Acute-phase protein profile in horses subjected to different exercise protocols

Pedrita Assunção, Tatiana Barbosa, Letícia Yonezawa, Luciano Barbosa, Marcus Watanabe, Aguemi Kohayagawa, Elizabeth Schmidt

Abstract

High-intensity exercise can be associated with the occurrence of muscle injury, as well as the induction of an acute-phase response (APR). The present study aims to investigate the synthesis and profile of serum proteins in horses before and after participating in 2 different exercise protocols and to relate this profile to the presence or absence of muscular injury caused by exercise. Ten purebred Arabian ($n = 5$) and Criollo ($n = 5$) horses were subjected to 2 different tests on a treadmill, one consisting of short-duration and rapid-acceleration training (TRA) that was mostly anerobic and the other of long-duration and slow-acceleration training (TLD) that was predominantly aerobic. Blood samples were obtained before the beginning of exercise (T0) and at 6 post-exercise time points: immediately after (T1) and 30 min (T2), 3 h (T3), 12 h (T4), 24 h (T5), and 48 h (T6) after exercise. Hematocrit was determined by the microhematocrit method. Plasma and serum samples were prepared by centrifugation ($1500 \times g$ for 5 min) for plasma concentrations of fibrinogen, total serum proteins (TP), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and creatine-kinase (CK) serum activity. Total protein concentration and CK serum activity were determined in an automated biochemistry analyzer. Fibrinogen was determined by the heat precipitation method in microhematocrit capillary tubes. Estimated concentrations of haptoglobin (Hp) significantly decreased after TRA and estimated concentrations of alpha-1 acid glycoprotein (AGP) significantly increased after both protocols at T2. Albumin increased after the TLD exercise protocol. Changes in hematocrit, haptoglobin, and albumin concentrations in horses subjected to different treadmill exercise protocols are related to a physiological response to hemoconcentration and hemolysis. Increases of AGP in the TLD protocol suggest the release of catecholamines as a response to avoid oxidative damage to tissue.

Résumé

*L'exercice est associé à l'induction d'une réponse inflammatoire en phase aiguë (APR). La présente étude vise à étudier la synthèse et le profil des protéines sériques avant et après la mise en pratique de deux protocoles d'exercices différents, et à et à établir un lien entre ce profil à la présence ou à l'absence de lésions musculaires causées par l'exercice. Dix chevaux, de race pure arabe (*n = 5*) et criollo (*n = 5*), ont été soumis à des tests sur un tapis roulant d'accélération rapide et de courte durée, principalement anaérobique (TRA), et des exercices de faible intensité et de longue durée (TLD), principalement aérobique. Des échantillons de sang veineux ont été prélevés avant le début de l'exercice (T0) et à six moments post-exercice : immédiatement après (T1), 30 minutes (T2), 3 h (T3), 12 h (T4), 24 h (T5) et 48 h (T6). L'hématocrite a été déterminé par la méthode du microhématocrite. Des échantillons de plasma et de sérum ont été préparés par centrifugation (1500 × g pendant 5 min) pour les concentrations plasmatiques de fibrinogène, les protéines sériques totales (TP), l'électrophorèse sur gel de polyacrylamide de sodium dodécylsulfate (SDS-PAGE) et l'activité sérique de créatine kinase (CK). La concentration en TP et l'activité sérique en CK ont été déterminées dans un analyseur automatique de biochimie. Le fibrinogène a été établi par la méthode de précipitation thermique dans des tubes capillaires à microhématocrite. Les niveaux estimés d'haptoglobine ont diminué de manière significative en TRA et les concentrations estimées d'AGP ont été significativement augmentées pour les deux protocoles à T2. L'albumine a augmenté considérablement dans le protocole TLD. Les variations des concentrations d'hématocrite, d'haptoglobine et d'albumine chez les chevaux soumis à différents protocoles d'exercice sur tapis roulant sont liées à la réponse physiologique à l'hémoconcentration et à l'hémolyse. L'augmentation de l'AGP dans le protocole TLD suggère la libération de catécholamines et une réponse permettant d'éviter les dommages oxydatifs aux tissus.*

(Traduit par les auteurs)

São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science (FMVZ), Department of Veterinary Clinical Sciences, Rua Prof. Doutor Walter Mauricio Correa, s/n bairro UNESP, Botucatu Campus, SP Botucatu, São Paulo 18618.618-681, Brazil.

