Effects of running speeds on some markers of muscular tissues and synovial fluid of Iraqi Arabian Horses

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Summary

Arabian horses are regarded as the most famous breed for speed for long distances due to their genetic superiority. The aims of the study were to focus on the effect of different running speeds on the muscular tissues (Myoglobin, creatine kinase, aspartate transaminase), lactate dehydrogenase and synovial fluid (creatine kinase, aspartate transaminase and lactate dehydrogenase). Non-trained eight local Iraqi Arabian horses were run on soil track for a distance (1) Km, walking, trot, canter, and gallop. Blood and synovial fluid samples were collected before and after running. The biochemical parameters of serum showed gradual increase according to each type of speed. All of them were significant at P≤0.01. The biochemical parameters of the synovial fluid also showed gradual increase according to each type of speed. Creatine kinase was significant at P≤0.05 in walking and trot but significant at P≤0.01 in canter and non-significant in gallop. While aspartate transaminase, and lactate dehydrogenase were significant at P≤0.01 at all speeds.

Keywords: Running, Speeds, Muscular tissue, Synovial fluid, Iraqi Arabian horses.

Introduction

Arabian horses are regarded the most famous speed breed for long distances due to their genetic superiority. The competing long – distances endurance rides under hard environment might lead to a lot of muscular disorders. The fundamental approach to diagnos muscle diseases in horses was based on a thorough history, careful physical examination, complete blood count, and serum biochemical profile (1). Intensive training of horses, on the border of their physical endurance, as well as extremely high effort during the races, could cause a number of pathological changes in the body, manifested in the form of so-called overtraining syndrome. It was noted that horses subjected to highly intensive training with short breaks for rest between training activity rounds achieve poor results. Unfortunately, the reasons for this situation were not sufficiently explained (2). Elevation in plasma/serum myoglobin concentrations indicated acute muscle damage. Normal serum concentrations in resting horses had been determined by nephelometry range= 0 – 9 µg/l, and measured concentrations from horses with rhabdomyolysis range from 10,000-800,000 µg/l (3 and 4). Daily training might result in persistent increase in serum creatine kinase (CK) (5), and resting (CK) values were higher in athletes (6 and 7). However, the marked increase in (CK) that occur after exercise were usually lower in trained compared with untrained subjects (8 and 9). Authors (10) in their study reported that, thoroughbreds were more likely to have high plasma (CK) activity when exercised at the trot or intervals of walk, trot and canter, compared with exercising at the gallop. One explanation for this might be that the development of rhabdomyolysis increases with exercise of prolonged duration. In muscle, CK function is making adenosine tri phosphate (ATP) available for contraction by the phosphorylation of adenosine phosphate (ADP) from creatine phosphate (11). Detection of intermittent large increases in serum or plasma (CK) and aspartate transaminase (AST) activity after exercise in horses could help clinicians in diagnosis the syndrome of exercise – associated muscle necrosis, commonly referred to as exertional rhabdo-myolysis (12). Serum skeletal muscle enzymes or proteins are markers of the functional status of muscular tissue, and vary widely in both pathological and physiological conditions. An increase in these enzymes might be an index of cellular necrosis or tissue damage following acute and chronic muscle injuries (13-15). Other researchers (16) conducted a study on Arabian horses in Brazil, peak levels, significantly different, were recorded,

immediately after rides the level of (CK) (413.591±50.75), 24 hours post rides lactate dehydrogenase (LDH) (628.61±33.30), and 48 hours after rides (AST) (389.89±16.96). Normal synovial fluid of domestic animals contains many enzymes, Alkaline and acid phosphatase, and lactic dehydrogenase (LDH) were present in detectable quantities. The ratios of enzyme level in the synovial fluid to their levels in the serum in same animal vary according to the presence and severity of a particular pathological condition (17-20). The aims of this research were to study the effect of different running speeds on some muscular (MG, CK, AST, and LDH) and synovial fluid (CK, AST, and LDH) enzymes in Iraqi Arabian horses.

