Research Article

Changes Induced by Exercise and Physical Training on Serum Biochemical Parameters in Arabian Horses after 15 kilometer running in a hot and dry climate

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Keywords
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Abstract

This study examines exercise-induced changes in serum biochemical parameters including calcium, phosphorus, sodium, potassium, chloride, magnesium, glucose, urea, creatinine, triglycerides, cholesterol, total protein, albumin, and total bilirubin, as well as serum enzyme activities such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in 22 Arabian horses aged 3 to 7 years. Blood samples were collected from the jugular vein one hour before (T0), immediately after (T1), six hours after (T2), and eighteen hours after (T3) a warm-up followed by maximum effort running over a 15-kilometer course. The obtained data were analyzed using one-way repeated measures ANOVA. For pairwise comparisons between measured values at each time point and baseline (T0), the Bonferroni corrected test was used. In all statistical comparisons, a p-value of <0.05 was considered significant. The results showed that the serum concentrations of urea, creatinine, triglycerides, calcium, phosphorus, and total bilirubin exhibited significant changes over time ($p < 0.05$). The results show that the mean concentrations of glucose, triglycerides, cholesterol, urea, phosphorus, sodium, chloride, and the serum activities of ALT, AST, and LDH (higher in mares), and magnesium (higher in stallions) were significantly different between sex ($p < 0.05$). Moreover, the results indicate that the mean concentrations of total protein and the mean serum activities of ALT, AST, ALP, GGT, and CPK were significantly different between age groups ($p < 0.05$).

Abbreviations

CPK: Creatine phosphokinase
LDH: Lactate dehydrogenase

GGT: gamma-glutamyl transferase
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase

Introduction

The Arabian horse is one of the oldest and most influential (and popular) horse breeds in the world [1]. Due to the considerable economic benefits associated with racing Arabian horses, monitoring disorders resulting from intense exercise and physical activity, as well as diseases that impact the performance of these horses, hold particular importance in this breed [1]. When racehorses display poor performance, blood sampling is performed to assess their health status and conduct biochemical tests [2]. Since biochemical parameters are influenced by exercise and physical training, blood collection at different time points can reveal the pattern of changes in serum parameter concentrations over time [2].

Horses are animals used in various sports such as racing, jumping, and equestrian competitions, requiring a high level of physical fitness. Regular and well-planned training can improve their physical abilities and athletic performance. However, intensive and prolonged training may lead to physiological and biochemical changes in the horses' bodies that could affect their health and performance. The ability of the horse to adapt to the physiological changes occurring during competition is an essential factor influencing its athletic success or failure [3].

The decrease in horses’ performance ability during exercise and physical activity is often due to changes in biochemical parameters. Therefore, measuring physiological exercise parameters in racehorses is of special importance for the optimal use of training programs [4].

The biochemical and physiological responses of horses during and after exercise are critical in equine sports medicine and veterinary practice. Blood biochemical markers, particularly those related to metabolism, muscle performance, and electrolyte balance, serve as important indicators for assessing athletic fitness, diagnosing exercise-related disorders, and optimizing training regimens [5,6,7].

Breed-specific differences can significantly influence these responses. For example, studies in Arabian horses have demonstrated distinct patterns of sweating and biochemical adaptations during endurance racing compared with other breeds such as the Žemaitukai horse [8,9]. Such findings highlight the importance of considering the horse's breed background when interpreting exercise-induced changes in serum parameters. In addition, factors such as sex, age, and environmental conditions have been reported to affect the dynamics of biochemical markers, including muscle enzymes (CK, LDH, AST), liver-related metabolites, electrolytes, and serum proteins [6,7,10]. Horses competing in hot and dry climates may be particularly vulnerable to dehydration and electrolyte imbalance, which can impair thermoregulation, metabolic processes, and athletic performance.

The present study was designed to investigate the effects of time (at multiple post-exercise sampling points), sex, and age on serum biochemical and electrolyte parameters in Arabian horses subjected to a 15-kilometer run under hot and dry climatic conditions. The findings are expected to contribute to the development of more precise training protocols and health monitoring strategies for this valuable breed. It is assumed that there is a correlation between changes in serum biochemical parameters and the intensity of physical training, which could help trainers assess the health status of their horses and adjust training programs according to their physical condition. Since all the horses in this study were kept in Yazd city, characterized by a hot and dry climate, environmental conditions may influence thermoregulation and energy expenditure [11]. Therefore, the effects of climate and weather were also considered in the present study.

Results

The results of the present study are presented in Tables 1 to 3. The concentrations of urea, creatinine, calcium, phosphorus, triglycerides, and total bilirubin showed significant changes over time as a result of physical exercise ($p < 0.05$), with these changes illustrated using line graphs at different time points (Figures 1-6).

A significant increase in the concentrations of total bilirubin and creatinine was observed from the pre-exercise to immediately after exercise ($p < 0.05$) (Figures 3 & 6). conversely, decreased calcium concentrations and increased phosphorus levels after exercise did not exhibit any significant changes compared to the pre-exercise stage . From time point T1 (immediately after exercise) to T2 (six hours post-exercise) there was a noticeable upward trend in calcium concentration, while phosphorus and creatinine concentrations demonstrated downward trend during the same period (Figures 3-5).

Total bilirubin concentration did not show a significant difference between T1 and T2 (Figure 6). Creatinine levels at T2 and T3 decreased compared to T1 and approached baseline levels (T0), resulting in no significant differences when compared to pre-exercise values (Figure 3).

Total bilirubin levels significantly increased immediately after exercise (T1) ($p < 0.05$) but then decreased again at T2 and T3, nearing the values observed at T0 (Figure 6).

Although time had a significant effect on the concentrations of calcium, urea and triglyceride ($p < 0.05$), no significant differences were observed between the various sampling times. An insignificant increase in phosphorus concentrations at T1 was followed by a significant decrease ($p < 0.05$) at T2, and then a non-significant increase at T3 (Table 1).

