

The effect of acute strenuous exercise on some physiological, blood and antioxidant system parameters in Kangal shepherd dogs with and without ankyloglossia

Mustafa KOÇKAYA¹, Mehmet EKİCİ¹

¹Department of Veterinary Physiology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140, Sivas/TURKEY

Key Words:

kangal shepherd dog
ankyloglossia
exercise
blood parameters

Received : 15.04.2020
Accepted : 01.07.2020
Published Online : 30.12.2020
Article Code : 719489

Correspondence:

M. KOÇKAYA
(vet_mustafakockaya@hotmail.com)

ORCID:

M. KOÇKAYA : 0000-0001-5173-0853
M. EKİCİ : 0000-0002-2163-6214

ABSTRACT

The aim of this study was to investigate the effect of acute strenuous exercise on some physiological, blood and antioxidant system parameters in Kangal shepherd dogs with ankyloglossia. In both groups, red blood cell, leukocyte, hemoglobin, mean corpuscular volume, hematocrit levels along with superoxide dismutase enzyme activity and malondialdehyde levels were increased after acute strenuous exercise compared to before exercise. In both groups, the biochemistry parameters of total protein, albumin, glucose levels were decreased after acute strenuous exercise compared to before exercise. In both groups, the heart rate, respiratory rate, body temperature physiological values were increased after acute strenuous exercise compared to before exercise. In comparison of the groups within themselves after acute strenuous exercise, the respiratory rate and body temperature values were increased more in the ankyloglossial group. When the data obtained were evaluated, it was found that acute strenuous exercise affected physiological data more in the dogs with ankyloglossia. This study is the first study on blood and antioxidant system parameters in Kangal shepherd dogs with ankyloglossia.

INTRODUCTION

Kangal shepherd dogs are preferred as guard and shepherd dogs around the world due to their majestic appearance, large figure, agility, bravery that enables them to stand against wild animals larger than themselves, loyalty to their owners and harmlessness and compassion toward children (10, 21, 23). The reasons behind the high preference rate of Kangal shepherd dogs are that they execute the assigned duty perfectly and have the skill to rapidly adapt to the environment. The thermoregulation mechanism plays a significant role in rapid adaptation of Kangal shepherd dogs to the environment, and their tongue plays a significant role in the adjustment of the thermoregulation mechanism (31). Ankyloglossia is a genetic disease seen in humans and rarely in Kangal shepherd dogs. Ankyloglossia is known as incomplete release of frenulum linguae, which ties the tongue to the mouth base, or thickens the tongue due to cell proliferation, and thus preventing the release of the tongue (2,16).

Increased oxygen consumption during exercise is the most obvious biological difference (3). In order to meet the oxygen consumption, some changes occur in the circulating blood with some physiological mechanisms in the body. As the intensity of exercise increases, oxygen consumption increases and the production of free radicals accelerates (27, 28). The cells have antioxidant systems against the destructive effects of free radicals and act by clearing the free radicals formed (8). Antioxidant enzymes effective at the cellular level during exercise

include superoxide dismutase (SOD) (1). It has been reported that acute strenuous exercise may negatively affect SOD activity (30). Exercise done regularly and at a certain intensity strengthens the antioxidant defense (17). Lipid peroxidation is thought to occur when the level of free oxygen radicals exceeds the level of antioxidants in the defense capacity of the cells during exercise. One of the substances that occur as a result of lipid peroxidation is malondialdehyde (MDA) and is an indicator of oxidative stress. The amount of resulting MDA is thought to increase in proportion to the intensity and duration of the exercise (32).

In the literature, there is no study on the effect of exercise status on blood and antioxidant system parameters in Kangal shepherd dogs with ankyloglossia. Therefore, the aim of this study was to investigate the effect of acute strenuous exercise on some physiological, blood and antioxidant system parameters in Kangal shepherd dogs with ankyloglossia.

MATERIAL and METHODS

Animal experimentations approval for the study was granted by the Local Ethics Committee for Animal Experimentations of Cumhuriyet University with issue number of 65202830050.04.04-250 in February 21, 2019

Animals

In the study, 8 Kangal shepherd dogs with ankyloglossia and 13 Kangal shepherd dogs without ankyloglossia between

the ages of 2-7 years, which were owned by animal breeders in Sivas, were used.

Physiological parameters and blood parameters

During the course of the study, the animals were kept in their familiar environment and allowed to perform their routines in order to prevent stress and potential alterations in their hematological parameters. Establishments were chosen according to having to offer a similar dietary provisions to dogs in order to prevent dietary changes in both hematological and biochemical parameters.

On days when the environment temperature was 20 °C, heart rate, respiratory rate, rectal temperature (°C) were measured and blood samples were taken from the dogs at rest and after two hours of acute strenuous exercise.