Address all correspondence to Dr. Pedrita Carvalho Ferreira Assunção; telephone: 005514 38802052; fax: 005514 38802052; e-mail: pedritacfassuncao@gmail.com

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Introduction

The increasing use of horses in sporting activities has led to a diversification of exercise in which they participate and to greater demands on their performance (1). Studies have demonstrated that such physical exercise induces alterations in blood markers in athletic horses when participating in field competitions, standardized treadmill exercises, or even exercises standardized by breed (2,3,4).

Exercise induces alterations in biochemical constituents of plasma in athletic horses (5,6) and their participation in increasingly exhausting sporting events requires an understanding of the physiological response in order to ensure their safety (7,4). High-intensity exercise can be associated with the occurrence of muscle injury, as well as the induction of an acute-phase response (APR) (8).

The physiological response to exercise differs in acute response and chronic adaptation. It has been shown that there is an analogous relationship between the APR to exercise and existing infectious and inflammatory processes so that the presence of any tissue injury promotes the release of pro-inflammatory cytokines, nitric oxide, and glucocorticoids that activate and modulate the APR and release of acute-phase proteins (APPs), primarily by the liver (9). The determination of these proteins is therefore useful in monitoring the health status of the animal (10). The APR related to tissue injury and inflammation as a result of exercise can be confirmed by increased creatine-kinase (CK) serum activity (11).

The participation of horses in exhaustive competitions and the need to establish safe protocols for training these animals require the monitoring and investigation of stress and possible inflammatory processes caused by exercise. The practice of physical exercise and the induction of a response similar to APR is controversial. Therefore, the objective of the present study was to investigate the profile of serum proteins, including APPs, in horses before and after participating in 2 different exercise protocols on a treadmill and to relate this profile to the presence or absence of muscular injury caused by exercise. The first protocol consisted of short-duration and rapid-acceleration training (TRA) that was primarily anerobic and the second was of long-duration and slow-acceleration training (TLD) that was predominantly aerobic.

Materials and methods

Animals

Ten adult horses were used, specifically 5 Arabian purebreds (2 male and 3 female), with a mean weight of 347.8 kg, and 5 of the Criollo breed (3 male and 2 female), with 391 kg mean weight, from 8 to 10 y in age, with no physical training for more than 1 y, and considered clinically healthy. The horses were maintained within picket fences under the same conditions of sanitation and feeding management. Their diet consisted of coast-cross hay *(Cynodon dactylon)*, 2 kg of commercial horse ration once daily (Royal Horse Sport, Socil, Brazil), 30 g of mineral supplement (Equifós, Matsuda, Brazil) mixed into the feed, and water *ad libitum*.

This experimental study was carried out in accordance with protocol nº 62/2012 of the Committee for Ethics in the Use of Animals (CEUA) at the Faculty of Veterinary Medicine and Animal Science, São Paulo State University (FMVZ-UNESP), Botucatu Campus.

Exercise protocols

The horses were subjected to the exercise protocols at the Center for Sports Medicine at the Faculty of Veterinary Medicine and Animal Science (FMVZ-UNESP), Botucatu Campus.

Pre-experimental training

For a period of 7 d, a standard test of progressive exercise (STPE) was conducted to determine the workload for each horse in order to calculate the individual velocities corresponding to the percentages of the maximum consumption of oxygen (VO_{2max}) according to the technique. The STPE was conducted on a high-velocity treadmill (Mustang 2200 AG; Kagra, Switzerland) inclined at +6%, with velocity gradually increased as described in a previous study (12). A mask was used to analyze gaseous exchanges and ventilatory data (Metavet; CORTEX Biophysik GmbH, Germany) and to determine the workload for each horse, based on (VO_{2max}), with the mean value for animals of 110.4 ± 20.4 mL/kg per minute in order to maintain a consistent level of oxygen consumption even when the exercise increased in velocity while the test was in progress.

