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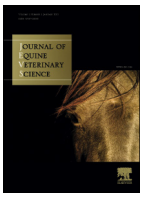

Original Research

Acute Phase Responses of Different Positions of High-Goal (Elite) Polo Ponies

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A B S T R A C T

The aim of this study was to investigate the acute phase response (APR) in 15 horses by quantifying physiological venous blood variables and serum acute phase proteins (APP) at 5 minutes and 6 and 12 hours after a training match of high-goal polo. The horses were divided into three experimental groups based on their team positions, including defense (n = 6), midfield (n = 5), and attack (n = 4). Serum proteinograms were obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Data were evaluated using analysis of variance for repeated measures. The match represented a high-intensity stimulus for all positions. Defenders appeared to use the anaerobic pathway more than the other positions, as shown by their lower pH and greater lactatemia. Alterations in muscle membrane permeability were observed in all horses, as seen by the increase in serum creatine kinase activity without a correlation with APR. Significant elevations in total serum protein, albumin, ceruloplasmin, haptoglobin, alpha-1 antitrypsin, and 23-kDa protein were seen only during the course of the physical exertion of the match, although there were no differences in these values among positions of the team. After 6 hours of the match, the concentration of transferrin declined, whereas that of alpha-1 acid glycoprotein remained unaltered at all assessed times. These results demonstrated that the defenders required the most use of the anaerobic pathway during the match, and that equestrian polo exercise triggers an acute phase response of relatively short duration; this APR is characterized as noninflammatory, as APR appears to be a physiological alteration related to the stress inherent in physical exercise.

1. Introduction

Physical exercise is one of the most physiologically stressful stimuli an animal can undergo; during physical exertion, the animal experiences reversible alterations in various homeostatic variables that are detectable by the quantification of laboratory variables.

The scientific community is currently interested in comparing the biochemical and hematological changes that result from physical exercise [1] with those that occur during an acute phase response (APR). Changes during APR occur in a quick, refined and nonspecific way and are caused by numerous diseases such as infection, inflammation, trauma, or immune disorders that result in

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various degrees of tissue damage [2]. Generally, acute phase proteins (APPs) are biomarkers [3], synthesized by the liver and mediated by cytokines; their concentration in the blood can increase (positive APP) or decrease (negative APP) as a consequence of an inflammatory stimulus.

The first response of an organism to an immunological stimulus is innate and nonspecific and precedes a specific immune reaction. Systemically, proinflammatory cytokines (mainly interleukin-6 [IL-6]) are released into the vascular system, where they activate inflammatory cells. The sympathetic adrenal and hypothalamic-pituitary-adrenal axis can have a significant impact on this process [4], by activating the production of more cytokines that when released, activate receptors in different target cells. This produces a systemic reaction resulting in the activation of physiological or pathological responses, with muscle catabolism being the most relevant to exercise [5]. However, during exercise, studies have reported that the role of IL-6 in intramuscular biological function is to promote glucose homeostasis. In this case, IL-6 is termed "myokine" (or a muscle cytokine) and essentially possesses anti-inflammatory activity [6,7].

There is currently a controversy whether physical exercise can provoke a response similar to the APR. A study of ultramarathon runners reported evidence that APR, as a consequence of exercise, can produce conditions similar to those observed in clinical or surgical morbid conditions [8]. It is well established in the literature that high-intensity exercise causes elevations in serum creatine kinase (CK) activity [9]. Evans and Cannon [10] affirmed that APR, as a result of exercise, can be related to skeletal muscle damage and its consequent inflammation, as shown by increase in serum CK activity.

Various sprinting sports such as soccer [11] and polo [12] are characterized by periods of high-intensity activity such as racing and body contact interspersed with periods of low-intensity activity such as walking or active or passive recovery periods. Monitoring the bodily responses produced by competition can determine the type of effort inherent in each equestrian discipline [13]. Various studies in humans have focused on determination of the metabolic demand of different positions of soccer players through quantification of physiological variables [14,15]. As mentioned previously, the positions in polo are similar to those in soccer, consisting of attack, midfield, and defense, also known as positions 1 and 2 and 3 and 4, respectively. In a brief review of the literature, almost no scientific works could be found investigating the equine metabolism of different polo positions and their relation to possible APR.

