Ankara Üniv Vet Fak Derg
47, 223-235, 2000

EFFECTS OF THE EXERCISE ON THE PERFORMANCE AND THE PLASMA BIOCHEMICAL VALUES OF GERMAN SHEPHERD DOGS FED WITH HIGH FAT DIET SUPPORTED WITH L-CARNITINE

Abdülkerim DENİZ[1] *Hamdi UYSAL*[2]
Serdar KARDEŞ[3] *Engin KIRMIZI*[4]

Egzersizin L-karnitin katkılı yüksek yağ diyeti ile beslenen Alman çoban köpeklerinin performansı ve plazma biyokimyasal değerleri üzerine etkisi

Özet: *Bu çalışmada, yaz mevsiminin sıcak günlerinde yapılan yürüyüş şeklindeki egzersizin L-karnitin katkılı yüksek yağ diyeti ile beslenen Alman çoban köpeklerinin performansı ve plazma biyokimyasal değerleri üzerine etkisi araştırıldı. Bu amaçla köpekler saatte 6,6 km hızla birinci hafta 7,5 km, ikinci hafta 15 km, üçüncü hafta ise 38 km'lik mesafelerde 5 gün süreyle yürütüldüler. Köpeklerin bir ve ikinci haftalardaki 7,5 ve 15 km'lik mesafeleri rahatlıkla yürümelerine rağmen, 16 adet köpekten 3 adedinin son 38 km'lik mesafe yürüyüşünü tamamlayamadıkları gözlendi. Yürüyüş egzersizlerinin öncesi ve sonrasında yapılan ölçümlerde biyokimyasal kan parametreleri yönünden farklılıklar bulundu. 15 km'lik mesafede yapılan yürüyüş egzersizleri süresince kan trigliserid konsantrasyonunun L-karnitin (100 mg/kg canlı ağırlık) ile desteklenen grupta düştüğü gözlendi. Bundan başka düşük egzersiz yoğunluğuna bağlı olarak gelişen kas hasarı durumunda CK aktivitesinin AST'ye göre daha duyarlı olduğu tespit edildi. 38 km mesafe yürüyüşünün üçüncü gününde performans yetersizliği görülen ve yürüyüşü tamamlayamayan 3 adet köpekte hemokonsantrasyon, CK, AST ve LDH'nın artan aktiviteleri ile kırmızı renkli kan plazması (myoglobinemi) gözlendi. Alman çoban köpekleri 7,5 ve 15 km'lik mesafeleri rahatlıkla yürürken, 38 km'lik mesafeyi yetersiz performansları nedeniyle % 35 protein ve % 25 yağ içeren bir diyet ile beslenmelerine rağmen 5 günden fazla yürüyemedikleri saptandı. Sonuç olarak önerilebilirki, hematokrit değeri ile laktat konsantrasyonu ve CK, AST, LDH aktiviteleri uzun mesafe egzersizleri yapan köpeklerin genel durumunu belirlemek için iyi bir gösterge olabilir.*

Anahtar kelimeler: *Egzersiz, kan parametreleri, köpek, L-karnitin, performans*

Summary: *In this study, effects of the exercise on the performance and the plasma biochemical values of German Shepherd dogs fed with high fat diet*

1. Dr., Vetifarm Veterinary Pharmaceuticals Products, Cumhuriyet Cad. 229/1, Harbiye, Istanbul, Turkey.
2. Dr., Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry, 06110, Ankara, Turkey.
3. Dr., Uludağ University, Department of Animal Nutrition, Faculty of Veterinary Medicine, Bursa, Turkey.
4. Dr., Gemlik Dog Breeding and Training Centre, Bursa, Turkey.

supported with and without L-carnitine was investigated at daily warm temperatures of the summer season. The dogs were walked to 7.5 km, 15 km and 38 km distances for five days at a speed of 6.6 km/h in the first, the second, and the third weeks of exercises programme respectively. They were completed to walk succesfully at 7.5 and 15 km distances. However, dogs were started to fail in walking to the last 38 km distance of exercising programme as three dogs were dropped out of sixteen dogs.

Measurements of the biochemical parameters before and after exercises were found to be different at all the distances of 7.5 km, 15 km and 38km. The triglyceride concentrations of the blood during the exercises at a 15 km distance were decreased in the group of dogs suplementated with L-carnitine (100mg/kg body weight). Moreover, CK activity was found to be more sensitive than AST activity for the low exercise-intencity induced muscle damage. In the third day of the exercises at a 38 km distance, hemoconcentration, increased activities of CK, AST and LDH and a red coloured blood plasma (myoglobinemia) were observed in three retired dogs with performance failure. As a result, German Shepherd dogs were able to walk happily at 7.5 and 15 km distances but, their body performance was not enough to walk such 38 km distances at least for five days with a diet containing 25 % of fat and 35 % of protein. Finally, it would be suggested that measurements of hematocrit value, lactate concentration and CK, AST and LDH activities can be useful markers for monitoring the general condition of exercising dogs in long distances.

Key words: *Blood parameters, dog, exercise, L-carnitine, performance*

Introduction

Proper diet is among the more important considerations in health maintenance and is essential in the management of body performance. There are various publications about performance and serum biochemical changes in the dogs during long distance training in a cold weather. These reports showed that feeding is very important in such long distance training of dogs (13). To complete such a long and heavy training, dogs were fed with high fat and protein diets to intake required metabolizable energy (13), and to increase free fatty acids mobilisation and utilization for the production of energy (22). Diets may also cause the pre- and postexercise changes on some serum parameters (22,15,23). Exercise-induced hematological and biochemical changes were reported for many serum paremeters (3,10,9,11). These reports indicated that the stress of prolonged and stroneuous running may induce physiologic changes and that affect serum biochemical values (22,10,9). Reports about the training of dogs pointed out that strong training can cause the death and dropped of dogs from training (9). There are contradictory reports about the changes on the blood parameters of retired and died dogs during long distance race. In one study about the training of dogs, strong training may cause the death and dropped of dogs without any changes on the blood biochemical values in the long distance race of the dogs (9). However, significant differences were found between some biochemical values of sled dogs that completed a long distance race and dogs that did not completed in an other research (14).