Experimental training I

Seven days after the pre-experimental training or adaptation period, the horses were subjected to short-duration and rapid-acceleration training (TRA), which is considered a predominantly anerobic exercise (lactate concentration greater than 4 mmol/L). This test consisted of a treadmill inclination of +6%, a warm-up period of 5 min for the workload of 50% of VO_{2max} (mean velocity: 3.7 ± 0.9 m/s), followed by 5 more minutes at a velocity of 1.5 m/s. The treadmill belt was then accelerated as rapidly as possible to obtain the individual velocity calculated, to achieve a workload of 105% of VO_{2max} (mean velocity: 9.2 ± 2.1 m/s) for 90 s. The horses were then subjected to a cooling-down period of 5 min at a velocity of 3 m/s with the treadmill in a horizontal position adapted from previous studies (12,13). After completion of this protocol, the horses rested for 7 d before participating in experimental training II.

Experimental training II

Fourteen days after the pre-experimental or adaptation period, the horses were subjected to long-duration and slow-acceleration training (TLD). This test is considered predominantly aerobic exercise (lactate concentration < 4 mmol/L) and was carried out on a treadmill inclined at +6%. This type of exercise was intended to achieve 35% of the VO_{2max} of each animal for 60 min, corresponding to a mean velocity of 2.3 ± 0.5 m/s, which was adapted from previous studies (12,13).

Laboratory analysis

Blood samples were obtained from the horse's jugular vein at 7 distinct time points: before exercise (T0); immediately after exercise (T1); and 30 min (T2), 3 h (T3), 12 h (T4), 24 h (T5), and 48 h (T6) after termination of the TRA and TLD protocols. On the day of the tests, a 14-G catheter (Angiocath 14G; BD Brazil, São Paulo, Brazil) was placed in the jugular vein of each horse and connected to an extensor

Table I. Median and interquartile range (25% to 75%) of concentrations of hematocrit, total serum protein, creatine kinase serum activity, and plasma concentrations of fibrinogen at 7 different time points in horses subjected to TRA (n = 10) and TLD (n = 10) exercise protocols.

	Time points						
	T0	T1	T2	T3	T4	T5	T6
Ht (%)							
TRA	37.5[a,c,d] (33 to 40.2)	49.5[a] (39.5 to 53)	38.5[a] (31.7 to 42.5)	35.5[b,c,d] (31.7 to 39)	35[b,c,d] (30.7 to 37.2)	34.5[b,d] (30 to 37)	33.5[b,c,d] (24.7 to 37)
TLD	33.5[a,b] (29.2 to 40.7)	42[a] (35.7 to 46)	32.5[b] (27.7 to 37)	35.5[a,b] (30 to 39.2)	35[b] (30.7 to 37.2)	33.5[b] (29 to 38.2)	33.5[b] (29.7 to 37)
TP (g/dL)							
TRA	7.2 (69 to 8.2)	7.4 (7.2 to 7.7)	7.2 (6.8 to 7.7)	7.4 (7.2 to 7.6)	7.1 (6.7 to 7.4)	7.3 (7 to 8)	7.3 (6.8 to 7.3)
TLD	7.8 (7 to 8.8)	7.6 (7.1 to 9.1)	7.1 (6.8 to 9.3)	8.1 (7.4 to 10)	7.7 (7 to 8.3)	7.0 (6.7 to 8.3)	7.1 (6.7 to 8.1)
CK (UI/L)							
TRA	250.5 (212.6 to 350.3)	278.6 (201.3 to 348.4)	251.1 (182 to 324)	294.2 (215.2 to 334)	264.4 (235 to 345.4)	300.1 (284.6 to 351)	283.3 (205 to 319.8)
TLD	278.4 (208 to 311.3)	334.8 (203.5 to 433.2)	238.2 (175.6 to 382.7)	238.2 (175.6 to 382.7)	303.6 (178.6 to 344)	264.6 (220 to 303)	256.9 (199 to 400)
Fb (g/dL)							
TRA	0.2 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.2 (0.2 to 0.4)	0.2 (0.2 to 0.2)	0.2 (0.2 to 0.4)	0.2 (0.2 to 0.4)	0.2 (0.2 to 0.4)
TLD	0.2 (0.2 to 0.4)	0.2 (0.2 to 0.4)	0.2 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.2 (0.2 to 0.2)	0.2 (0.2 to 0.2)	0.2 (0.2 to 0.4)