Blood samples from each dog were collected from antebrachial cephalic vena into two tubes which were lithium heparin containing tubes and yellow capped anticoagulantfree gel coated biochemistry tubes. Blood samples were delivered into the laboratory in shortest time possible. Blood samples were centrifuged for 10 min at 4000 rpm for obtaining serums. Obtained serums were stored at -20°C until the analysis. Biochemistry analyzer device (Mindray BS200, Mindray, P.R.C.) was

Table 1. Determined hematological values in study groups

		Group		
		With ankyloglossia	Without ankyloglossia	P value
		Mean ± SEM (Median)	Mean ± SEM (Median)	(with in group)
RBC	Resting	6.11±0.028 (6.13)	6.10±0.029 (6.11)	>0.05
	Exercise	6.23±0.007 (6.23)	6.22±0.007 (6.23)	>0.05
	P value (with in group)	<0.001		
HCT	Resting	35.83±0.24 (35.75)	36.07±0.17 (35.99)	>0.05
	Exercise	36.83±0.039 (36.85)	36.78±0.024 (36.78)	>0.05
	P value (with in group)	<0.001		
HGB	Resting	10.88±0.09 (10.80)	10.88±0.06 (10.80)	>0.05
	Exercise	12.63±0.29 (13.00)	12.08±0.25 (12.35)	>0.05
	P value (with in group)	<0.001		
MCV	Resting	64.87±0.14 (64.80)	65.03±0.11 (65.00)	>0.05
	Exercise	65.64±0.27 (65.70)	65.37±0.11 (65.50)	>0.05
	P value (with in group)	<0.01		
WBC	Resting	12.60±0.02 (12.60)	12.60±0.02 (12.60)	>0.05
	Exercise	12.70±0.04 (12.70)	12.69±0.03 (12.70)	>0.05
	P value (with in group)	<0.05		

RBC: Red blood cell, HCT: Hematokrit, HGB: Hemoglobin, MCV: Mean corpuscular volume, WBC: White blood cell, SEM: Ortalama standart hata

used to determine serum total protein, albumin and glucose levels. Automatic hemocytometer device (Hematologic Analyzer System 9000, Sero Diagnostics) was used to determine hematological parameters. Malondialdehyde (MDA) measurement was performed as described by Janero (19). This method is based on the spectrophotometric measurement of the absorbance at 535nm of the color that MDA forms with thiobarbituric acid (TBA) in acidic media. The results were calculated using a standard graph. Serum Superoxide Dismutase (SOD) levels were determined by the xanthine/xanthine oxidase method (12). The resulting superoxide radicals (O_2^-) reduce the nitro blue tetrazolium (NBT) and form a colored formazan. This complex yields maximum absorbance at 560 nm. When SOD is present in the medium, no NBT reduction occurs and it does not turn into a blue-violet color, and a light color is formed depending on the amount and activity of the enzyme.

Statistical Analyses

IBM SPSS Statistics 25.0 software was used for the statistical analysis of the data (18). While the statistical difference between the groups was analyzed by the Mann Whitney U test, the statistical analysis of the intragroup change in different conditions was performed using the Wilcoxon test. The results were presented as Mean \pm SEM (Median). The level of significance was set at $p < 0.05$.

RESULTS

Red blood cell, leukocyte, hemoglobin, mean corpuscular volume (MCV), hematocrit blood parameters levels were increased in the dogs with and without ankyloglossia after acute strenuous exercise compared to before exercise ($p < 0.001$, $p < 0.01$) and the values of these parameters are shown in Table 1.

There was a decrease in serum total protein (TP), albumin, glucose levels in the dogs with and without ankyloglossia after acute strenuous exercise compared to before exercise ($p < 0.001$, $p < 0.01$) and these values are shown in Table 2.

Serum SOD and MDA levels were increased in the dogs with and without ankyloglossia after acute strenuous exercise compared to before exercise ($p < 0.001$) and these values are shown in Table 3.

It was found that the physiological values of heart rate (HR), respiratory rate (RR), body (rectal) temperature (RT) increased in the dogs with and without ankyloglossia after acute strenuous exercise compared to before exercise ($p < 0.001$). When the groups were compared within themselves after acute strenuous exercise, it was found that the RR and RT values were increased more in the ankyloglossia group ($p < 0.01$, $p < 0.001$). The values for these parameters are shown in Table 4.