L-carnitine is a derivative of amino acids (18), and it is an important compound required for the transport of long chain fatty acids into mitocondrial matrix for β-oxidation (4,19). The effects of L-carnitine on the serum biochemical parameters during exercise are asserted in such increase of the aerobic capacity due to glycogen sparing effect, delaying and onset of fatigue and hypoglycemia and decreasing production of lactic acid (20). The other reports also show that L-carnitine causes better utilisation of lipids in dogs during exercise (6). Most of the exercise related researches in dogs were performed on Alaskan Husky dogs at temperature below zero.

The purpose of this study reported here was to investigate the performance and changes in plasma biochemical parameters in German Shepherd dogs supported with- and without L-carnitine during long distance walking exercises at warm temperatures of the summer season. This study was necessary to performe due to observed performance failure of German Shepherd dogs used for several purposes, like long patrolling along the boarders of Turkey for tracing illegal movements, by the Turkish army.

Materials and Methods

This study was performed at Gemlik Military Dog Breeding and Training Centre in Bursa/Turkey. Sixteen German Shepherd dogs were used in the study. These dogs were 17-24 months old and in equal sex dispersal. All dogs were inspected clinically, hematologically and biochemically in a veterinarian clinic. They were all found to be healthy to performe this study.

Feeding

The dogs were fed with a commercial extruded dog food. This diet contained 35% of protein, 25% of fat, 22% of carbohydrate, 7.5% moisture. Four weeks before the exercise periods start, the dogs fed with this extrude dog food for a adaptation and reaching a good condition. L-carnitine and the exercise was not applied to the dogs during the adaptation time of animals for the food. 100 mg L-carnitine /kg body weight (Carniking, Charce No. 0000131; Lonza AG, Basle) were mixtured with a small amount of feed to give 8 dogs (group 1) during exercise periods. Therefore, L-carnitine was provided separately from the daily diet of the dogs. The food of other 8 dogs were not supported with L-carnitine as control group (group 2). The dogs were fed for 2 hours before and after the exercise. Feeding time of the dogs was 20 minutes in each add libitum feeding period. Plenty of water was provided for the dogs in each 40 minutes during exercise periods.

Exercise programme of the dogs

In the first and the second week of the exercise periods, the dogs were walked on a plain ground from Monday to Friday for 5 days at 7.5 and 15 km distances respectively. In the third week of the exercise period, the dogs were walked on the same ground from Monday to Wednesday for three days at 38 km. The dogs were rested for five minutes in each 40 minutes of exercise period. At the weekends of each exercise programme of different distances, the dogs were rested without having L-carnitine in the food.

Analysis of the blood

Blood samples were taken three times a week from the cephalic vein at the beginning of exercise, and 40 minutes after the exercise of each distance into tubes containing EDTA and Lithium Heparin. The plasma samples for biochemical measurements were collected from the tubes containing blood mixed with Lithium Heparin after centrifugation at 10000g for 15 minutes. The blood in EDTA tubes was used for the measurements of the erytrocyte counts, hematocrit values and hemoglobine concentrations. The erytrocytes were counted manually, and hematocrit values and hemoglobine concentration were measured using by QBC-Vet autoreader (idexx GmbH). The biochemical blood parameters (aspartate aminotransferase, creatine kinase, lactate dehydrogenase, alkaline phosphotase activities, glucose, urea, albumine, triglycerides, total protein, creatinine, cholesterol, and total bilirubine concentration) were measured in plasma using an autoanalyzer

Vettest 8008 (idexx GmbH). Lactate concentrations were measured using an Accusport Lactat Messer (Boehringer Mannheim) calibrated before use in the whole blood collected directly by a injection of a cephalic vein using an injector. Daily temperature measurements were performed everyday during the exercise periods.

Statistical analysis

Significance of differences between pre- and postexercise was determined using by Wilcoxon Matched-Paires Signed-Ranks test. The mean values of the biochemical parameters in each pre-and postexercises between three exercise periods were compared with one-way ANOVA, and in the presence of existed significance, duncan- test was used to identify spesific differences between three exercise periods. Comparison of mean values of total protein, cholesterol, alkaline phosphatase, creatinine, and total bilirubine concentrations between 15 and 38 km exercises in each groups supported with and without L-carnitine was performed using Mann-Whitney U-Wilcoxon Rank Sum W Test.

Results

The daily temperatures were between 20 - 26 °C during exercise periods of this study. Dogs were completed the exercise at an average speed of 6.6 km/h. They were found to be successful in completing the 7.5 and 15 km exercise periods for five days in a week. Although all dogs were completed 38 km exercise period successfully first two days of the week, thirteeen dogs were only managed to complete 38 km exercise period in the third day of the week. A dog (the diet was supported with no L-carnitine, Dog No. 5) retired at the 29th km of the last 38 km exercise period. The other two dogs (the diet was supported without no L-carnitine (Dog No. 15), and supported with L-Carnitine (Dog No. 3) were also retired at the 26th km of the last 38 km exercise period. These 3 dogs that retired from last exercise of 38 km showed high body temperatures (41-42 °C) with certain clinical signs such as polypnoe and limping due to possible myopathy. Appetite of the Dogs were decreased markedly (70% in comparison to sedentary period) during the last exercise days of 38 km. The water requirements of the dogs were also decreased in the last two - three days of the 38 km exercise period.