T0 — before exercise; T1 — immediately after exercise; T2 — 30 min; T3 — 3 h; T4 — 12 h; T5 — 24 h; T6 — 48 h; TRA — short-duration and rapid acceleration training; TLD — long-duration and slow-acceleration training; Ht — hematocrit; TP — total serum protein; CK — creatine-kinase; Fb — fibrinogen.

Lowercase letters in the same line indicate significant difference between time points ($P < 0.05$).

to collect blood through a 25-G × 8-G needle coupled to tubes with a vacuum system (Vacuum II; Labnew, Campinas, São Paulo, Brazil) containing potassium ethylene diamine tetraacetic acid (EDTA) for the hematocrit and determination of plasma concentration of fibrinogen. Serum samples were obtained with vacuum tubes (Vacuum II; Labnew) and were prepared by centrifugation (1500 × g for 5 min) and stored in plain microtubes (Eppendorf) at −20°C (Eppendorf, Hamburg, Germany).

Hematocrit was determined by the microhematocrit method (14). The hematocrit and biochemical analytes were determined at the Veterinary Clinical Laboratory at the School of Veterinary Medicine and Animal Sciences (FMVZ-UNESP), Botucatu Campus and the serum protein sodium dodecyl sulfate electrophoresis (SDS-PAGE) was carried out at the Research Support Laboratory at the Department of Veterinary Medicine and Surgery, FCAV/UNESP, Jaboticabal Campus, São Paulo, Brazil.

Plasma fibrinogen concentration was determined by the heat precipitation method (56°C) in microhematocrit capillary tubes and the reading was done by refractometry (15). The serum activity of creatine kinase (CK) was determined by the kinetic method using commercial kits (Katal, São Paulo, Brazil) in an automated spectrophotometer (CobasMira Plus; Roche Diagnostic Systems, Indianapolis, Indiana, USA) within 24 h after sample collection.

The concentration of total serum protein was determined by the biuret method using a commercial kit (Katal) in an automatized spectrophotometer (Cobas Mira; Roche). Serum protein fractions were determined by unidimensional electrophoresis by means of SDS-PAGE, as described by Laemmli et al (16) and modified using the vertical electrophoresis system (BioRad Laboratories, Hercules, California, USA) in 10% acrylamide gels. Electrophoretic analyses were conducted in the Research Support Laboratory at the Department of Veterinary Medicine and Surgery, FCAV/UNESP, Jaboticabal Campus, São Paulo, Brazil. Serum samples (5 μL) were prepared with 5% mercaptoethanol and 0.001% bromophenol blue in a reducing condition (5 min at 95°C) and 5% glycerol was added afterwards. The electric current for the 8-in × 8-in vertical gel electrophoresis system was programmed at 35 and 50 mA, while samples were in the stacking and running gel, respectively. The gels were stained with 0.2% Coomassie Brilliant Blue solution for 15 min and destained in a solution of 7% acetic acid until the gel background was completely clear.

The estimated concentration of serum protein band or peaks was determined by computer-assisted densitometry (CS 9301; Shimadzu Scientific Instrument, Kyoto, Japan) according to a method described in a previous study (17). Protein peaks were identified using reference markers (SDS6H2; Sigma-Aldrich, St. Louis, Missouri, USA)

with a molecular mass of 200 000, 116 000, 97 000, 66 000, 55 000, 45 000, 36 000, 29 000, 24 000, and 20 000 Da. Protein concentration in each single electrophoretic band (mg/dL) was estimated when surface under specific peak (%) on an electrophoretogram was multiplied with total protein concentration (g/dL) and divided by 100.