Table 1.
The Concentration of measured variables (Mean ± SE) in the serum of Arabian horses before and after exercise. In each row, values with different superscripts are significantly different ($p < 0.05$).

Parameters	Unit	Before exercise (T_0)	Immediately after exercise (T_1)	6h after exercise (T_2)	18h after exercise (T_3)	P-value
Glucose	mg/dl	91.4±1.7	92.2±2.4	93.2±1.7	92.7±1.7	0.74
Triglyeride	mg/dl	38.6 ± 2.75^{a}	45.3 ± 4.4^{a}	36.5 ± 2^{a}	43.4 ± 3^{a}	0.01
Cholestrol	mg/dl	72.1±2.5	73.2±2.5	74±2.2	74±2.4	0.39
Total Protein	mg/dl	6.6±0.1	6.7±0.1	6.7±0.1	6.7±0.1	0.65
Albumin	mg/dl	3.1±0.4	3.1±0.45	3.1±0.5	3.1±0.3	0.27
Urea	mg/dl	34.8 ± 1.15^{a}	35.5 ± 1.2^{a}	37.9 ± 1.2^{a}	35.5 ± 1.1^{a}	0.02
Creatinine	mg/dl	1.05 ± 0.05^{a}	1.2 ± 0.05^{b}	1.05 ± 0.05^{a}	1.05 ± 0.06^{ab}	0.008
T.Bili	mg/dl	1.05 ± 0.05^{a}	1.13 ± 0.04^{b}	1.12 ± 0.05^{ab}	1.1 ± 0.04^{ab}	0.05
ALT	U/L	9.4±0.95	9±1.15	11.1±1.9	12.2±2.5	0.17
AST	U/L	279.2±19.2	293.7±21.5	286.9±16.6	273.8±17.8	0.35
ALP	U/L	364.5±20.1	372.7±20.5	367.8±20.8	369.2±22.3	0.64
GGT	U/L	23.5±1.1	23.1±1.3	22.5±0.9	23.2±1.1	0.58
LDH	U/L	215.5±12.7	234.3±15.3	247.4±19.5	245.6±23.3	0.17
CPK	U/L	200.1±41.9	265.3±68.9	232.6±52.8	187.9±25.6	0.142
Calcium	mg/dl	10.9 ± 0.1^{a}	10.4 ± 0.1^{a}	11 ± 0.2^{a}	10.9 ± 0.1^{a}	0.01
Phosphorus	mg/dl	3.9 ± 0.1^{ab}	4.2 ± 0.2^{a}	3.7 ± 0.2^{b}	4 ± 0.1^{ab}	0.001
Magnesium	mg/dl	2.45±0.1	2.45±0.1	2.5±0.1	2.4±0.1	0.57
Sodium	mEq/L	136.8±1.3	138±1.4	136.8±1.2	137.2±1.2	0.86
Potassium	mEq/L	3.8±0.07	3.7±0.07	3.8±0.15	3.9±0.15	0.62
Chloride	mEq/L	99.4±0.9	99.5±1	100.1±0.8	99.8±0.8	0.86

Upon visiting the equestrian clubs, data regarding each horse — including age, sex, weight, and administered medications — were recorded. The mean and median ages of the horses in this study were 4 years. The mean, median, minimum, and maximum ages for stallions were 4.3, 4, 3, and 7 years, respectively, and for mares were 3.6, 3, 3, and 6 years, respectively.

The results also show that the mean concentrations of glucose, triglycerides, cholesterol, urea, phosphorus, sodium, and chloride, as well as the mean serum activities of ALT, AST, and LDH, were significantly higher in mares than in stallions ($p < 0.05$). In contrast, the mean serum concentration of magnesium was significantly higher in stallions compared to mares ($p < 0.05$) (Table 2).

Table 2.
The concentration of measured variables (Mean ± SE) in the serum of Arabian racehorses for different sexes.

Parameters	Unit	Stallion (n=14)			Mare(n=8)			P-value
		Mean ± SE	Min	Max	Mean ± SE	Min	max	
Glucose	mg/dl	89.6±1.6	73	110	97.2±2.1	82	112	0.01
Triglyeride	mg/dl	36.6±3.1	21	80	48.6±4.1	24.5	111	0.029
Cholestrol	mg/dl	68.6±2.3	46.5	91	81.5±3.1	68	100	0.003
Total Protein	mg/dl	6.7±0.1	5.8	7.7	6.6±0.1	5.5	7.7	0.698
Albumin	mg/dl	3.1±0.04	2.55	3.56	3.1±0.05	2.68	3.47	0.479
Urea	mg/dl	34.4±1.2	22.5	41.5	38.6±1.5	28	51	0.042
Creatinine	mg/dl	1.1±0.6	0.37	1.6	1.1±0.8	0.47	1.62	0.855
T.Bili	mg/dl	1.2±0.05	0.76	1.79	1±0.06	0.69	1.41	0.061
ALT	U/L	7.7±1.6	1.26	18.1	15.2±2.2	3.26	61	0.012
AST	U/L	255.5±19.5	166	374	332.1±25.8	173	578	0.028
ALP	U/L	354.9±25.9	196	497	392.3±34.3	257	604	0.395
GGT	U/L	22.2±1.2	14.5	35.5	24.7±1.6	12.6	38.5	0.23
LDH	U/L	208.9±17.6	134	352	282.6±23.2	166	658	0.02
CPK	U/L	157.5±53.1	69.5	444	342.6±75.1	113	1399	0.059
Calcium	mg/dl	10.8±0.1	8.9	12.5	10.7±0.1	9.5	12.1	0.703
Phosphorus	mg/dl	3.7±0.15	2.57	5.2	4.3±0.2	3.09	6.4	0.038
Magnesium	mg/dl	2.6±0.12	1.59	3.62	2.15±0.15	1.85	2.56	0.03
Sodium	mEq/L	135.5±0.8	118	148	140.1±1.1	124	149	0.003
Potassium	mEq/L	3.8±0.07	2.9	5.1	3.7±0.09	2.3	5.2	0.237
Chloride	mEq/L	98.4±0.5	88	107	102±0.7	92	108	0.00

Moreover, the results indicate that the mean concentrations of total protein and the mean serum activities of ALT, AST, ALP, GGT, and CPK were significantly higher ($p < 0.05$) in the age group of less than 4 years compared to the group aged 4 years and older. Conversely, the mean concentration of creatinine in horses younger than 4 years was significantly lower than in horses aged 4 years and older ($p < 0.05$) (Table 3).