From a clinical perspective, documentation of the extent and nature of APR in the play of various equestrian disciplines is important. Other studies along this line have monitored acute phase proteins in different equestrian disciplines, including endurance [16,17] and racing [1]. Quantification of the proteins resulting from the exertion of these horses can provide valuable information to identify the possible occurrence of APR in a game of polo. This study tested the hypothesis that the physical exertion produced in a training match can induce an APR in healthy horses and sought to determine whether the response differed by position on the of high-goal polo team.

2. Materials and Methods

2.1. Horses

Fifteen clinically healthy “high-goal” polo ponies of a Brazilian elite polo team (10 geldings and 5 females) were used. The horses had a mean ± SE body weight of 442 ± 28 kg and were 7.4 ± 2.2 years old. These animals had participated in another study [10] conducted by our laboratory. All horses began the 2009 season in the month of March; they underwent the same weekly training program, consisting essentially of aerobic exercises (taqueio and vareio), 6 times a week. A training match, the target activity of our study, was played once a week. The animals were kept in individual stalls, with free access to water and supplemented with *Medicago sativa* hay and mineralized salt. The concentrate diet was composed of 30% ration (Supra-Tonnus, São Leopoldo, Rio Grande do Sul, Brazil) and 70% oats, and each horse was furnished with 6-8 kg/day of this ration. All riders had international experience and a mean body weight of 83 ± 3 kg. These horses had previously participated in another study [12].

2.2. Groups

The horses were divided into three groups according to their positions on the team, namely, defense (n = 6), midfield (n = 5), and attack (n = 4).

2.3. Training Match and Sampling

After 6 weeks of training, the horses were entered into a training match in preparation for a tournament of 25 goals; the training match consisted of six 7-minute periods (chukkas). Each horse participated in only one chukka. The match was played outdoors on a grassy area 275 m by 180 m in dimension, located at south latitude −23°05′ 25″, west longitude 47°13′ 05″, and altitude 624 m. A standard operating procedure for blood sample collection was established to ensure proper procedures for collection, processing, and storage. After a period of 48 hours of inactivity (18 hours before the training match), blood was collected from the jugular vein; blood was then collected again at 5 min and 6 and 12 hours after the match.

2.4. Blood Analysis

Immediately after blood samples were collected, a portable chemical analyzer (Heska Corp., Fort Collins, CO) was used to determine the pH, total carbon dioxide (TCO_2), and base excess (BE). An electroenzymatic method was used to determine lactatemia of the whole blood in duplicate with an automated lactate analyzer (YSI Inc., Yellow Springs, OH). Ten milliliters of blood was collected in tubes without anticoagulant for later analysis of CK activity and immediately centrifuged under refrigeration (multispeed refrigerated centrifuge PK121R model; ALC, Princeton, NJ)

and analyzed by spectrophotometry (Quick Lab chemistry analyzer, Hameln, Germany); an aliquot of serum was placed in a 1.5-mL Eppendorf tube and stored frozen at −18°C until electrophoretic analysis for quantification of total proteins and APP.