Table 2. Serum biochemical levels between groups

		Group		
		With ankyloglossia	Without ankyloglossia	P value
		Mean \pm SEM (Median)	Mean \pm SEM (Median)	(with in group)
TP	Resting	5.68 \pm 0.01 (5.70)	5.66 \pm 0.01 (5.68)	>0.05
	Exercise	5.62 \pm 0.01 (5.61)	5.63 \pm 0.006 (5.63)	>0.05
	P value (with in group)		<0.01	
Albumin	Resting	3.98 \pm 0.004 (3.99)	3.98 \pm 0.002 (3.99)	>0.05
	Exercise	3.97 \pm 0.009 (3.98)	3.96 \pm 0.005 (3.98)	>0.05
	P value (with in group)		<0.05	
Glucose	Resting	93.98 \pm 0.41 (94.07)	94.20 \pm 0.22 (94.49)	>0.05
	Exercise	91.00 \pm 0.39 (90.89)	91.45 \pm 0.26 (91.28)	>0.05
	P value (with in group)		<0.001	

TP: Total protein, SEM: Ortalama standart hata

Table 3. Serum SOD and MDA levels between groups

		Group		
		With ankyloglossia	Without ankyloglossia	P value
		Mean ± SEM (Median)	Mean ± SEM (Median)	(with in group)
MDA	Resting	0.63±0.004 (0.63)	0.63±0.003 (0.63)	>0.05
	Exercise	1.26±0.06 (1.29)	1.12±0.04 (1.05)	>0.05
	P value (with in group)	<0.001		
SOD	Resting	86.64±0.89 (86.93)	86.66±0.65 (86.68)	>0.05
	Exercise	133.79±0.70 (133.92)	132.50±0.46 (132.48)	>0.05
	P value (with in group)	<0.001		

MDA: Malondialdehyde, SOD: Superoxide dismutase, SEM:Ortalama standart hata

Table 4. Determined physiological parameters in study groups

		Group		
		With ankyloglossia	Without ankyloglossia	P value
		Mean ± SEM (Median)	Mean ± SEM (Median)	(with in group)
HR (n/min)	Resting	94.12±0.29 (94.00)	94.00±0.22 (94.00)	>0.05
	Exercise	116.87±0.47 (116.5)	114.53±0.24 (114.0)	>0.05
	P value (with in group)	<0.001		
RR (n/min)	Resting	22.12±0.29 (22.0)	22.07±0.24 (22.0)	>0.05
	Exercise	36.00±0.26 (36.0)	35.15±0.27 (35.0)	<0.01
	P value (with in group)	<0.001		
RT (°C)	Resting	37.6±0.03 (37.64)	37.56±0.015 (37.56)	>0.05
	Exercise	37.86±0.006 (37.86)	37.78±0.006 (37.78)	<0.001
	P value (with in group)	<0.001		

HR: Heart rate, RR: Respiratory rate, RT: Rectal temperature, SEM: Ortalama standart hata

DISCUSSION

The oxygen requirement of the skeletal muscles increases during exercise. Mobilization of red blood cells from the spleen occurs to meet the requirement for oxygen. This increases the RBC, HCT, HGB and MCV values. During exercise, some fluid leaves the vessels and goes between the tissues. This increases the red blood cell and hemoglobin density in the blood. Many studies have reported increased RBC, HCT, MCV, HGB values in the case of acute exercise (7, 8, 14, 24, 29, 33). In the present study, there was an increase in RBC, HCT, MCV, HGB values in the dogs with and without ankyloglossia after acute strenuous exercise compared to resting state, which is consistent with other studies.

It has been reported that white blood cell count is increased during acute exercise, and the reason for this is related to the change in catecholamines and hemodynamics (8, 13, 25, 34). In the present study, there was an increase in WBC values in the dogs with and without ankyloglossia after acute strenuous exercise compared to resting state, which is consistent with other studies.

It has been reported that the serum glucose level is decreased during acute strenuous exercise and accordingly, protein breakdown (proteolysis) increases and total protein and albumin levels are decreased (5). It has been reported that protein catabolism slightly increases and total protein level is decreased during acute strenuous exercise (4, 9, 11, 15). In the present study, the serum glucose, albumin and TP levels were lower in the dogs with and without ankyloglossia after acute strenuous exercise compared to resting state, which is consistent with other study results.

It has been reported that oxidative stress is increased in cases of acute exercise and intense exercise, and SOD and MDA levels are increased due to increased oxidative stress (1, 6, 26, 32). In the present study, the SOD and MDA values were higher after acute strenuous exercise compared to resting state, which is consistent with other study results.

The exercise studies on Kangal shepherd dogs found that HR, RR, RT values were increased after exercise compared to resting state (20, 22). In the present study, these values were reported to be increased in the Kangal shepherd dogs with and without ankyloglossia after acute strenuous exercise compared to the resting period. However, when the groups were evaluated within themselves, it was found that the RR and RT values were increased more in the Kangal shepherd dogs with ankyloglossia. This increase is due to the fact that the body temperature cannot be lowered since the tongue does not completely come out in animals with ankyloglossia and the respiratory rate is increased to provide thermoregulation.