Statistical analysis

Analyses were carried out using statistical software GraphPad, Version 6 for Windows (GraphPad Software, San Diego, California, USA). All data were first assessed for normality using the Shapiro-Wilk test. As the data did not meet the normal distribution criteria, the non-parametric 1-way analysis of variance (ANOVA) and the Friedman test were used for multiple comparisons (among the time points), and the Wilcoxon-signed rank test to compare the quantitative variables between the 2 protocols. The correlation between the quantitative variables (total serum proteins, fibrinogen, APP, and CK activity) was evaluated by Spearman's rank-order correlation test. The significance level considered for all the tests was $P < 0.05$.

Results

The hematocrit, serum activity of CK, and plasma concentrations of fibrinogen, as well as the total serum protein concentration and its fractions, were determined in all horses, in each experimental protocol. As there were no significant differences in the variables between the 2 breeds of horses, the data are presented separately by exercise protocol.

Hematocrit, total serum protein concentrations, CK serum activity, and plasma concentration of fibrinogen of horses subjected to the exercise protocols are shown in Table I. Except for hematocrit values, there were no significant alterations in the concentrations of these analytes between the 2 exercise protocols or among the different time points. When comparing the time points within the TLD protocol, a significant decrease ($P < 0.05$) was observed in hematocrit between T1 and T2, T4, T5, and T6. Within the TRA protocol, hematocrit values were significantly decreased between T1 and T3, T4, T5, and T6. In this same protocol, hematocrit was significantly decreased between T2 and T5.

The SDS-PAGE technique allowed the fractionation of protein bands (Figure 1). When comparing the time points within the exercise protocols, a significant decrease ($P < 0.05$) was observed in estimated concentration of haptoglobin (Hp) (50 kDa band) in the TRA protocol between T1 and T2 and between T1 and T4 (Table II). The Hp concentration in the TLD protocol did not differ significantly among the time points. There was a significant increase ($P < 0.05$) in the estimated concentration of albumin (65 kDa band) between T2 and T3 and a significant decrease between T3 and T5 in the TLD protocol.

Comparison of the 2 exercise protocols revealed a significant difference ($P < 0.05$) in the estimated concentration of AGP (45 kDa band) 30 min (T2) after the exercises (Table II). Concentration of AGP was not significantly different, however, compared with the other time points.

The correlation between total serum protein concentrations, plasma concentration of fibrinogen, and estimated concentrations of haptoglobin and AGP and CK serum activity are presented in Table III. There was a positive weak correlation ($P < 0.05$; $r = 0.3$) between CK serum activity and total serum protein concentrations and between CK serum activity and estimated concentration of haptoglobin in exercise protocol TRA and between CK serum activity and estimated concentration of haptoglobin in protocol TLD ($P < 0.05$; $r = 0.3$).

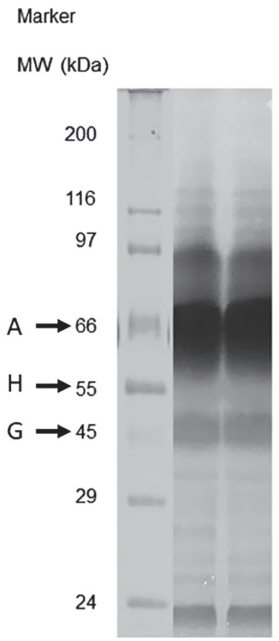

Figure 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of 2 horse's serum on a pore gradient gel (10%) under reducing conditions. Molecular mass markers are shown on the left side of the gel. Arrows indicate A — albumin; H — haptoglobin; G — alpha-1 acid glycoprotein (AGP).

Discussion

In athletic horses, physical exercise induces alterations in blood markers that are related to participation in field competitions or standardized and controlled exercises on a treadmill (5,18). The stress caused by practising physical exercise can trigger an acute-phase response and the synthesis of acute-phase proteins, which associated with other markers, identifies the presence of tissue injury caused by physical activity (3).

At all time points in this study (T0 to T6), the CK serum activities in all horses increased to above the reference interval for the species (2.3 to 23.4 IU/L) (15). Horses could have increased serum activity of this enzyme under certain physiological conditions, which suggests that CK could be removed more slowly from the circulation or that some animals are more sensitive to membrane permeability of the muscle cell when stimulated (19). Hemolysis may also falsely elevate the activity of this enzyme, as the erythrocytes contain adenilatequinase and glucose-6-phosphate that interfere with the serum activity of CK (20). In humans, carriers of CK isoenzymes in the bloodstream indicated chronically elevated CK serum activity, without a specific cause (21).