2.5. Determination of APPs with SDS-PAGE

Total serum protein (TSP) was determined by means of spectrophotometry, with the help of an assortment of diagnostic reagents (Labtest; Sistema de Diagnósticos Ltda, Lagoa Santa, Brazil). SDS-PAGE was performed in 10% acrylamide gels. Electrophoretic analyses were conducted in the Research Support Laboratory of the Department of Veterinary Medicine and Surgery, FCAV—Unesp, Jaboticabal, SP. The serum protein profile was obtained in a polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE). Electrophoretic fractionation was conducted according by the technique described by Laemmli [18] and modified using the vertical electrophoresis system (Bio-Rad Laboratories Inc.). The electrophoretic fractionation was performed by a unidimensional electrophoresis system on 4%-10% gradient acrylamide gels. Serum samples (5 mL) were prepared in 40 mL of Dulbecco phosphate-buffered saline solution and 10 mL of gel mixture (10% water, 2% SDS, 5% 2-mercaptoethanol, 10 mM ethylenediaminetetraacetic acid, 20 mM Tris phosphate [pH 7.4], 5% glycerol, and 0.001% bromophenol blue as the dye). The electric current for the 8 × 8-inch vertical gel electrophoresis system was programmed at 35 and 50 mA, while samples were in the stacking and running gel, respectively. After fractionation, the gel was stained in 0.2% Coomassie Brilliant Blue solution for 10 minutes. Next, the gel was destained in a solution containing 250 mL of methanol, 100 mL of acetic acid, and 650 mL of water until protein fractions appeared clear. Concentrations of these protein fractions were determined according to the method described by Fagliari et al. [19], using a digital densitometer (9301PC Shimadzu, Tokyo, Japan). Proteins were identified using reference markers (Sigma Chemical Co., St. Louis, MO) with molecular mass of 200,000; 116,000; 97,000; 66,000; 55,000; 45,000; 36,000; 29,000; 24,000; 20,000; 14,200; and 6,500 Da. In addition, the purified proteins (Sigma Chemical Co., St. Louis, MO) albumin (ALB), haptoglobin (HP), ceruloplasmin (CER), and transferrin (TRANS) were also used.

2.6. Statistical Analysis

Normality of data (means ± SD) was verified using the Kolmogorov-Smirnov test. The effect of exercise on physiologic variables was evaluated by ANOVA for repeated measures using the general linear models procedure of SAS software (SAS Institute Inc., 1988, Cary, North Carolina) followed by the Tukey test when necessary, with the aim of determining significant differences for each sampling time. Because the protein α_1-antitrypsin (α_1-AnT) did not show normality, the Friedman test for repeated measures analysis of variance was performed. However, when this parameter was assessed for values for the attack position, the data showed normality and were analyzed in a parametric manner. Relationships between the variables lactate, pH, CK, and the APP were determined using the Pearson correlations coefficient *r*. Correlations were also examined between 23-kDa protein and the other APP. A *P* value of ≤.05 was considered significant.

3. Results

Table 1 shows the physiological variables related to the acid-base balance and the characterization of the metabolic pathways for ATP production obtained after the match. Immediately after the end of the chukka, a decrease in pH, TCO_2 and BE was observed in horses of all positions, with a return to normal values 6 hours after the effort. The pH of the blood of the horses that played defense was lower ($P < .05$) than that of those who played the MF and A positions. There were no differences in TCO_2 or BE between the groups. Lactate increased after the exercise in all of the experimental groups. At the time of evaluation lactatemia was 34% and 30% greater in defenders than in horses from the MF and A groups, respectively. The effect of the exercise on the individual positions was the same as that described for the overall mean effects. An increase in CK activity was observed after match for the horses in the D, MF and A positions: 6 hours after the effort, increases of 19.0%, 31% and 37%, respectively, occurred. There were no differences in values of CK between the horses of the different positions at any of the times assessed. The effort resulted in an increase in CK activity after 6 hours in horses of all positions.

Table 1
General mean (GM) ± SD pH, TCO_2, BE, and lactate values for different positions, namely defense (D, n = 6), midfield (MF, n = 5) and attack (A, n = 4) before and 5 minutes (after) and 6 and 12 hours after a polo training match for a championship of 25 goals