Results of the analysis of the plasma for the biochemical parameters during the long distance exercises of the dogs are presented in tables 1-4. Significant changes were observed in the postexercise CK, LDH and urea values amongst 7.5, 15 and 38 km exercise periods. Postexercise CK values of 38 km distance was found to be increased ($P<0.01$) in comparison to the 7.5 and 15 km exercise distances in both groups 1 and 2 (see tables 1, 2 and 3). Postexercise LDH activities of first experimental group at 38 km distance were higher ($P<0.05$) than 7.5 and 15 km distances. However, postexercise urea values were found to be lower ($P<0.001$) in the first group when the exercise distances are increased each week (see tables 1, 2 and 3). ALP and total protein values of groups 1 and 2 after the 38 km distance exercises were found to be significantly decreased ($P<0.05$) when compared with 15 km distance exercises (see tables 2 and 3). The plasma biochemical parameters of dogs retired from training during last 38 km exercise were given in table 4.

Unfortunately, statistical comparison of the results of the dogs retired from the exercises with other exercise completed dogs at the last 38 km was not possible as their number (n=3) was not enough. As seen in table 4, the postexercise triglyceride concentrations (1.19 to 1.75 fold) of these dogs were high. Triglyceride concentration in all dogs was tended to decrease during the 15 km (significant in group 1) and 38 km exercises (not significant). However, plasma triglyceride concentrations of these three dogs tented to increase markedly at the time when they retired. The postexercise erytrocyte caunt, hemoglobin concentration and hematocrit value of these three dogs were found

Table 1. A comparison of pre- and postexercise hematological and biochemical values (mean±SE, minimum and maximum values) of dogs in the L-carnitine supplemented (group 1) and non L-carnitine supplemented (group 2) groups during 7.5 km exercise period.

Tablo 1. 7.5 km'lik egzersiz periyodunda L-karnitin ile beslenen (grup 1) ve beslenmeyen (grup 2) köpeklerin egzersiz öncesi ve sonrasındaki hematolojik ve biyokimyasal değerlerinin (ortalama ± standart sapma, minumum ve maksimum değerler) karşılaştırılması.

	Group 1 (n=8)			Group 2 (n=8)		
	Preexercise	Postexercise	P Value	Preexercise	Postexercise	P Value
RBC $x10^9/\mu l$	6.13 ± 0.26 (4.93-7.06)	6.38 ± 0.37 (5.08-7.92)	>0.05	5.72 ± 0.16 (5.2-6.28)	5.97 ± 0.19 (5.3-6.68)	>0.05
HMG g/dl	15.71 ± 0.48 (13.4-18.1)	15.62 ± 0.41 (13.5-17.5)	>0.05	15.22 ± 0.51 (13.2-16.8)	15.02 ± 0.49 (12.8-16.5)	>0.05
Hematocrit %	49.38 ± 1.46 (41.7-55.4)	48.37 ± 1.14 (41.9 -55.4)	>0.05	46.63 ± 1.38 (40.2-50.8)	45.67 ± 1.48 (38.3-50.4)	>0.05
AST IU/L	28.77 ± 3.97 (13-47)	31.13 ± 2.09 (22.3-39.6)	>0.05	27.03 ± 2.42 (17.3-41.3)	32.75 ± 4.07 (16.6-45.0)	>0.05
CK IU/L	92.28 ± 2.87 (79.3-107.3)	129.33 ± 10.94 (86.66-183.0)	**0.0117**	103.7 ± 15.33 (59.66-196.3)	139.08 ± 24.4 (70-252.7)	**0.0117**
LDH IU/L	155.0 ± 5.03 (141.6-181)	170.30 ± 7.43 (148.6-205)	**0.0173**	156.22 ± 7.60 (107-178.6)	165.83 ± 9.68 (131.3-224.6)	>0.05
Urea mg/dl	20.33 ± 0.86 (16.8-24.1)	22.50 ± 1.02 (19.1-22.9)	>0.05	21.76 ± 1.30 (16-28.2)	22.28 ± 1.36 (16.8-28.1)	>0.05
Albumin g/dl	3.17 ± 0.05 (2.98-3.36)	3.12 ± 0.05 (2.98-3.35)	>0.05	3.12 ± 0.04 (2.96-3.3)	3.13 ± 0.04 (2.93-3.27)	>0.05
Glucose mg/dl	107.53 ± 2.01 (99.2-117.1)	102.03 ± 1.94 (94.3-107.9)	**=0.05**	104.41 ± 2.09 (94.7-112.5)	99.18 ± 2.58 (88.1-109.2)	**0.0117**
Lactate mmol/l	2.37 ± 0.14 (1.9-2.96)	1.93 ± 0.12 (1.33-2.36)	0.0117	2.12 ± 0.09 (1.76-2.46)	1.83 ± 0.09 (1.33-2.13)	**0.0117**
TRGL mg/dl	41.98 ± 4.06 (29.4-59.6)	45.27 ± 3.01 (36.3-62.5)	>0.05	37.72 ± 3.46 (27.6-51.1)	41,88 ± 3,14 (28.3-55.3)	>0.05

Abbreviations: RBC: red blood cells count, HMG: hemoglobin concentration, AST: aspartate aminotrasferase, CK: creatine kinase, LDH: lactate dehydrogenase, TRGL: triglyceride concentration.

Table 2. A comparison of pre- and postexercise hematological and biochemical values (mean±SE, minimum and maximum values) of dogs in the L- carnitine supplemented (group 1) and non L-carnitine supplemented (group 2) groups during 15 km exercise period.

Tablo 2. 15 km'lik egzersiz periyodunda L-karnitin ile beslenen (grup 1) ve beslenmeyen (grup 2) köpeklerin egzersiz öncesi ve sonrasındaki hematolojik ve biyokimyasal değerlerinin (ortalama ± standart sapma, minumum ve maksimum değerler) karşılaştırılması.

