:348–55.

[16] Kitaoka Y, Endo Y, Mukai K, Aida H, Hiraga A, Takemasa T, et al. Effect of acute exercise on monocarboxylate transporters 1 and 4 in untrained and trained Thoroughbreds. Am J Vet Res 2013;74:642–7.
[17] Väihkönen LK, Pösö AR. Interindividual variation in total and carrier-mediated lactate influx into red blood cells. Am J Physiol 1998;274:R1025–30.
[18] Evans JW. Horses: a guide to selection, care and enjoyment. 2nd ed. Texas: W.H. Freeman; 1989. p. 707.
[19] Koho NM, Väihkönen LK, Pösö AR. Lactate transport in red blood cells by monocarboxylate transporters. Equine Vet J Suppl 2002;34:555–9.
[20] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[21] Koho NM, Mykkänen AK, Reeben M, Raekallio MR, Ilves M, Pösö AR. Sequence variations and two levels of MCT1 and CD147 expression in red blood cells and gluteus muscle of horses. Gene 2012;491:65–70.
[22] Sampaio IBM. Estatística aplicada à experimentação animal. Belo Horizonte: Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia; 1998. p. 221.
[23] Feringer Junior WH, Carvalho JR, Almeida ML, Queiroz-Neto A, Ferraz GC. Lactate transport in red blood cells by monocarboxylate transporter MCT1 and its accessory protein CD147 in Brazilian sport horses of different performance levels. Equine Vet J 2014;46:17.
[24] Baker SK, McCullagh KJ, Bonen A. Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. J Appl Physiol (1985) 1998;84:987–94.
[25] Bonen A, McCullagh KJ, Putman CT, Hultman E, Jones NL, Heigenhauser GJ. Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. Am J Physiol Endocrinol Metab 1998;274:E102–7.
[26] Dubouchaud H, Butterfield GE, Wolfel EE, Bergman BC, Brooks GA. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. Am J Physiol Endocrinol Metab 2000;278:E571–9.
[27] Eydoux N, Dubouchaud H, Py G, Granier P, Prefaut C, Mercier J. Lactate transport in rat sarcolemmal vesicles after a single bout of submaximal exercise. Int J Sports Med 2000;21:393–9.
[28] Green H, Halestrap A, Mockett C, O'Toole D, Grant S, Ouyang J. Increases in muscle MCT are associated with reductions in muscle lactate after a single exercise session in humans. Am J Physiol Endocrinol Metab 2002;282:E154–60.
[29] Tonouchi M, Hatta H, Bonen A. Muscle contraction increases lactate transport while reducing sarcolemmal MCT4, but not MCT1. Am J Physiol Endocrinol Metab 2002;282:E1062–9.
[30] Coles L, Litt J, Hatta H, Bonen A. Exercise rapidly increases expression of the monocarboxylate transporters MCT1 and MCT4 in rat muscle. J Physiol 2004;561:253–61.
[31] Thomas C, Perrey S, Lambert K, Hugon G, Mornet D, Mercier J. Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans. J Appl Physiol (1985) 2005;98:804–9.
[32] Bickham DC, Bentley DJ, Le Rossignol PF, Cameron-Smith D. The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. Eur J Appl Physiol 2006;96:636–43.
[33] Bishop D, Edge J, Thomas C, Mercier J. High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. J Appl Physiol (1985) 2007;102:616–21.
[34] Takimoto M, Takeyama M, Enoki T, Hamada T. Effect of acute high-intensity intermittent exercise on MCT1 and MCT4 expression in rat epitrochlearis. Adv Exer Sport Physiol 2011;17:60.
[35] Thomas C, Bishop DJ, Lambert K, Mercier J, Brooks GA. Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. Am J Physiol Regul Integr Comp Physiol 2012;302:R1–14.
[36] Hamada T, Takimoto M. Regulation of the exercise-induced expression of the monocarboxylate transporters MCT1 and MCT4 in skeletal muscle. J Phys Fitness Sports Med 2013;2:85–92.
[37] Wilson JR, McCully KK, Mancini DM, Boden B, Chance B. Relationship of muscular fatigue to pH and diprotonated Pi in humans: a 31P-NMR study. J Appl Physiol (1985) 1988;64:2333–9.
[38] Väihkönen LK, Ojala M, Pösö AR. Age-related changes and inheritance of lactate transport activity in red blood cells. Equine Vet J 2002;34:568–72.