Variable	Steps of Assessment			
	Before	Postexercise	6 h	12 h
pH				
D	7.42 ± 0.01	7.14 ± 0.01*	7.40 ± 0.01	7.42 ± 0.01
MF	7.41 ± 0.01	7.17 ± 0.01	7.41 ± 0.01	7.41 ± 0.01
A	7.42 ± 0.01	7.17 ± 0.01	7.41 ± 0.01	7.42 ± 0.01
GM	7.42 ± 0.01^A	7.16 ± 0.02^B	7.41 ± 0.01^A	7.41 ± 0.02^A
TCO_2 (mM)				
D	34 ± 1	12 ± 1**	32 ± 1	35 ± 4
MF	33 ± 1	15 ± 4**	31 ± 1	32 ± 1
A	34 ± 2	14 ± 2**	32 ± 1	32 ± 2
GM	33 ± 1^A	14 ± 3^B	32 ± 1^A	34 ± 3^A
BE (ecf) (mM)				
D	8 ± 1	−16 ± 4**	6 ± 2	7 ± 2
MF	7 ± 1	−12 ± 1**	6 ± 2	8 ± 1
A	8 ± 1	−13 ± 2**	6 ± 1	6 ± 2
GM	7 ± 1^A	−14 ± 4^B	7 ± 1^A	7 ± 2^A
Lactate (mM)				
D	0.66 ± 0.2	23 ± 5*,**	0.74 ± 0.15	0.73 ± 0.23
MF	0.68 ± 0.1	15.4 ± 3**	0.80 ± 0.08	0.75 ± 0.22
A	0.70 ± 0.1	16.1 ± 3**	0.69 ± 0.04	0.93 ± 0.15
GM	0.68 ± 0.1^A	18.5 ± 5^B	0.75 ± 0.11^A	0.79 ± 0.21^A
CK (IU/L)				
D	264 ± 50	258 ± 21	326 ± 65**	226 ± 64
MF	251 ± 25	255 ± 38	369 ± 72**	207 ± 48
A	216 ± 11	273 ± 69	344 ± 97**	242 ± 48
GM	247 ± 30^A	261 ± 41^A	343 ± 94^B	225 ± 53^A

BE, base excess; CK, creatine kinase; ecf, extracellular fluid; TCO_2, total carbon dioxide.
Different letters (A and B) in the same line indicate differences ($P < .05$).
* Indicates difference in comparison to the values of the other positions for the same variable.
** Indicates difference in comparison to the values before the match.

After the preparation of the electropherogram, the mean concentrations of the APP were determined before and after the polo training match and are summarized in Table 2. There was a mean increase of 17.5% ($P < .001$) in TSP and ALB 5 minutes after the match; this increased persisted up to 6 hours after the match, and values were similar for all groups at all times studied. No differences in CER levels were seen between the groups, and CER levels increased ($P < .05$) only 6 hours after the exercise, returning to normal 12 hours after the match. The same pattern was seen for TRANS: levels of this protein increased by 17.5% 5 minutes after the match and decreased 6 hours after the match ($P < .05$). HP was increased ($P < .05$) by 29.7% only 5 minutes after the exercise. After the exercise, mean α1-AnT levels were elevated ($P < .001$), and remained so for 6 hours; after 12 hours, α1-AnT levels returned to baseline, and no differences were observed between the members of the different groups at any of the assessment times. A marked increase of the α1-AnT levels occurred in the attackers 5 after exercise. Neither exercise nor position had any effect on the concentrations of α_1-acid glycoprotein (α1-GlycA). In addition to the above-mentioned proteins, a nonidentified 23-kDa protein (23-kDa-p) was encountered. The analysis in Table 1 indicates that the levels of this protein increased ($P < .001$) up until 6 hours after the exercise for horses of all positions. The correlations between the APP and the physiological variables lactate and pH are shown in Table 3. High correlations ($0.6 < r < 1.0$) were observed for lactate, pH, 23-kDa-p, and ALB. Moderate correlations ($0.4 < r < 0.5$) were observed for lactate, pH, and TRANS and 23-kDa-p. Low correlations were detected for CK and all proteins.