	Group 1 (n=8)			Group 2 (n=8)		
	Preexercise	Postexercise	P Value	Preexercise	Postexercise	P Value
RBC x10^9/μl	6.09 ± 0.19 (5.02-6.7)	6.25 ± 0.27 (5.03-7.4)	>0.05	5.88 ± 0.24 (5.03-7.08)	6.00 ± 1.18 (5.23-6.8)	>0.05
HGM g/dl	15.11 ± 0.43 (12.7-16.6)	15.18 ± 0.41 (12.9-16.4)	>0.05	15.23 ± 0.29 (13.7-16)	15.15 ± 0.37 (12.9-16.4)	>0.05
Hematocrit %	47.57 ± 1.25 (40-51)	47.32 ± 1.13 (40-49.7)	>0.05	46.47 ± 0.99 (40.2-49.3)	45.68 ± 1.17 (38.9-50.1)	>0.05
AST IU	22.13 ± 1.81 (17-31.6)	30.90 ± 2.76 (20-40)	**0.0117**	28.31 ± 8.39 (8-70.6)	41.70 ± 12.78 (18.6-127.6)	>0.05
CK IU/L	92.20 ± 4.28 (79.3-114)	133.45 ± 11.11 (89.7-167.7)	**0.0117**	81.33 ± 5.87 (56-115.7)	119.74 ± 6.71 (83-142)	**0.0117**
LDH IU/L	162.05 ± 12.9 (119.3-226.6)	172.30 ± 6.94 (146-202)	>0.05	180.56 ± 22.14 (133-331)	175.50 ± 14.60 (143-272)	>0.05
ALP IU/L	36.0 ± 1.83 (29-43)	35.12±1.77§ (31-46)	>0.05	42.75 ± 5.52 (19-63)	47.62 ± 6.19 (19-64)	**0.0425**
Urea mg/dl	18.48 ± 0.94 (12.5-21.2)	18.88 ± 1.07 (13.7-22.3)	>0.05	21.27 ± 1.51 (15.3-27.7)	22.28 ± 1.13 (17.4-27.9)	>0.05>
Albumin G/dl	3.13 ± 0.05 (2.93-3.25)	3.17 ± 0.05 (3.02-3.23)	>0.05	3.07 ± 0.05 (2.73-3.27)	3.12 ± 0.05 (2.9-3.31)	>0.05
Glucose mg/dl	110.86 ± 1.12 (105.2-114.2)	102.62 ± 1.09 (98.1-106.8)	**0.0117**	108.12 ± 1.89 (103.2-116.2)	99.96 ± 2.64 (85.5-110.6)	**0.0117**
Lactate mmol/L	2.35 ± 0.12 (1.93-3.0)	1.80 ± 0.09 (1.56-2.26)	0.0117	2.18 ± 0.12 (1.53-2.63)	1.80 ± 0.09 (1.46-2.16)	0.0117
TRGL mg/dl	58.35 ± 5.60 (34.1-78.3)	46.21 ± 4.15 (33.4-70.8)	**=0.05**	46.30 ± 4.81 (27.7-67.2)	39.88 ± 4.07 (23.6-57.2)	>0.05
Cholesterol mg/dl	206.6 ± 11.28 (177-261)	180.0 ± 9.5 (177-268)	>0.05	212.50 ± 9.74 (183-254)	211.62 ± 8.43 (183-239)	>0.05
Tot. Protein g/dl	6.41 ± 0.93 (6.1-6.8)	6.39 ± 0.13 (6.3-7.1)	>0.05	6.52 ± 0.20 (6.1-7.1)	6.48 ± 0.17 (5.6-7.0)	>0.05
Tot.Bilirub. mg/dl	0.04 ± 0.007 (0.03-0.13)	0.05 ± 0.09 (0.01-0.09)	>0.05	0.06 ± 0.01 (0.02-0.08)	0.06 ± 0.01 (0.02-0.08)	>0.05
Creatinine mg/dl	1.35 ± 0.65 (1.12-1.68)	1.35 ± 0.84 (1.06-1.75)	>0.05	1.39 ± 0.03 (1.25-1.54)	1.46 ± 0.07 (1.21-1.75)	>0.05

Table 3. A comparison of pre- and postexercise hematological and biochemical values (mean±SE, minimum and maximum values) of dogs in the L-carnitine supplemented (group 1) and non L-carnitine supplemented (group 2) groups during 38 km exercise period.

Tablo 3. 38 km'lik egzersiz periyodunda L-karnitin ile beslenen (grup 1) ve beslenmeyen (grup 2) köpeklerin egzersiz öncesi ve sonrasındaki hematolojik ve biyokimyasal değerlerinin (ortalama ± standart sapma, minumum ve maksimum değerler) karşılaştırılması.