Table 3.
The concentration of measured variables (Mean ± SE) in the serum of Arabian horses for different age groups

Parameters	Unit	< 4 years old (n=10)			≥ 4 years old (n=12)			P-value
		Mean ± SE	Min	Max	Mean ± SE	Min	max	
Glucose	mg/dl	94.3±2.1	75.5	112	90.8±1.9	73	111	0.246
Triglyeride	mg/dl	46.4±3.8	24.5	111	36.4±3.4	21	73	0.067
Cholesterol	mg/dl	75.6±3.3	46	100	71.3±3	52	87	0.36
Total Protein	mg/dl	6.9±0.1	5.9	7.7	6.5±0.1	5.5	6.8	0.008
Albumin	mg/dl	3.1±0.05	2.67	3.48	3.1±0.04	2.55	3.56	0.614
Urea	mg/dl	35.8±1.5	22.5	48.5	35.9±1.4	26	51	0.959
Creatinine	mg/dl	0.95±0.06	0.37	1.38	1.2±0.05	0.56	1.62	0.013
T.Bili	mg/dl	1.02±0.06	0.71	1.25	1.17±0.05	0.69	1.79	0.077
ALT	U/L	14±2	1.33	61	7.4±1.8	1.26	17.1	0.024
AST	U/L	333.3±21.2	173	578	241.8±19.4	166	391	0.005
ALP	U/L	428.9±25.3	314	604	318.2±23.1	196	483	0.004

GGT	U/L	25.4±1.3	14.5	38.5	21.1±1.2	12.6	28	0.025
LDH	U/L	266.6±22	141	658	210±20.1	134	361	0.073
CPK	U/L	332.2±64.4	100	1399	134.5±55.7	69.5	248	0.032
Calcium	mg/dl	10.7±0.13	9.2	12.1	10.9±0.12	8.9	12.5	0.183
Phosphorus	mg/dl	4.1±0.2	2.82	6.4	3.8±0.2	2.57	5.2	0.228
Magnesium	mg/dl	2.3±0.15	1.96	3.35	2.6±0.15	1.59	3.62	0.134
Sodium	mEq/L	138.6±1.2	124	149	136±1.1	118	146	0.113
Potassium	mEq/L	3.8±0.1	2.5	5.2	3.8±0.1	2.3	4.9	0.873
Chloride	mEq/L	100.6±0.8	90	107	99±0.7	88	108	0.127

Discussion

Studies show that exercise and physical activity in horses can affect certain serum biochemical parameters, which are associated with factors such as individual differences, breed, and exercise intensity [12].

In this study, a decrease in calcium concentration between T0 and T1 was observed, though it was not statistically significant (Figure 4). The binding of calcium ions (Ca^{2+}) to phosphate ions, albumin, and lactate can be considered one reason for this decreased calcium amount. Furthermore, ionized calcium enters actively working muscles for storage in the sarcoplasmic reticulum, which could also account for the observed decrease in calcium levels [13]. Other studies suggest hypocalcemia could also result from continuous calcitonin activity or severe sweating during physical activity [14]. In a study by Martins et al. on 24 Brazilian Mangalarga horses, a slight decrease in calcium concentration was observed after physical activity in stallions, attributed either to calcium loss through sweating or to calcium influx into muscle cells for binding to troponin molecules, facilitating actin-myosin interaction for muscle contraction during exercise [15].

In the present study, The overall effect of time is significant for calcium ($p < 0.05$), but calcium concentrations do not show a significant difference between the post-exercise sampling time (T1,T2,T3) and the pre-exercise time (T0). Similarly, in a study by Inoue et al. on changes in serum minerals and electrolytes during exercise in Thoroughbred horses over five days using a treadmill, the effect of time on calcium concentration was significant ($p < 0.05$), but no significant difference was found between pre-exercise and immediately post-exercise calcium levels [16].

Grimston et al. demonstrated that exercise in humans increases blood calcitonin levels, and this increase is associated more with hypercalcemia-induced stimulation rather than dehydration [17]. Another human study found that increased calcitonin could partially prevent hypercalcemia caused by dehydration [18].

In the present study, no increase in calcium concentration was observed immediately after exercise (T1); instead, a non-significant decrease compared to pre-exercise levels (T0) was noted. Additionally, Inoue et al. suggested that hypocalcemia during exercise could be attributed to sweating, which significantly ($p < 0.05$) counteracts blood concentration-induced hypercalcemia [16]. Kanungo et al. evaluated the intermittent effects of exercise on serum metabolites in racehorses under hot and humid conditions, finding that calcium concentration changes at intervals of 30 minutes, four hours, and eight hours after exercise were not significantly different [19]. Similarly, Zobba et al. observed a downward trend in calcium concentration following intense exercise in ponies, which returned close to baseline values within 30 minutes after exercise [20].