Table 2
General mean (GM) ± SD concentrations of total serum protein and acute phase proteins for different positions, namely defense (D, n = 6), midfield (MF, n = 5), and attack (A, n = 4) before and 5 minutes after and 6 and 12 hours after a polo training match

Variables	Steps of Assessment			
	Before	PostExercise	6 h	12 h
TSP				
D	7.00 ± 0.17^{a}	8.60 ± 0.23^{a}	7.42 ± 0.15^{a}	7.11 ± 0.19^{a}
MF	6.50 ± 0.12^{a}	7.36 ± 0.13^{a}	6.90 ± 0.28^{a}	6.81 ± 0.13^{a}
A	6.70 ± 0.03^{a}	7.90 ± 0.20^{a}	7.20 ± 0.30^{a}	7.42 ± 0.33^{a}
GM	6.85 ± 0.13^{C}	7.79 ± 0.13^{A}	7.19 ± 0.13^{B}	7.09 ± 0.13^{CB}
ALB				
D	3.9 ± 0.8^{a}	5.1 ± 1.1^{a}	4.5 ± 1.1^{a}	4.3 ± 0.7^{ab}
MF	4.0 ± 0.6^{a}	4.5 ± 0.6^{b}	4.1 ± 1.2^{a}	4.0 ± 1.4^{b}
A	4.2 ± 1^{a}	5.1 ± 0.2^{a}	4.3 ± 0.9^{a}	4.5 ± 1.2^{a}
GM	4.0 ± 0.6^{C}	4.9 ± 0.9^{A}	4.3 ± 0.7^{B}	4.2 ± 0.8^{CB}
CER				
D	6.95 ± 0.29^{a}	6.40 ± 2.6^{a}	8.32 ± 0.84^{a}	7.30 ± 1.04^{a}
MF	3.32 ± 0.25^{b}	5.79 ± 1.21^{a}	7.70 ± 1.41^{a}	8.07 ± 1.66^{a}
A	4.03 ± 0.56^{ab}	9.14 ± 2.34^{a}	8.16 ± 0.72^{a}	8.33 ± 1.09^{a}
GM	4.85 ± 0.52^{B}	6.80 ± 1.18^{AB}	8.07 ± 0.57^{A}	7.83 ± 0.70^{AB}
TRANS				
D	349 ± 14^{a}	407 ± 23^{a}	280 ± 14^{a}	331 ± 12^{a}
MF	347 ± 3^{a}	409 ± 17^{a}	323 ± 27^{a}	336 ± 30^{a}
A	334 ± 29^{a}	376 ± 30^{a}	255 ± 26^{a}	358 ± 29^{a}
GM	339 ± 10^{B}	399 ± 12^{A}	288 ± 14^{C}	340 ± 12^{B}
HP				
D	16.8 ± 1^{a}	23.5 ± 1^{a}	15.9 ± 3^{a}	15.3 ± 2^{a}
MF	18.2 ± 2^{a}	22.0 ± 3^{a}	16.7 ± 2^{a}	15.7 ± 1^{a}
A	15.2 ± 1^{a}	19.3 ± 2^{a}	16.0 ± 1^{a}	15.4 ± 2^{a}
GM	16.8 ± 1^{B}	21.8 ± 1^{A}	16.2 ± 1^{B}	15.5 ± 1^{B}
α1-AnT				
D	248 ± 52^{a}	55 ± 55^{a}	64 ± 64^{a}	140 ± 140^{a}
MF	172 ± 58^{a}	487 ± 90^{a}	468 ± 110^{a}	286 ± 27^{a}
A	0 ± 0^{a}	$698 \pm 141^{a\#}$	206 ± 125^{a}	269 ± 181^{a}
GM	155 ± 38^{A}	329 ± 92^{B}	268 ± 76^{B}	250 ± 72^{AB}
α1-GlycA				
D	13.0 ± 1^{a}	12.4 ± 4^{a}	12 ± 2^{ab}	11 ± 2^{a}
MF	12.5 ± 1^{a}	10.9 ± 3^{a}	10 ± 1^{b}	13 ± 2^{a}
A	14.7 ± 1^{a}	11.3 ± 1^{a}	16 ± 1^{a}	8 ± 2^{a}
GM	13.2 ± 1^{A}	11.6 ± 1^{A}	13 ± 1^{A}	11 ± 1^{A}
23-kDa-p				
D	390 ± 18^{a}	483 ± 22^{a}	402 ± 15^{a}	391 ± 16^{a}
MF	362 ± 8^{a}	431 ± 13^{a}	409 ± 12^{a}	402 ± 4^{a}
A	374 ± 20^{a}	429 ± 18^{a}	379 ± 27^{a}	388 ± 20^{a}
GM	377 ± 10^{C}	451 ± 12^{A}	398 ± 10^{B}	394 ± 8^{CB}