	Group 1 (n=8)			Group 2 (n=8)		
	Preexercise	Postexercise	P Value	Preexercise	Postexercise	P Value
RBC x10⁹/µl	6.34 ± 0.51 (4.23-9.15)	6.42 ± 0.30 (5.0-7.87)	>0.05	6.22 ± 0.41 (5.33-8.85)	6.14 ± 0.20 (5.54-7.12)	>0.05
HGM g/dl	15.46 ± 0.46 (12.8-16.9)	15.20 ± 0.73 (11.6-18.5)	>0.05	15.57 ± 0.23 (14.9-18.2)	15.27 ± 0.49 (13.5-18.2)	>0.05
Hematocrit %	47.86 ± 1.35 (39.4-52)	46.76 ± 2.27 (35-55.90)	>0.05	47,12 ± 0.68 (44.9-49.6)	46.62 ± 1.42 (41.1-54.5)	>0.05
AST IU/L	26.00 ± 7.32 (6.5-70.5)	86.68 ± 33.31 (25.5-311.5)	**0.0117**	22,56 ± 4.94 (1-41.5)	52.62 ± 10.33 (4-84.5)	**0.0117**
CK IU/L	146.6 ± 52.86 (51.5-508.5)	484.8 ± 145.79 (95-1135.5)	**0.0117**	98.93 ± 15.06 (57-188)	837.81 ± 245.68 (116-3418)	**0.0117**
LDH IU/L	166.18 ± 7.07 (138-204)	397.8 ± 116.44 (171.5-1158.5)	**0.0117**	156.81 ± 14.38 (80-223.5)	484.81± 188.96 (201-1786)	**0.0117**
ALP IU/L	33.25 ± 2.24 (25-43)	23.87 ± 2.48 (18-40)	**0.0117**	35.37 ± 4.04 (18-53)	29.75 ± 3.35 (19-45)	>0.05
Urea mg/dl	15.10 ± 1.03 (9.6-22.2)	15.78 ± 0.67 (13.6-18.6)	>0.05	17.48 ± 1.55 (8.7-21.9)	19.66 ± 1.42 (12.1-23.9)	=0.05
Albumin g/dl	3.25 ± 0.11 (3.2-4.0)	3.16 ± 0.11 (2.9-3.6)	**0.0180**	3.38 ± 0.72 (3.1-3.7)	3.10 ± 0.85 (2.7-3.7)	0.0180
Glucose mg/dl	119.66 ± 1.92 (113-130.1)	102.23 ± 3.05 (87.4-114.3)	0.0117	117.72 ± 2.74 (106.6-130.3)	96.06 ± 5.54 (69.3-116.7)	0.0117
Lactate mmol/L	2.08 ± 0.91 (1.6-2.4)	1.83 ± 0.13 (1.3-2.35)	>0.05	1.98 ± 0.11 (1.6-2.45)	1.76 ± 0.132 (2.45-2.6)	>0.05
TRGL mg/dl	44.40 ± 4.56 (30.7-65.9)	41.96 ± 7.13 (22.6-1134)	>0.05	41.06 ± 3.64 (30.6-58)	35.77 ± 3.01 (27.2-51.10)	>0.05
Cholesterol mg/dl	206.75 ± 15.4 (168-303)	174.75 ± 14.75 (137-193)	**0.0117**	205.37 ± 9.64 (169-244)	172.12 ± 8.28 (143-207)	0.0117
Tot. Protein g/dl	6.10 ± 0.93 (5.7-6.4)	5.10 ± 0.13 (4.6-5.4)	**0.0117**	6.19 ± 0.10 (5.6-6.5)	5.20 ± 0.17 (4.5-6.1)	**0.0117**
Tot. Bilirub. mg/dl	0.07 ± 0.02 (0.0-0.1)	0.06 ± 0.02 (0.0-0.1)	>0.05	0.09 ± 0.01 (0.0-0.1)	0.06 ± 0.02 (0.0-0.1)	>0.05
Creatinine mg/dl	1.23 ± 0.32 (1.1-1.3)	1.16 ± 0.42 (1.0-1.3)	>0.05	1.24 ± 0.05 (1.0-1.4)	1.28 ± 0.13 (1.1-2.1)	>0.05

Table 4. Hematological and biochemical values of the dogs that retired from the last 38 km distance exercise period.
Tablo 4. Son 38 km mesafelık egzersiz periyodunu tamamlayamayan köpeklerdeki hematolojik ve biyokimyasal değerler

	Retired Dog (No. 3) supported with L-Carnitine		Retired Dog (No.5) supported without L-Carnitine		Retired Dog (No. 15) supported without L-Carnitine	
	Preexercise	Postexercise	Preexercise	Postexercise	Preexercise	Postexercise
RBC $x10^9/\mu l$	7.60	**12.2**	8.02	**11.20**	5.38	**5.90**
HGM g/dl	17.3	**21.9**	18	**20.9**	14.3	**15.0**
Hematocrit %	51	**64.9**	53	**61.5**	43.9	**48.2**
AST IU/l	93	**589**	64	**155**	16	**52**
CK IU/L	927	**2036**	104	**3418**	79	**677**
LDH IU/L	138	**2098**	209	**3432**	153	**401**
ALP IU/L	25	**20**	41	**37**	18	19
Urea mg/dl	7.6	**16.7**	13.8	**22.1**	4.9	**11.9**
Albumin g/dl	3.7	3.4	3.6	3.5	3.4	3.0
Glucose mg/dl	147.4	**106.2**	122.7	**37.9**	134.6	**124.1**
Lactate mmol/L	1.8	**3.7**	1.8	**3.5**	1.9	**2.1**
TRGL mg/dl	64.9	**113.4**	41.6	**50.8**	44.9	**53.4**
Cholesterol mg/dl	184	**156**	187	**166**	222	**184**
Tot. Protein g/dl	6.0	**5.2**	6.5	**6.1**	6.4	**5.4**
Tot. Bilirub. mg/dl	0.1	0.1	0.1	0.1	0.1	0.1
Creatinine mg/dl	1.2	1.3	1.3	1.4	1.1	1.0

Abbreviations: RBC: red blood cells count, HMG: hemoglobin concentration, AST: aspartate aminotrasferase, CK: creatine kinase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, TRGL: triglyceride concentration, Tot.Protein: total protein concentration, Tot.Bilirub: total bilirubine concentration to be high at the last 38 km (table 4). Moreover, there was no significant changes in erytrocyte counts, hemoglobin concentration and hematocrit values during all exercise periods of all dogs (tables 1, 2, 3). Interestingly, two of the dogs retired at the last 38 km after postexercise were shown higher amounts of hematocrit values seen in table 4. It was also noted that the color of plasma in these three retired dogs was rather red in comparison to the normal colored plasmas of the other thirteen dogs completed the 38 km distance exercises.