In this study, phosphorus concentrations showed a trend of increase immediately after physical exercise (T1) compared to pre-exercise levels (T0), although this change was not statistically significant (Figure 5). Previous research on Thoroughbred horses indicated a significant rise in phosphorus levels five minutes post-exercise [21]. This increase may be linked to phosphate release from skeletal muscles during the breakdown of high-energy phosphate stores, such as ATP [22, 23]. Studies on Arabian horses participating in endurance races revealed significant increases in phosphorus levels immediately after the race compared to pre-race level, with further studies revealing similar trends in phosphorus concentration just before and after races of varying distances [24]. In the current study, the non-significant increase in phosphorus at T1 was followed by a significant decrease at T2 (six hours post-exercise), corroborating findings from earlier literatures [24]. Additionally, other studies have noted a non-significant increase in serum phosphorus immediately after exercise, which aligns with the present findings regarding phosphorus changes from T0 to T1 [10].

In this study, mean serum creatinine concentrations remained within the normal range [25] at all time points but increased significantly ($p < 0.05$) immediately after exercise (T1) compared to pre-exercise (T0). This rise was followed by a significant decrease ($p < 0.05$) at T2 (six hours after exercise), likely due to rehydration during rest, and continued to decline at T3, approaching baseline (Figure3). The initial increase in creatinine at T1 is consistent with pre-renal azotemia secondary to reduced blood volume from excessive sweating [26, 27]. The subsequent decline and return toward baseline suggest that other causes, such as renal failure or exertional rhabdomyolysis, are unlilely [3]. Similar patterns have been reported in various equine exercise studies. Poskiene et al. observed a 21-30% post-race increase in creatinine in 112 endurance horses, followed by a decline [8]. In Standardbred horses competing over 1600-2000 m, creatinine rose significantly ($p < 0.05$) up to 60 min post-race, then decreased [6]. Thoroughbreds showed peak creatinine 5 min after a 1600 m race, normalizing within 60 min [21]. In endurance races 0f 65-120 km, creatinine increased by about 19% post-race and decreased by 13% within 12-18 hours [24]. Conversely, a short-distance study in Standardbreds found no significant change 48-72 h after exercise, supporting the view that creatinine returns to baseline within days [2]. The present findings align with these observations, showing a transient post-exercise creatinine rise followed by recovery within 18 h, reflecting hydration changes rather than persistent renal dysfunction.

In the present study, mean serum urea concentrations remained within the normal range [25] at all sampling times. Although an upward trend was observed immediately after exercise (T1) and persisted at six hours post-exercise (T2), these increases were not statistically significant, and values declined toward baseline from T3 (Figure 2). Such transient, non-significant rises may be related to mild dehydration, hemoconcentration from sweating, or increased protein catabolism for energy during exercise [4]. Similar patterns have been reported in previous studies on horses subjected to varying exercise intensities. In ponies without regular training, urea increased significantly ($p < 0.05$) post-exercise and returned rapidly to baseline, whereas trained ponies showed smaller changes; this was attributed to fluid loss and reduced renal blood flow [28]. Endurance race studies in Arabians, Zemaitukai, warmbloods, and mixed breeds consistently demonstrated post-exercise elevations in serum urea, sometimes reaching about 40%, particularly after longer distances. These increases have been linked to both dehydration-related hemoconcentration and adaptive renal mechanisms that enhance urea reabsorption to support water and sodium conservation during prolonged exertion [9]. Taken together, the literature suggests that post-exercise urea changes-whether significant or not- are generally short-term, reflecting hydration status and metabolic protein turnover rather than persistent renal dysfunction. The present findings, showing only mild, non-significant fluctuations, are consistent with these physiological responses.

In this study, mean triglyceride concentrations stayed within the normal range [25], with a slight, non-significant rise immediately post-exercise (T1) followed by a decline at six hours (T2) and a modest, non-significant increase at 18 hours (T3) (Figure 1). Such fluctuations likely reflect transient changes in lipid mobilization for muscle energy supply [5]. Previous equine studies show variable findings: some report significant post-exercise increases, particularly after prolonged or high-intensity phases such as cross-country or 120-km endurance rides [5, 24], while others found no significant increases after shorter endurance events or standardized training [7, 29]. Collectively, these results suggest that exercise-related changes in triglycerides are influenced by exercise type, duration, and intensity, and in most cases remain transient without exceeding physiological limits.

In the present study, mean total bilirubin concentration remained within the normal range [25], but a significant increase ($p < 0.05$) occurred immediately post-exercise (T1) compared to baseline (T0), with no further significant changes at six (T2) or 18 hours (T3) post-exercise (Figure 6). Such transient rises are consistent with previous reports showing marked bilirubin elevations after both short-distance races and prolonged endurance events, often returning toward baseline within hours [10,21, 24]. In endurance horses, increases of 60-184% have been observed within hours after competition, followed by partial recovery within 12-18 hours [24], whereas Thoroughbreds in sprint races showed rapid normalization within 60 minutes [21], These changes are attributed to factors including hemoconcentration from fluid loss, reduced renal clearance, and increased bilirubin production from enhanced hemolysis during exercise, with hydration status inversely correlated with bilirubin concentration [24]. Collectively, evidence suggests that post-exercise hyperbilirubinemia in horses is a reversible physiological response to physical activity, influenced by exercise duration, intensity, and hydration rather than an indicator of persistent hepatic dysfunction.

Differences in serum biochemical parameters between stallions and mares after training were observed in the present study. Regarding the effect of sex on parameters measured before and after exercise, the following possible reasons can be suggested to researchers for future studies: The lower concentrations of glucose, triglycerides, cholesterol, and phosphorus in stallions during exercise may be due to their more efficient energy metabolism, such that the consumption of these variables by muscle cells occurs at a greater rate and with higher efficiency. The lower urea concentration in stallions may be related to more efficient protein anabolism. The lower activities of transaminases (ALT and AST) and lactate dehydrogenase

(LDH) in stallions may result from better muscular adaptability during exercise, reducing leakage of these enzymes into the bloodstream. The higher sodium and chloride concentrations in mares following exercise may be due to greater dehydration experienced by mares during physical activity. The reason for the higher magnesium concentrations in stallions remains unknown.