23-kDa p, 23-kDa protein (MW = 23,000; mg/dL); ALB, albumin (molecular weight [MW] = 63,000; mg/dL); CER, ceruloplasmin (MW = 102,000; mg/dL); HP, haptoglobin (MW = 40,000; mg/dL); TRANS, transferrin (MW = 85,000; mg/dL); TSP, total serum protein (g/L); α1-AnT, alpha-1 antitrypsin (MW = 57,000; mg/dL); α1-GlycA, alpha-1 acid glycoprotein (MW = 36,000; mg/dL).
Different upper-case letters on the same line indicates statistical difference. Different lower-case letters in the same column indicates statistical difference between the positions in the same assessment time and variables.
#Indicates an increase 5 minutes after the match for the attackers compared to the values before the match.

4. Discussion

Currently, there is limited information on the performance and inflammatory responses that can occur in horses after a polo match. The horses that played in this training match had concentrations of APP consistent to an APR; this study therefore provided some evidence to support the hypothesis that an APR produced by a stressing stimulus such as intense exercise is equivalent to that observed in tissue insults. Of the eight acute phase reactive biomarkers studied, the levels of six of these were consistent with an APR. These results are similar to those reported by Piñeiro et al. [20] who studied APR in human ultramarathons competitors. During high-intensity exercise, there is a shift of protein-poor fluid from the intravascular space to the interstitial and intracellular spaces. This fluid shift could lead to an increase in TSP and ALB concentrations following exercise, as was found in our study. Any changes in APP concentrations, especially those observed at the 5-minute postexercise time point, should be interpreted in light of this fluid shift. This fluid shift is less important for the 6 and 12 hours postexercise, as TSP and ALB had returned to values close to their the preexercise values.

Table 3
Correlations between variables concentrations of horses submitted to a preparatory polo training match

	CK	Lactate	pH	23- kDa-p
Albumin	0.025	0.667*	−0.694*	0.618*
Ceruloplasmin	0.258	−0.172	−0.099	0.138
Transferrin	−0.188	0.485*	−0.514*	0.501*
Haptoglobin	−0.069	0.461*	−0.442*	0.262*
α_1-Antitrypsin	0.174	0.162	−0.044	0.043
α_1-Glycoprotein acid	−0.055	−0.091	0.059*	−0.292*
23-kDa-p	0.033	0.570*	−0.536*	

CK, creatine kinase.
* $P < .05$.

Our group has been quantifying the APP in equine serum by using SDS-PAGE [19]. Furthermore, the values obtained before exercise in the present study were consistent with those of a study recently published by our laboratory [21]. Other authors who monitored acute phase proteins during a training program in athletic horses also found similar values as those reported in the present study, despite the use of a different method [22]. Thus, it is likely the alterations observed after the match in the current experimental model were not due to drift.

The physiological response observed in these horses and the trends in values of pH, TCO_2, BE, and lactate suggest that this training match was high-intensity exercise, as was reported earlier [12]. The physiological variables revealed that the defenders had the highest rate of utilization of the anaerobic metabolic pathway, as they had a lower blood pH and greater lactatemia. Similarly, in soccer, it has been show that there are considerable individual differences in physiological demand between players, related in part to their position on the team [15].

An elevation in serum CK activity similar to that observed in the occurrence of muscle microtraumas [11] that occur after a soccer match was observed in horses of all positions. Some studies have suggested that the increase in this serum enzyme activity occurs due to the transitory increase in the permeability of the sarcolemma [23] in the absence of histological lesions [24].