Purebred Arabian and Thoroughbred horses submitted to different athletic modalities, e.g., endurance/long-distance, racing, and

Table II. Median and interquartile range (25% to 75%) of the estimated concentrations of haptoglobin (Hp), albumin (Alb), and alpha-1 acid glycoprotein (AGP) at 6 different time points in horses subjected to TRA (n = 10) and TLD (n = 10) exercise protocols.

	Hp (TRA) (g/dL)	Hp (TLD) (g/dL)	Alb (TRA) (g/dL)	Alb (TLD) (g/dL)	AGP (TRA) (mg/dL)	AGP (TLD) (mg/dL)	
T0	2.0[a,b] (1.4 to 2.5)	2.1 (1.7 to 3.4)	4.8 (4.4 to 5.2)	4.9[a,b] (4.3 to 5.8)	3.6 (3.4 to 4.9)	4.6 (2.9 to 5.5)	
T1	1.9[a] (1.4 to 2.9)	2.2 (1.1 to 3.3)	4.7 (4.3 to 4.9)	4.8[a,b] (4.6 to 5.8)	3.4 (3 to 5.6)	4.3 (3.5 to 4.8)	
T2	1.6[b] (1.4 to 2.3)	2.1 (1.2 to 3.4)	4.5 (4.3 to 4.8)	4.7[b] (3.8 to 5.6)	3.4[A] (2.8 to 3.9)	5.5[B] (4 to 6.1)	P = 0.002
T3	1.8[a,b] (1.4 to 2.7)	2.2 (1.6 to 2.9)	4.9 (4.4 to 5.2)	5.5[a] (4.5 to 5.9)	3.6 (2.3 to 4.4)	4.6 (3.5 to 5.9)	
T4	1.5[b] (1.1 to 2.4)	2.1 (1.2 to 3.2)	4.4 (4.2 to 4.8)	4.8[a,b] (4.2 to 5.4)	3.3 (2.4 to 5)	5.0 (3.5 to 5.9)	
T5	1.7[a,b] (1.3 to 2.7)	2.3 (1.1 to 3.7)	4.6 (4.4 to 5.3)	4.6[b] (4.2 to 4.7)	4.1 (3 to 4.5)	4.6 (3 to 5.8)	
T6	1.8[a,b] (1.5 to 2.6)	2.4 (1.0 to 3.9)	4.6 (4.0 to 5.3)	4.6[a,b] (4.3 to 5.5)	3.7 (2.8 to 4.5)	4.2 (3.5 to 7.1)	
	P = 0.02	P = 0.23	P = 0.08	P = 0.03	P = 0.72	P = 0.44	

T0 — before exercise; T1 — immediately after exercise; T2 — 30 min; T3 — 3 h; T4 — 12 h; T5 — 24 h; T6 — 48 h; TRA — short-duration and rapid-acceleration training; TLD — long-duration and slow-acceleration training.
Lowercase letters in the same column indicate significant difference between time points ($P < 0.05$). Capital letters on the same line indicate significant difference between exercise protocols.

high-goal polo, presented hematological and biochemical changes directly related to the type of training, presence or absence of tissue injury, and adaptation to the sporting activity (18,22,23).

The CK serum activity and plasma concentration of fibrinogen did not change significantly among the time points or between the protocols evaluated. One study reported that the serum activity of CK in horses subjected to high-goal polo remained within the reference interval for the species because the activity of this enzyme changes due to the temporary permeability of the muscle's cellular membrane, as a direct physiological response to the type and intensity of physical exercise (3). As the exercise protocols used in the present study did not promote changes in the permeability of the muscle's cellular membrane, the serum activity of CK did not change.