There are also differences between serum biochemical parameters in horses under four years of age compared to horses four years of age and older following exercise. Regarding the effect of age on the parameters measured before and after exercise, the following possible reasons can be suggested to researchers for future studies: total protein concentrations were higher in horses younger than four years compared to those aged four years or older, which is likely related either to greater protein production in the body or to greater dehydration. Creatinine concentrations in horses younger than four years were lower than in horses older than four years, which may be attributed to smaller muscle mass or higher renal clearance in this age group. Higher serum activities of ALT, AST, and CPK in horses under four years of age are likely associated with greater muscle damage during exercise and the leakage of these enzymes into the bloodstream in younger horses. ALP activity was also higher in horses younger than four years, probably due to growing animals in this age range. The higher GGT activity in horses younger than four years compared to those older than four years has no clearly identified cause.

In conclusion, this study highlights the significant biochemical alterations in serum parameters of Arabian horses following a 15-kilometer run in hot and dry conditions. Assessing the effect of time, sex and age on serum biochemical parameters alongside electrolyte levels, provides important insights into the physiological responses induced by exercise. Our findings emphasize the importance of monitoring biochemical markers at multiple time points post-exercise to gain a comprehensive understanding of their dynamics and implications for equine health and performance. This research contributes to the broader field of equine exercise physiology, facilitating the development of tailored training regimens that optimize the well-being and athletic capabilities of Arabian horses. However, the need for further studies regarding the duration and type of training in horses of this breed is still felt.

Materials and Methods

Horses and Physical Training

In this study, 22 apparently healthy Arabian horses (14 stallions and 8 mares) aged between three and seven years were selected from two equestrian clubs located in the Ashkezar and Zarach regions of Yazd Province. All of the horses in this study are indigenous Arabian horses and have the Arabian horse markings, which is a sign of their authenticity. All horses had similar conditions in terms of diet and training protocols. Yazd is one of the three main centers for Arabian horse breeding in Iran, characterized by a hot and dry climate. Each horse underwent a thorough physical examination by a specialist in internal medicine to ensure its health status throughout the study, including history-taking and clinical examination. It should be noted that the horses at these breeding centers were maintained under a unified vaccination and deworming program. During this study, none of the mares were pregnant. All horses were housed individually in stalls where the ambient temperature ranged between 25–27°C, and uniform photoperiod conditions were maintained during the summer (sunrise at 5:30 a.m. and sunset at 5:45 p.m.). The horses' diet was formulated according to the National Research Council (NRC) Nutritional Requirements table (1989). Their diet consisted of dry alfalfa hay (approximately 1.5% of body weight) and concentrate feed (approximately 1% of body weight). The horses were trained under conditions similar to racing six days a week, with one day of rest. Training sessions began at sunrise (5:30 a.m.) and lasted two hours. All horses followed a standardized daily training program, although the trainer could adjust the training regimen for individual horses based on age, physical ability, and performance. However, the overall duration and intensity of training were generally similar among all horses. The training included 30 minutes of warming up (Warm-Up), 45 minutes of walking (Pacing), 20 minutes of trotting, and 25 minutes of cantering at a moderate pace. The total distance covered during training was approximately 15 kilometers, with an average speed during the canter of about 12 meters per second.

Blood Sampling

All stages and procedures of this study followed the ethical standards of the 1975 Helsinki Declaration (revised in 2000 and 2008). The study was also approved under national regulations (Ethics Code: IR.UM.REC.1400.219).

Blood was collected from the jugular vein of each horse at four-time points:

- Before the start of exercise and physical activity (T0)
- Immediately after exercise and physical activity (T1)
- Six hours post-exercise (T2)
- Eighteen hours post-exercise (T3)

Sampling was carried out in the summer season from June 22 to August 22, 2019, in Yazd province. Blood was collected into tubes without anticoagulant and centrifuged at 2000g for 20 minutes. The serum was separated within 20 minutes after blood collection and transferred to a freezer at −20°C. The serum samples were transported from Yazd to Ferdowsi University of Mashhad's Faculty of Veterinary Medicine using a nitrogen tank at −196°C and subsequently stored at −80°C. The concentrations of glucose, urea, triglycerides, cholesterol, creatinine, total bilirubin, calcium, phosphorus, total protein, albumin, sodium, potassium, chloride, and magnesium, as well as the serum activities of ALT, AST, ALP, GGT, LDH, and CPK, were measured using commercial kits produced by Pars Azmoon Company (Tehran, Iran) and an Alpha Classic refrigerated biochemical autoanalyzer (Esfahan, Iran). The concentrations of sodium, potassium, and chloride were measured using an ion specific electrode device (STARLYTE Ⅲ, Alpha Wasserman, Netherlands). The accuracy of biochemical parameter measurements was ensured using control serum (Centronorm, Centronic GmbH Ltd., Wartenberg, Germany).

Statistical Analysis

Data analysis was performed using SPSS statistical software version 16. One-way repeated measures ANOVA was used to compare the concentrations of parameters across consecutive time points. When significant differences were found, pairwise comparisons were conducted using the Bonferroni corrected test. The effects of time, group (age and sex), and time-group interaction were assessed. Changes in each parameter over time and among age and sex groups were considered statistically significant at $p < 0.05$.

References

1. Ropka-Molik K, Stefaniuk-Szmukier M, Musiał A, Velie BJI. The genetics of racing performance in Arabian horses. Int J Genomics. 2019;2019(1):9013239.

2. Bos A, Compagnie E, Lindner AJ. Effect of racing on blood variables in Standardbred horses. J Vet Clin Pathol. 2018;47(4):625-8.