Materials and Methods

The study was conducted on 8 non-trained local Iraqi Arabian horses in the Arabian horses center, University of Al-Qadisyah. Their ages 5 to 6.5 years old, two stallions and six mares. All animals were kept on same feeding (grains, alfalfa and hay) with water *ad libitum*. They were housed in individual stalls in the stables, providing with bedding of straw. Clinical examination was done for one week apparently were healthy, they had no previous exertional rhabdomyolysis and their history were recorded. All horses were run on soil track for a distance about 1 Km, each different hors speed was studied for 3 days interval (walking 3-5 km/hr – trot 20 km/hr- canter 30 km/hr – gallope 50 km/hr). The speed of running was calculated by dividing the distance on the time measured by sport time calculator. Blood samples were taken from the jugular vein, before and after 6 hours of speeds by using 18-gauge sterile needles and syringes. Blood sera were separated by centrifugation to collect serum. Also synovial fluid samples (2-3 ml) were collected from the left mid- carpal joint before and after each speeds, all sites of arthrocentesis were aseptically prepared. All samples transfer to EDTA vacutainer tubes and send to laboratory analysis. (MG), (CK), (AST) and (LDH) were measured by Reflotron® plus (Roche Diagnostics GmbH Mannheim Germany) using commercial reagent kits (Roche Diagnostic GmbH Sandhofer Strasse 116D-68305 Mannheim, Germany). The results were analyzed statistically by using (T- student test) at level P≤ 0.01 and P≤ 0.05 (21).

Results and Discussion

The strenuous physical exercise was one of the most common causes of muscle damage which led to disability in affected horses. The design of the study included many degrees of running speeds, walking, trot, canter, and gallop. (Table, 1 and 2) were included to be compared with our results.

Table, 1: The normal mean values ± SE of MG, CK, AST, and LDH of the serum.

Parameters	Mean values± SE	References
MG (g/dl)	4.25±0.12	(22)
CK (U/L)	148.61±9.30	=
AST (U/L)	93.26±11.00	=
LDH (U/L)	109.21±11.00	=

Table, 2: The normal mean values± SE of CK, AST, and LDH of the synovial fluid.

Parameters	Mean values± SE	References
CK(U/L)	Less than 148.61±9.3	(22)
AST(U/L)	62.34±3.20	=
LDH(U/L)	179.23±12.90	=

The biochemical parameters of serum MG, CK, AST, and LDH showed clear gradual increase according to the increase of the speed. All the results were significant at P≤0.01 as showed at (Table, 3), the mean values ± SE of MG, CK, AST, and LDH before walking were 4892. ± 1059.3, 242.475 ± 9.612, 349.125 ±7.021, and 453.925 ± 9.172 respectively, and after walking were 5513.125 ± 1020.720, 330.837 ± 13.035, 359.887 ± 6.612, and 467.275 ± 11.210 respectively. Also the mean values±SE of MG, CK, AST, and LDH before trot were 5027.250 ±9 94.407, 255.287 ± 6.536, 360.437 ± 7.414 and 466.562 ± 9.068 respectively and after trot were 7676.125 ± 1042.170, 497.475 ± 28.600, 373.600 ± 7.378, and 483.300 ± 10.536 respectively. And the mean values± SE of MG, CK, AST, and LDH before canter were 4971.125 ± 985.299, 261.875 ± 5.657, 372.062 ± 8.107 and 480.712 ± 7.676 respectively, and after canter were 9567.125 ± 1016.432, 659.262 ± 40.875, 388.650 ± 6.514 and 498.225 ± 10.530 respectively. While the mean values± SE of MG, CK, AST, and LDH before gallop were 5034.750 ± 1006.786, 264.012 ± 6.359,

387.050 ± 7.306 and 501.437 ± 8.393 respectively, and after gallop were 11308.00 ± 1012.767, 956.637 ± 46.232, 427.987 ± 13.598 and 536.950 ± 12.896, respectively.

Table, 3: The mean values±SE of biochemical parameters of serum (MG, CK, AST and LDH) at different speeds.