Discussion

In this study, effects of heavy training on the performance and the plasma biochemical paremeters of dogs fed with high fat diet supported with and without L-carnitine was investigated at daily warm temperatures. Used food for feeding the dogs contained 25% of fat, 35% of protein and 22% of corbohytrate. These ingredients of the food were also used for long distance training of the dogs by other researchers (13,21). The proportion of the fat and carbohydrates used differs markedly between trained and untrained individuals. In trained individuals, fat is the primary fuel, and muscle and hepatic glycogen depletion are slower than in untrained individuals (8).

The dogs were walked to 7.5 km, 15 km and 38 km distances in the first, the second and the third weeks of exercise programme respectively. Clinically and hematologically, there was no performance failure in the dogs in the first and second week of the exercise programme as they walked totally 113 km at 23 °C in average. However, three dogs were retired at the 26th and 29th km of the last 38 km exercises. Two retired dogs had a high postexercise erytrocyte count (11.2-12.2 _ 10^9/µl), hemoglobine concentration (20.9 - 21.9 g/dl) and hematocrit values (61.5 - 64.9%) (table 4), which were not appered so dramaticaly in other dogs during all three week exercises (tables 1, 2, 3, 4). Therefore, the hemaconcentration was not formed in thirteen dogs during all exercise periods apart from three retired dogs. Hinchcliff et al. (10) reported that erytrocyte count in sled dogs during 575 miles exercise at –7 to –30 °C was were also not changed. However, other researchers were indicated the decreased erytrocyte counts during a 300 miles exercise at –10 to –35 °C (11). In our study, the exercise distance was shorter than other reported studies, but the daily temparature in our study was quite higher than other studies. It could be speculated that the higher body temparatures (41-42 °C) measured in retired dogs may be result of the higher ambient temperatures during the exercises. For the thermoregulation of body temperature, loss of water in these dogs via respiratory, renal or saliva may cause the declined plasma volume. Dogs loose water through expired air and saliva during exercise (2). In addition, the running dogs for their large metabolizable energy rerquirements and due to their great water turnover may loose their water through respiratory and renal ways (12).

The total plasma protein concentrations in dogs decrease during long distance exercises in temperatures below 0 °C (6,8,18). This is presented by the increased urea concentration in plasma as result of amino acid oxidation during exercise (10). The dogs does not become dehydrated during the long distance race as they have decreased total plasma protein and creatinine concentrations, and not changed erytrocyte count (10). In our study, the plasma total protein and albumine concentrations were decreased significantly ($p<0.05$) in the group 1 and group 2 during 38 km exercises, however the creatinine concentration was not changed significantly (table 3). That may be a result of proteins used for energy during 38 km exercises. Generally, the results obtained from the three week exercise programme explained that the dogs have not shown hemaconcentration except two retired dogs. Study in horses during exercise training revealed an exercise induced hypervolamia (17). Moreover, a long distance running in horses results in hemaconcentration presented by increased erytrocyte counts and plasma total protein concentration (24). Although creatinine concentration was not changed in the plasma, the total protein concentration showed a decreasing tendency in three dogs retired during the last 38 km distance exercises, In spite of these results, increased erytrocyte counts and hematocrit values of these dogs pointed out the hemaconcentration. The total protein concentration may not have been increased because of the consumption of protein fractions such as albumine and globuline for energy requirements (11). Surprisingly, concentrations of urea in the postexercise measurements of 7.5 km, 15 km and 38 km exercises in group 1 were found to be decreased significantly ($p<0.001$). This may be explained by that L-carnitine promotes the excretion of urea via urination. Hinchcliff et al. (12) reported that dogs had a great water turnover during long distance training. This may be a reason for urea excretion via increased urination.

The concentration of plasma triglyceride was not changed significantly during the 7.5 km and 38 km exercise periods. Significant ($p<0.05$) decrease of plasma triglyceride concentration was only found in postexercise measurement in the group 1 during 15 km exercise period, however, there was no significant change in the group 2 as control.

These results may indicate that L-carnitine in the group 1 promotes the oxidation of long chain fatty acids. The plasma free carnitine levels in German Shepherd dogs were found to be decreased after four hours of intensive training (3). Therefore, the decrease of plasma tryglyceride concentration during 15 km exercise period may be explained by improved β-oxidation of long chain fatty acids due to effect of L-carnitine supplementation. Triglicerides are important as a fuel of muscular activity. It is required for the replenisment of intramuscular triglycerides after training (26). Serum triglycerides concentrations were found to be decreased after a long distance training by others (10). There was no given an exact explanation for the useage of serum triglycerides, as it was asserted that triglycerides may have been decreased because of the longer time between the recent meal and the sampling time or the effect of the exercise induced increase in triglycerides oxidation (10). In an other research (22), pre- to postexercise plasma triglycerides was not changed in the dogs fed with high fat diets (60% fat). That was explained by the exercise induced increase of lipoprotein lipase activity reesterificated free fat yacids (22). Our results explained that the dropped dogs from the training had an increasing tendency for postexercise plasma triglyceride concentration (1.19-1.75 fold). There was no significant differences between pre-to postexercise trigliceride concentrations of other dogs during 38 km exercise period. This was a possible result of the increased lipoprotein lipase activity or no useage of triglycerides in muscular activity. In agreement with our results, a report in dogs retired and finished from a long distance sled dog race revealed the higher plasma triglyceride concentrations in the retired dogs than the finished dogs (9).