3. Ghadrdan Mashhadi AR, Razi Jalali M, Fatemi Tabatabaei SR, Arjmand Nezhad M. The effect of exercise on concentration of some serum biochemical parameters in Arabian horses. J Vet Clin Pathol. 2018;12:145-55.

4. Pourmohammad R, Mohri M, Seifi H, Sardari K. Effect of exercise on some minerals, metabolites and enzyme activities in the serum of trained Arabian horses. Turk J Vet Anim Sci. 2019;43(6):791-9.

5. Rose RJ, Ilkiw JE, Arnold KS, Backhouse J, Sampson D. Plasma biochemistry in the horse during 3-day event competition. Equine Vet J. 1980;12(3):132-6.

6. Piccione G, Casella S, Giannetto C, Monteverde V, Ferrantelli V. Exercise-induced modifications on haematochemical and electrophoretic parameters during 1600 and 2000 meters trot races in Standardbred horses. J Appl Anim Res. 2009;35(2):131-5.

7. Kedzierski W, Bergero D, Assenza A. Trends of hematological and biochemical values in the blood of young race horses during standardized field exercise tests. Acta Vet. 2009;59(5-6):457-66.

8. Poškienė I, Gruodytė R, Autukaitė J, Juozaitienė V, Antanaitis R. Speed and blood parameters differ between Arabian and Žemaitukai horses during endurance racing. Animals (Basel). 2021;11(4):995.

9. Poškienė I, Juozaitienė V, Antanaitis R. A comparison of haematological and biochemical blood indices between the Žemaitukai and Arabian horses participating in endurance competitions. Acta Vet Brno. 2021;90(2):159-69.

10. Kupczyński R, Śpitalniak K. Analysis of acid–base balance as well as hematological and biochemical parameters in horses of combined driving discipline. Arch Anim Breed. 2015;58(1):221-8.

11. Backhouse J. The implications of heat generation and electrolytes in feed and competition in athletic horses. Asian-Australas J Anim Sci. 2000;13(3):394-9.

12. Padilha FGF, Dimache LAG, Almeida FQ, Ferreira AMR. Blood biochemical parameters of Brazilian sport horses under training in tropical climate. Rev Bras Zootec. 2017;46:678-82.

13. Vervuert I, Coenen M, Wedemeyer U, Chrobok C, Harmeyer J, Sporleder HP. Calcium homeostasis and intact plasma parathyroid hormone during exercise and training in young Standardbred horses. Equine Vet J. 2002;34(7):713-8.

14. Allaam M, Elseady Y, Nayel M, Elsify A, Salama A, Hassan H, et al. Physiological and hemato-chemical evaluation of Thoroughbred race horses after exercise. Int J Anim Vet Med Sci. 2014;8(3):81-93.

15. Martins LP, Di Filippo PA, Meireles MAD, Peçanha RMS, Macêdo Mello L, Ribeiro LMF, et al. Effect of marcha exercise on serum electrolytes and acid–base balance in Mangalarga Marchador horses. J Equine Vet Sci. 2017;49:108-12.

16. Inoue Y, Osawa T, Matsui A, Asai Y, Murakami Y, Matsui T, et al. Changes of serum mineral concentrations in horses during exercise. Asian-Australas J Anim Sci. 2002;15(4):531-6.

17. Grimston SK, Tanguay KE, Gundberg CM, Hanley DA. The calciotropic hormone response to changes in serum calcium during exercise in female long distance runners. J Clin Endocrinol Metab. 1993;76(4):867-72.

18. Aloia JF, Rasulo P, Deftos LJ, Vaswani A, Yeh JK. Exercise-induced hypercalcemia and the calciotropic hormones. J Lab Clin Med. 1985;106(3):229-32.

19. Kanungo S, Pradhan C, Babu L, Behera K, Palei A, Jena B, et al. Effect of periodicity of exercise on serum metabolites of stall-housed competition horses under climatic conditions of Odisha. Int J Curr Microbiol Appl Sci. 2018;7:3338-47.

20. Zobba R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, et al. Physical, hematological, and biochemical responses to acute intense exercise in polo horses. J Equine Vet Sci. 2011;31(9):542-8.

21. Hassan HY, Aly MA, Elseady Y, Nayel MA, Elsify AM, Salama AA, et al. The effect of race on clinical, hematological and biochemical biomarkers in Thoroughbred horses. Eur J Psychol. 2015;11(3).

22. Yamada MO, Minami T, Ichii M, Okazaki Y, Utsumi M, Tohno S, et al. An improved method for estimating original mineral contents in excavated bone using sulfur. Biol Trace Elem Res. 1996;52:155-61.

23. Arslan S, Erdem S, Sivri A, Hasçelik Z, Tan E. Exercise-induced apoptosis of rat skeletal muscle and the effect of meloxicam. Rheumatol Int. 2002;21:133-6.

24. Larsson J, Pilborg P, Johansen M, Christophersen M, Holte A, Roepstorff L, et al. Physiological parameters of endurance horses pre- compared to post-race, correlated with performance: a two race study from Scandinavia. ISRN Vet Sci. 2013;2013(1):684353.

25. Latimer KS. Duncan and Prasse's veterinary laboratory medicine: clinical pathology. 5th ed. Ames (IA): Wiley-Blackwell; 2011.

26. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th ed. San Diego (CA): Academic Press; 2008.

27. Nelson RW, Couto CG. Small animal internal medicine. 6th ed. St. Louis (MO): Elsevier Health Sciences; 2019.

28. Soroko M, Śpitalniak-Bajerska K, Zaborski D, Poźniak B, Dudek K, Janczarek I. Exercise-induced changes in skin temperature and blood parameters in horses. Arch Anim Breed. 2019;62(1):205-13.