Another concept to consider is the possibility that the stressful stimulus can provoke a noninflammatory and psychophysical response that can induce APR in healthy domestic animals due to the production of catecholamines that are detectable in the laboratory [4,20]. Because APPs are considered biomarkers almost exclusive for inflammation and/or infection, the present study, which considered physical exercise as an experimental model, could have revealed a possible essential relationship between APR and a noninflammatory stimulus. Our hypothesis is reinforced by the high correlation between the production of lactate and adrenaline/noradrenaline [25] which regulates muscle glycogenolysis during intense exercise. Additionally, blood lactate concentrations and venous pH showed a high or moderate correlation with ALB, TRANS, HP, and 23-kDa-p.

An elevation of 12% and 4.7% in total protein was observed 5 minutes and 6 hours, respectively, after the match, most likely reflecting a certain degree of dehydration, despite the fact that this level of alteration is sometimes considered insufficient to cause hemoconcentration as observed in endurance horses [26]. Indeed, this alteration was attributed to a slight reduction in plasma volume, and not to actual dehydration caused by sweating; where the hemoconcentration observed in polo horses could be due to fluid intercompartmentalization instead of a true loss of fluid via perspiration. This mechanism can partially explain the increases found in APP, especially for ALB, as ALB is considered a negative acute phase protein [27].

This is the first report to provide information on the dynamics of CER, a protein that acts in the cellular oxidative metabolism of cooper and iron, after physical exercise in the polo game. By definition, when there are changes greater than 25% in the concentrations of APP, an APR occurs [27]. Here, a 40% increase in CER was observed. The elevation of the concentration of this protein can be attributed to an inflammatory or noninflammatory response [4,5] of acute phase induced by exercise [28].

Human runners of 1600-km ultramarathon races [29] were found to have a 15% reduction in levels of TRANS, a negative acute phase protein. This pattern is expected in the acute phase response, as this decrease can be related to the increase in bioavailability of some hormones such as catecholamines that are ligands of this protein [30] and are elevated after intense exercise [25]. This effect makes this protein one of the "acute-booster reactants" (ABRs) [8].

During intense exercise, a release of hemoglobin caused by partial hemolysis [17, 31] due to an increase in erythrocyte permeability can occur. This hemoglobinemia is neutralized by HP, a positive APP, which responds moderately to inflammatory stimuli in horses [32]. These authors concluded that the hemolysis that occurs due to induced splenic contraction from exercise elevates the production of hepatic HP. In this way, this protein can be used as a clinical indicator of erythrocyte fragility as well as a marked of the hemolysis that occurs in blood vessels during exercise. In the experimental model used in this work, there was a 23% increase in HP 5 minutes after the match.

While the serum concentrations of α1-AnT were not modified in ultramarathon athletes [8], our results demonstrated a large variation in the levels of this protein. Before the exercise, the attackers had an α1-AnT concentration of zero; 5 minutes after the match, they showed a significant increase in the level of this protein. Further studies are necessary to elucidate the interaction of the α1-AnT-exercise-acute phase response triad.

Alpha-1 acid glycoprotein, classified as a moderate APP in horses, was constant at all times assessed. One possible explanation for this finding is that increases in the levels of this protein are probably related to chronic processes [3].

In the present study, one biomarker was identified only as a "23-kDa protein." Despite the lack of information on the sequence of this protein, the 23-kDa protein has been described as a monomer of the α chain of HP in cattle [33]. In the present study, this protein had a high correlation with ALB and TRANS, although more studies are necessary to elucidate the true biological role of this protein.

5. Conclusions

It is important to point out a study in the literature that examined certain blood proteins and the inflammatory response in horses found moderate alterations in the serum protein profile related to APR after intense exercise. It is clear that this phenomenon occurred in the horses of the polo team studied, after participating in a match that demanded both the aerobic and anaerobic pathways, because the concentrations of the multiple components of the APR increased concomitantly, although not uniformly, in all the horses and positions. This paper contributes new findings on APR stimulated by this equestrian sport.

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