There were no significant differences in the glucose concentrations between the group 1 and the group 2 pre- and postexercises. This was revealed that glucose was used in the same rate by two groups. The preexercise plasma glucose concentration of dogs at 7.5 and 15 km exercises did not show a significant differences, but the preexercise glucose concentration of 38 km period was significantly ($p<0.001$) higher than those of 7.5 and 15 km exercises. These increased preexercise glucose concentration of 38 km exercise may pointed out the beginning of gluconeogenesis, and the mobilisation of glycogen in the muscle and the liver cells.

Lactate is produced from pyruvate by skeletal muscle and erytrocytes in anaerobic glycolysis (25). Plasma lactate concentration were decreased in postexercise measurements of 7.5 and 15 km exercise periods in both groups. Similar results were obtained by Biagi et al. (3) after a long distance training of German Shepherd dogs. This may explain that pyruvate was oxidazed aerobicly in the citric acid cycle and did not converted into lactate because of enough oxygen supplied into muscle cells. Another explanation for the decrease of lactate is converted into glucose in liver by cori cycle (25). In contrast, lactate concentration did not change significantly during 38 km distance exercise period. This may be the result of increased lactate concentration in a tendency due to pyruvate converted into lactate in an inadequete oxygene supply in the citric acid cycle. There was no significant differences between blood lactate concentration of group 1 and group 2, however, opposite results were reported by Pelletier (20) and Grandjean et al. (6). These reports showed that receiving supplements of L-carnitine reduced blood lactate concentration and regulated gluconeogenesis. These contradictory reports may be the result of the different L-carnitine supplementation. L-carnitine did not effect the blood lactate concentration reported in exercising human (7). Increased (1.11-2.1 fold) lactate concentration was detected in dropped dogs from last 38 km exercise. In contrary, there was no elevated lactate concentration in other dogs during all exercises. Postexercise increased lactate concentrations in the dogs retired during 38 km distance exercises were likely the yield of anaerobic glycolysis.

Muscle damage causes an increase in the plasma CK activity in dogs and CK activity has a specivity of 98% for skelet muscle diseases in dogs (1). Several studies reported an increased CK activity in dogs with skeletal muscle damage induced by long distance training (14,16). Significant high CK activity in postexercise measurements were detected during 38 km exercise period in comparison to 7.5 and 15 km distance exercises. The increased CK activity found in postexercise measurements in given exercise distances may indicate the different levels of the muscle damage. This may be a good indicator as a sign of myopathies in the dogs in the different exercise intencities.

Plasma AST activity increases also during the long distance training (10,14). Hence, it also indicates the musculer damage particulary at 38 km exercise distance together with the increased CK activity. AST activity was not changed significantly ($p>0.05$) during 7.5 km exercise period, but the slight increase in CK activity may present at the low exercise intencity-induced myopathy better than plasma AST activity. Dramatic increase of AST activity may be the sign of a serious action in muscle. 38 km exercise period showed to induce a dramatic increase in CK and AST activity in dogs, this was also reported in dogs retired from a long distance race (14). In another study, there was no significant difference in CK activities between finished and retired dogs from a long distance sled dogs race (9). However, there is an other report that showed significant difference between finished and retired dogs from heavy training (14). Exercise induced rhabdomyolysis as a muscle disease in Greyhounds (16,5) and sled dogs (14) is a serious cause of death in these dogs. The dogs that retired during last 38 km exercise programme were expressed a dramatic increase in CK, AST, and LDH activities in postexercise measurements. But, such result was not obtained in other thirteen dogs that completed the exercises at 38 km. It may be speculated that these dogs might have had performance failure due to rhabdomyolysis. Moreover, the colour of the plasma was rather red that might be the result of a myoglobinemia in these dogs. A typical muscle soreness was also easly visualised in retired dogs at 38 km than the other thirteen dogs that completed the exercise. Similarly, muscle soreness and myoglobunuria were recognized by Gannon (5) in the hyperacute and acute forms of rhabdomyolysis in Greyhounds.

Exercise induced decrease in plasma cholesterol concentrations were recognised during the 38 km exercise period in both groups. These decreasing tendency was likely in dogs that retired from exercise at 38 km distance. Cholesterol is eliminated by liver as bile salts, and it is a component of all eucaryotic plasma mambranes and most required for synthesis of steroids (25). Decrease of cholesterol concentration was noticed by Hinchcliff et al. (10) after a long distance exercise, but explanation for that was not given by these researchers. High metabolic rate in exercised dogs might cause the elimination of plasma cholesterol in its increased rate by liver, or it may have used for various metabolic functions. Reports on racing sled dogs showed that dogs need a high metabolisable energy (45000 kj/day) for supporting the large metabolic rate (13), which is induced by great water turnover (12), physical and metabolic changes and thermoregulatory effort (13). In conclusion, these results showed that German Shepherd dogs fed with high fat diet at daily warm temperatures are able to walk 7.5 to 15 km in a day for at least five days without pathological changes such as muscle injury and hemaconcentration. 100 mg/kg L-Carnitine supplementation of the food for feeding the dogs may help to improve the energy production in their requirements during middle distance (about 15 km) training. However, if the dogs have to walk 38 km distances after they walked over 100 km distance in 10 days, the performance of the dogs may be forced to end up with the hemaconcentration, muscle injury, and performance failure. Hence, it would finally suggested that measurements of the hematocrit value, lactate concentration and CK, AST and LDH activities can be useful markers for monitoring the general condition of the dogs exercising at long distances.

Acknowledgments

The authors wish to thank Dr Duygu Fatura and Burcu Ince, DVM, for their technical assistance in the laboratory of Pets' Veterinary Clinic in Bursa. The authors also thank Dr. Mehmet Orman for his excellent technical assistance in statistical analysis of the data.