29. Klobučar K, Vrbanac Z, Gotić J, Bojanić K, Bureš T, Brkljača Bottegaro N. Changes in biochemical parameters in horses during 40 km and 80 km endurance races. Animals (Basel). 2019;9(10):742.

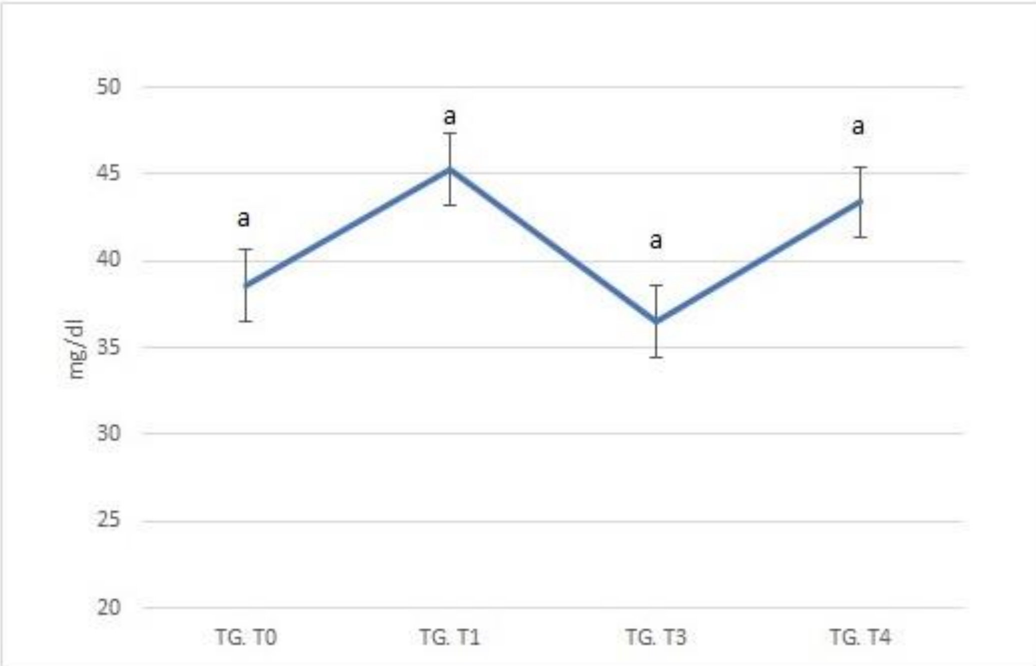

Figure 1. Triglyceride Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

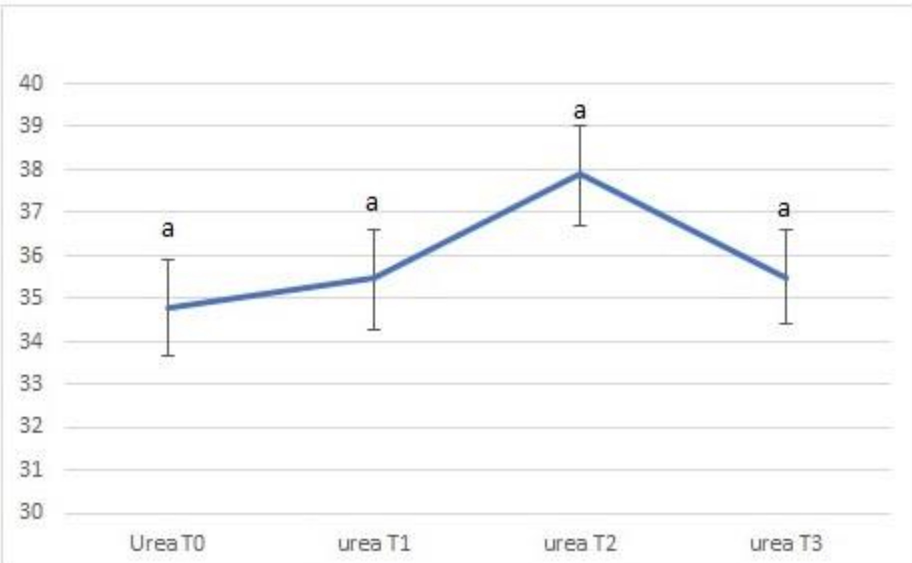

Figure 2. Urea Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

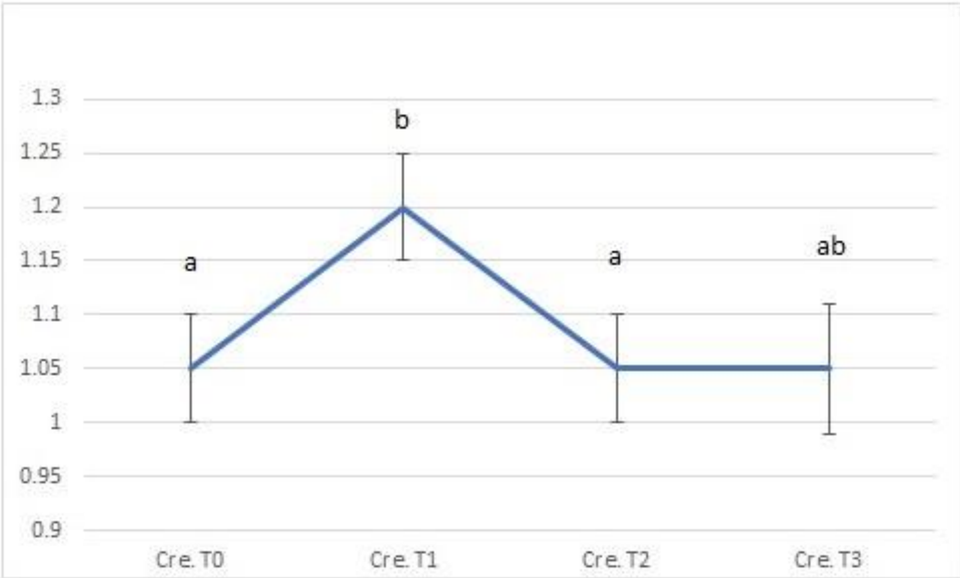