Sample collection time	Walking				Trot				Canter				Gallop			
	MG g/l	CK u/l	AST u/l	LDH u/l	MG g/l	CK u/l	AST u/l	LDH u/l	MG g/l	CK u/l	AST u/l	LDH u/l	MG g/l	CK u/l	AST u/l	LDH u/l
Before	4892 ± 1059.3 A	242.4 ± 9.6 B	349.1 ± 7.0 A	453.9 ± 9.1 A	5027.2 ± 994.4 A	255.2 ± 6.5 B	360.4 ± 7.4 A	466.5 ± 9.0 A	4971.1 ± 985.2 B	261.8 ± 5.6 A	372.0 ± 8.1 A	480.7 ± 7.6 A	5034.7 ± 1006.7 A	264.0 ± 6.3 B	387.0 ± 7.3 B	501.4 ± 8.3 B
After	5513.1 ± 1020.7 A	330.837 ± 13.035 A	359.8 ± 6.6 A	467.2 ± 11.2 A	7676.1 ± 1042.1 A	508.7 ± 20.9 A	373.6 ± 7.3 A	483.3 ± 10.5 A	9567.1 ± 1016.4 A	659.2 ± 40.8 B	388.6 ± 6.5 A	498.2 ± 10.5 A	33808 ± 2237.4 A	981.1 ± 46.5 A	427.9 ± 13.5 A	536.9 ± 12.8 A

All results are significant at P<0.01.

It was believed that this increase was due to direct damage of the gluteus medius muscle which was exposed to gradual elevation of physical work; this would lead to direct damage of the muscle membrane and might lead to leakage of intracellular muscle components into the extracellular fluid. Serum skeletal muscle enzymes or proteins were markers of the functional status of muscle tissue, and widely in both pathological and physiological conditions. An increase in these enzymes might be an index of cellular necrosis or tissue damage following acute and chronic muscle injuries (13 and 15).

After exertion, myoglobin might increase within 30 minutes (22), and remain increase for 5 days; probably due to low-grade inflammation (23) so that, each type of horses running speeds were studied on three days interval in this research and myoglobin was a useful marker to monitor the effectiveness of work load on muscle tissue in training (24). The time of CK release into and clearance from plasma depended primarily on the level of training, type, intensity, and duration of exercise. Peak serum CK approximately two fold above baseline occurred 8 hours after strength training (25), while the decrease in serum enzymes depended on the period of rest after exercise, as short-term physical inactivity might reduce both the lymphatic transport of CK and its release from the muscle fibers (26).

The results of our study showed that, AST activities increased significantly immediately after muscular exertion, It was believed this might be due to the muscular necrosis of gluteus medius muscle. As well, this increase might remain for 24 hours. Also (27) mentioned that AST increase was also related to the duration of exercise. Exercise induced a significant increase in LDH (28 and 29). The degree of increase depended on the intensity and duration of the effort (30-33). These results agreed with our results as showed in (Table, 3), this might be due to muscular necrosis.

After a prolong endurance exercise, the activities of LDH were doubled and remained in an increase for 2 weeks (34). An eccentric bout of exercise induced much greater increase in serum enzyme activity than a concentric bout, and LDH increased between the third and fifth day after exercise (35 and 36). Enzymes were released into the synovial fluid from damaged tissue during phagocytosis and from the general circulation about the inflamed joint.

The results of (Table, 4) showed that, the mean values± SE of MG, AST, and LDH before walking were 222.825 ± 9.701, 327.125 ± 9.028 and 424.200 ± 11.280 respectively, and after walking were 245.775 ± 12.092, 344.513 ± 9.752 and 438.850 ± 11.663 respectively. Also the mean values±SE of CK, AST and LDH before trot were 235.863 ± 6.295, 338.875 ± 10.073 and 434.688 ± 10.412 respectively, and after trot were 263.950 ± 14.417, 367.313 ± 14.233 and 463.012 ± 10.971 respectively, and the mean values±SE of CK, AST and LDH before canter were 238.138 ± 5.980, 351.375 ± 8.743 and 459.538 ± 6.782 respectively, and after canter were

286.663 ± 19.242, 391.438 ± 11.828 and 509.388 ± 13.213 respectively. While the mean values±SE of CK, AST and LDH before gallop were 246.425 ± 9.191, 362.875 ± 10.090 and 481.150 ± 14.679 respectively, and after gallop were 406.300 ± 90.886, 425.988 ± 18.020 and 547.100 ± 20.505 respectively.