References

1. **Aktaş M, Auguste D, Lefebre HP et al** (1994) *Creatine kinase in the dog:* A review. Vet Res Commun. **17**, 353-369.
2. **Baker MA** (1980) *Anatomical and Physiological Adaptations of Panting Mammals to Heat and Exercise.* 121-146, In: A Horvat, M K Yousef (Ed.): Environmental Physiology: Aging, Heat and Attitute. Elsevier North Holland. New York.
3. **Biagi G, Luddi R, Maccari F, Ambrogi C, Nannipieri S, Cardini G** (1992) *Variations in plasma carnitine levels and in other parameter concentrations related to training in German Shepherd dog.* Annali, Universita Degli Studi Di Pisa Della, Facolta Di Medicina Veterinaria Di Pisa. Giardini. XLIV. 469-478.
4. **Borum P** (1983) *Carnitine.* Annu Rev Nutr. **3**, 233-259.
5. **Gannon JR** (1980) *Exertional rhabdomyolisis (myoglobuniria) in the racing Greyhound.* 783-787. In: Kirk, R.W (Ed.): Current Veterinary Therapy VII. Small Animal Practice, First edition: W.B. Saunders Co. Philadelphia.
6. **Grandjean D, Valette JP, Jouglin M, Gabillard C, Bacque H, Bene M, Guillaud JP** (1993) *Use of nutritional supplement with L-Carnitine, vitamin C and vitamin B12 in sporting dogs.* Experimental study in sled dogs. Recl Med Vet, **169**, 543-551.
7. **Greig C, Finch D, Jones A, Cooper M, Sargeant, AJ, Forte C.A.** (1987) *The effect of oral supplementation with L-Carnitine on maximum and submaximum exercise capacity.* Eur J Appl Physiol, **56**, 457-460.
8. **Hickson RC** (1983) *Carbohydrate metabolism in exercise.* 1-8. In: Fox, E. (Ed.): Nutrient Utilization During Exercise. Ross Laboratories, Columbus, OH.
9. **Hinchcliff KW** (1996) *Performance failure in Alaskan sled dogs: biochemical correlates.* Res Vet Sci, **61**, 271-272.
10. **Hinchcliff KW, Olson J, Crusberg C, Kenyon J, Long R, Royle W, Weber W, Burr J** (1993) *Serum biochemical changes in dogs, competing in a long-distance sled race.* JAVMA, **202**, 401-405
11. **Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA** (1997) *Effect of racing on serum sodium and potassium concentrations and acid-base status of Alaskan sled dogs.* JAVMA, **210**, 1615-1618.
12. **Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA** (1997) *Exercise-associated hyponatremia in Alaskan sled dogs: urinary and hormonal responses.* Am Philos Soc, **83**, 824-829.
13. **Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA** (1997) *Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold.* Am J Vet Res, **58**, 1-6.

14. **Hinchcliff KW, Shaw LC, Vukich NS, Schmidt KE** (1998) *Effect of distance traveled and speed of racing on body weight and serum enzyme activity of sled dogs competing in a long-distance race.* JAVMA, **213**, 639-644.

15. **Kronfeld DS, Hammel EP, Ramberg CF, Dunalp HL** (1977) *Hematological and metabolic responces to training in racing sled dogs fed diets containing medium, low, or zero carbonhydrate.* Am J Clin Nutr, **30**, 419-430.

16. **Lassen ED, Craig AM, Blythe LL** (1986) *Effects of racing on hematological and serum biochemical values in Greyhounds.* JAVMA, **188**, 1299-1303.

17. **McKeever K, Schrug W, Jarrett S, et al** (1987) *Exercise training-induced hypervolemia in the horses.* Med Sci Sports Exerc, **19**, 21-27.

18. **Neu H** (1995) *Carnitine:Function und klinische Bedeutung bei Herzerkrankunken (Kardiomyopathien) des Hundes-eine Literaturübersicht.* Kleintier Prax, **40**, 157-248.

19. **Ohlenschlager G, und Berger I** (1990) *L-Carnitine, Eine Körpereigene Sustanz von interessanter, vielfältiger therapeutischer Wirkung.* Heil Prax Mag, **4**, 1-5.

20. **Pelletier B** (1992) *L-Carnitine or vitamin Bt, of interest in the dog.* Act Vet, **1210**, 19-21.

21. **Reinhart G** (1995) *Developing a manageable feeding program. In: Performance of Dog Nutrition Symposium.* The Iams Company, 43-49.

22. **Reynolds AJ, Fuhrer L, Dunlap HL, Finke MD, Kallfelz FA** (1994) *Lipid metabolite responses to diet and training in sled dogs.* Am Inst Nutr, **22**, 2754S-2759S.

23. **Reynolds AJ, Fuhrer L, Dunlap HL, Finke MD, Kallfelz FA** (1995) *Effect of diet and training on muscle glycogen storage and utilization in sled dogs.* J Appl Physiol, **79**, 1601-1607.

24. **Snow DH, Kerr M, Nimmo, MA, et al** (1982) *Alterations in the blood, sweet, urine, and muscle composition during prolonged exercise in the horse.* Vet Rec. **384**, 110-377.

25. **Stryer L** (1988) *Gluconeogenesis and biosythesis of membrane lipids and steroid hormones.* 427-570. In: Stryer, L (Ed.) Biochemistry. third edition. W.H. Freeman and Company, New York.

26. **Terjung RL, Budohoski L, Nazar K, Kobryn A, Kaciuba-Vacilko H** (1982) *Chylomicron tryglyceride metabolism in resting and exercising fed dogs.* J Appl Physiol, **52**, 815-820.

Correspondence address :

Hamdi UYSAL, D.V.M., Ph.D.
Ankara University,
Faculty of Veterinary Medicine,
Department of Biochemistry,
06110-Dışkapı, Ankara
TURKEY