The fibrinogen results obtained in this study were not significantly changed, which suggests that the horses adapted to both exercise protocols because the values of fibrinogen were within the reference interval. This indicates that the exercise did not promote enough physiological stress to cause an increase in the concentration of fibrinogen. According to Fazio et al (5), fibrinogen is an important marker for evaluating training in athletic horses and is closely linked to the intensity of the workload, the duration of the exercise, and the distance involved. The length of time and rate of acceleration of the running treadmill in these 2 exercise protocols may not have been sufficient to cause changes.

The electrophoretic fractionation of protein bands in this study was determined according to a previous study with SDS-PAGE in ponies (17), which suggests that the haptoglobin fraction could be considered with an MW of 50 kDa and the AGP fraction of 45 kDa.

Table III. Correlation between the concentrations of variables in horses subjected to TRA and TLD exercise protocols.

	CK	
	CK (TRA) (r)	CK (TLD) (r)
Total serum protein (TP)	0.3[a] ($P < 0.01$)	−0.17
Plasma concentration of fibrinogen	0.14	0.09
Haptoglobin estimated concentration	0.3[a] ($P < 0.04$)	0.3[a] ($P < 0.04$)
Alpha-1 acid glycoprotein (AGP) estimated concentration	−0.07	−0.16

[a] $P < 0.05$.
CK — creatine-kinase serum activity; TRA — short-duration and rapid acceleration training; TLD — long-duration and slow-acceleration training.

For both exercise protocols (TRA and TLD), hematocrit was significantly increased immediately after the exercise (T1). In horses, it is known that exercise causes concentrations of catecholamines

and cortisol to increase temporarily, causing mobilization of spleen erythrocytes and contraction of the spleen to guarantee oxygen supply, which determines the potential for athletic development (3,24). Thus, these horses had hemoconcentration immediately after the exercises, but hematocrit levels returned to baseline values 30 min after both protocols (T2). Significantly, decreases of hematocrit (Table I) in protocol TRA after 3, 12, 24, and 48 h, and after 30 min and 12, 24, and 48 h in the TLD protocol could confirm the hypothesis of exercise-induced intravascular hemolysis, as was observed in Standardbred horses subjected to exercises on race tracks (25). During exercise in horses, fluid is displaced from the intravascular space to the interstitial and intracellular space, which may lead to an increase in albumin concentrations and is related to the length and intensity of the type of exercise (3). In those horses subjected to protocol TLD, the significantly increased albumin concentrations at T3 suggest that profuse sweating could markedly cause the fluid shift and that albumin concentrations could remain increased for a prolonged time (26).

Changes in acute-phase proteins (APPs) in athletic horses remain poorly understood (27,28,29). Haptoglobin was identified as a hemoglobin-binding protein and is the principal scavenger of free hemoglobin in blood (30). In this study, plasma-free hemoglobin decreased estimated concentrations of haptoglobin in protocol TRA (Table II), which is mainly an anerobic exercise. As such, this protocol requires a high level of oxygen supply as the increased hematocrit level was higher than the maximum reference interval (RI) (32% to 47%) for this species (15), with significantly decreased hematocrit values at T3, T4, T5, and T6 and significantly decreased haptoglobin concentrations at T2 and T4.

Alpha-1 acid glycoprotein (AGP) acts as a natural anti-inflammatory and is associated with the release of catecholamines and glucocorticoids to reestablish homeostasis (10). This prevents oxidative damage because AGP inhibits the activation of neutrophils, increases the secretion of interleukin-1 (IL-1), and acts in the clearance of lipopolysaccharides, thus neutralizing their toxicity (31). Concentrations of AGP did not change in ponies subjected to different positions in high-goal polo (3). In the present study, the significantly different estimated concentrations of AGP at T2, when comparing TRA and TLD protocols, could suggest that catecholamines and this protein were released to prevent tissue injuries and avoid oxidative damage, which is different than the findings of previous studies of AGP in horses (28,31).

In conclusion, changes in hematocrit, estimated concentrations of haptoglobin, and albumin concentrations in horses subjected to different exercise protocols on a treadmill are not caused by an inflammatory response, but rather are related to a physiological response to hemoconcentration and hemolysis. Increases of AGP in the TLD protocol suggest the release of catecholamines as a response to avoid tissue oxidative damage.

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