Table, 4: The mean values ± SE of biochemical parameters of synovial fluid (CK, AST and LDH) at different speeds.

Sample collection time	Walking			Trot			Canter			Gallop		
	CK u/l	AST u/l	LDH u/l	CK u/l	AST u/l	LDH u/l	CK u/l	AST u/l	LDH u/l	CK u/l	AST u/l	LDH u/l
Before	222.825 ± 9.701 A	327.125 ± 9.028 B	424.200 ± 11.280 B	235.863 ± 6.295 A	338.875 ± 10.073 B	434.688 ± 10.412 B	238.138 ± 5.980 B	351.375 ± 8.743 B	459.538 ± 6.782 B	246.425 ± 9.191 C	362.875 ± 10.090 B	481.150 ± 14.679 B
After	245.775 ± 12.092 A	344.513 ± 9.752 B	438.850 ± 11.663 B	263.950 ± 14.417 A	367.313 ± 14.233 B	463.012 ± 10.971 B	286.663 ± 19.242 B	391.438 ± 11.828 B	509.388 ± 13.213 B	406.300 ± 90.886 C	425.988 ± 18.020 B	547.100 ± 20.505 B

A: Significant at P<0.05. B: Significant at P<0.01. C: Non- significant.

The results of (Table, 4) showed that, the mean value±SE of CK, AST and LDH in synovial fluid were slightly below their limits in serum and this might be due to the degree of permeability of the joint capsule but there was a gradual increase according to each type of running speed. It was believed that this might be due to the gradual severity and increase of permeability of joint capsule. These results were accompanied with the results of (37). CK is significant at P≤0.05 in walking and trot, significant at P≤0.01 in canter but non-significant in gallop, while AST and LDH were significant at P≤0.01 in all speeds. A gradual increase of running speed might lead to increase the joint capsule permeability. Our research was the first study in our country. It focused on the effect of running speeds on some important muscular and synovial enzymes in Iraqi Arabian horses. Its results were very useful for future researches that might be about other pathological changes specially those of the joint structures which would have direct effect on the horse performance, in addition their usefulness in preparation (training) programs design of sports horses.

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تاثير السرع المختلفة في بعض مؤشرات الأنسجة العضلية والسائل المفصلي في الخيول العربية العراقية

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الخلاصة

تعد الخيول العربية النسل الأسرع في العالم في المسافات الطويلة بسبب تفوقها الوراثي. تهدف الدراسة إلى تسليط الضوء لتاثير السرع المختلفة على بعض مؤشرات العضلات والسائل المفصلي. أجريت الدراسة على ثمانية خيول عربية عراقية غير مدربة تم جريها على أرضية ترابية لمسافة 1 كم مسير و خبب وهذب وحضر. اخذت نماذج الدم والسائل المفصلي قبل وبعد الجري. بينت المعايير الكيموحيوية لمصل الدم زيادة تدريجية بحسب درجة السرعة وكانت جميعها ملحوظة إحصائياً بمستوى 0.01، أما المعايير الكيموحيوية للسائل المفصلي فقد أظهرت أيضاً زيادة تدريجية بحسب درجة السرعة وكان انزيم الكرياتين كاينيز ملحوظاً احصائياً بمستوى 0.05 بسرعة المسير والخبب ولكنه ملحوظ احصائياً بمستوى 0.01 عند الهذب وغير ملحوظ احصائياً عند الحضر. بينما كانت معايير انزيم الاسبرتيت ترانسامنيز وهرمون لاكتيت ديهيدروجنيز ملحوظة إحصائياً بمستوى 0.01 بجميع درجات السرع.

الكلمات المفتاحية: جري، سرع، نسيج عضلي، سائل مفصلي، خيول عربية عراقية.