Figure 3. Creatinine Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

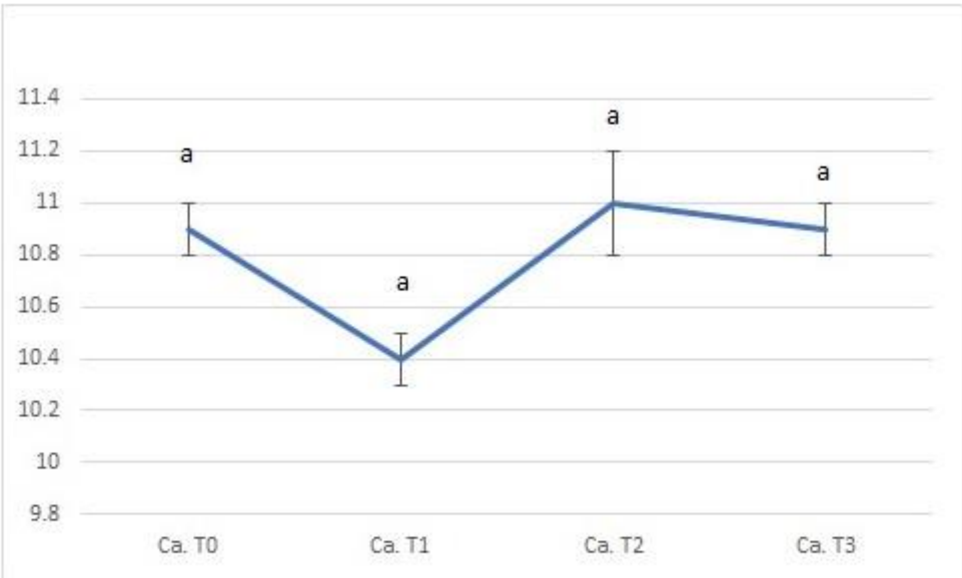

Figure 4. Calcium Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

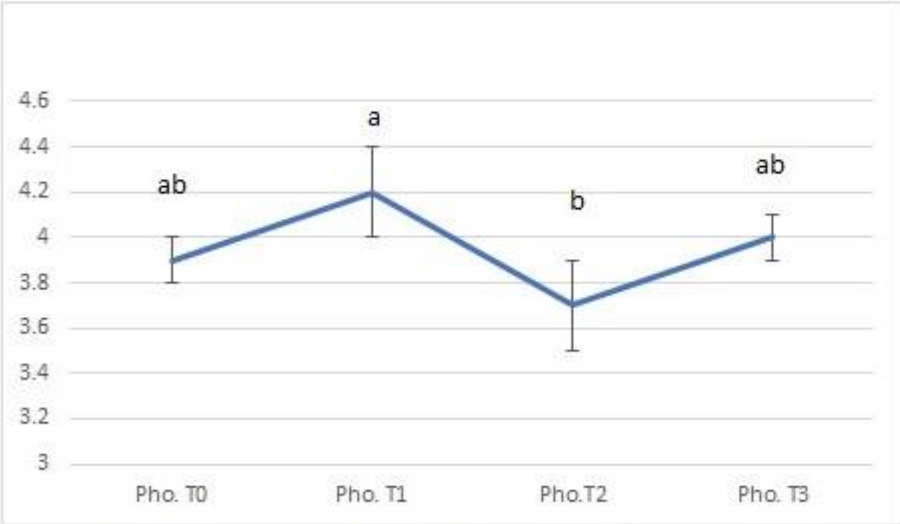

Figure 5. Phosphorus Values (Mean ± SE) in the serum of Arabian horses before and after exercise.

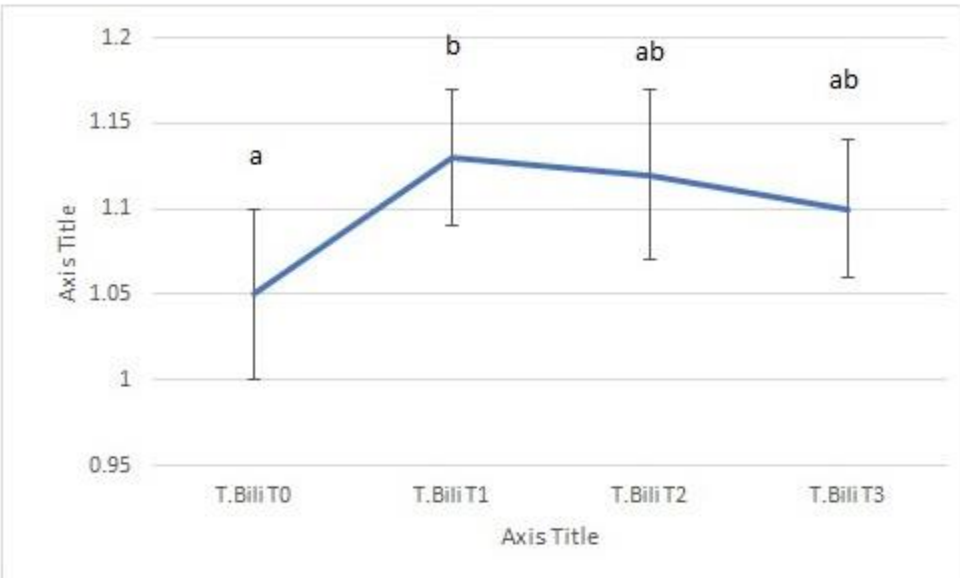

Figure 6. Total Bilirubin Values (Mean ± SE) in the serum of Arabian horses before and after exercise.