In conclusion, acute strenuous exercise affects physiological data more in dogs with ankyloglossia and increased respiratory rate in dogs with ankyloglossia during exercise is not sufficient to adjust the thermoregulation.

CONCLUSION

Ankyloglossia condition affects physiological data more in Kangal shepherd dogs during exercise and causes the body temperature to increase more. It will be useful to consider these parameters in exercise types and treatments for dogs with ankyloglossia.

CONFLICTS of INTEREST

The authors declare no conflicts of interest with respect to the publication of this manuscript.

REFERENCES

1. Abed KE, Rebai H, Bloomer RJ, Trabelsi K, Masmoudi L, Zbidi A, Sahnoun Z, Hakim A, Tabka Z. Antioksidant status and oksidative stres at rest and in response to acute exercise in judokas and sedantary men. *Journal of Strength & Conditioning Research*. 2011; 25(9):2400-2409.
2. Alkan F, Koç Y, Tepeli C, Albasan H, Altan S. Management of complete and partial ankyloglossia in kangal shepherd dogs. *Research Opinions in Animal and Veterinary Sciences*. 2013; 3:462-465.
3. Arslan R. Sedanterlerde Akut ve Programlı Submaksimal Egzersizin Eritrosit Membran Lipit Peroksidasyonu ve Antioksidan Savunma Sistemi Üzerine Etkilerinin Araştırılması. Doctoral thesis, Van Yüzüncü Yıl University. 1997.
4. Astrand PO, Rodahl K. *Textbook of Work Physiology*, McGraw- Hill Inc. 3rd. Edt. New York. 1986.
5. Blomstrand E, Saltin B. Effect of muscle glycogen on glucose, lactate and amino acid metabolism during exercise and recovery in human subjects. *J Physiol*. 1999; 514 (1): 293-302.
6. Bloomer RJ, Goldfarb AH. Anaerobic exercise and oxidative stres. *Canadian Journal of Applied Physiology*. 2004; 29(3): 245-63.
7. Convertino A. Blood volume: its adaptation to endurance training; *Med. Sci. and Exerc*. 1991; 23: 1338-1348.
8. Çelik A, Varol R, Onat T, Dağdelen Y, Tugay Y. Akut egzersizin futbolcularda antioksidan sistem parametrelerine etkisi. *Spor metre Beden Eğitimi ve Spor Bilimleri Dergisi*, 2007; 5(4):167-172.
9. Çevik C, Günay M, Tamer K, Sezen M, Onay M. Farklı aerobik antrenman programlarının serum enzimler, serum elektrolitler, üre, ürik asit, kreatin, total protein ve fosfor üzerindeki etkileri ve ilişki düzeylerinin belirlenmesi. *Gazi Beden Eğitimi ve Spor Bilimleri Dergisi*. 1996; 1(2): 37-46.
10. Çetin O, Tepeli C. Türkiye'de Türk çoban köpeği yetiştiriciliği. In: *Uluslararası Türk Çoban Köpeği Sempozyumu*. Konya, Turkey. 1996; pp. 217-225 (in Turkish).
11. Devries AH. *Physiology of Exercise for Physical Education and Athletics*. Wm. C. Brown Publ. Dubuque. 1986.
12. Durak I, Yurtaslan Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity

assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clin Chim Acta*, 1993; 214: 103-104.

13. Dzhelebov PV, Gundasheva DI, Andonova MY, Slavov EP. Changes in serum cortisol and some innate immunity parameters after exhaustive exercise in male dogs. *Bulgarian Journal of Veterinary Medicine*. 2019; 22(3): 275–284.

14. Fellman N. Hormonal and plasma volume alterations following endurance exercise. *Sports Med*. 1992; 13: 37-49.

15. Fox E, Bowers R, Foss M. *The Physiological Basis of Physical Education and Athletics*. Saunder College Publ. 4rd ed. Philadelphia. 1988.

16. Fulton AJ, Fiani N, Verstraete FJM. Canine pediatric dentistry. *Vet Clin North Am Small Anim Pract*. 2014; 44: 303-324.

17. Giovanelli G, Buratti S. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chemistry*. 2009; 112: 903–908.

18. IBM SPSS Statistics Inc. SPSS 25.0 for Windows, Evaluation Version. Armonk, NY, USA: IBM Corp. 2019.

19. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med*. 1990; 9: 515-540.

20. Koçkaya M, Oğrak YZ, Urošovic M. Kangal Irkı Türk Çoban Köpeklerinin Bazı Fizyolojik Özelliklerinin Belirlenmesi. *Journal of Agricultural Faculty of Gaziosmanpaşa University*. 2013; 30 (1):91-95.

21. Koçkaya M, Şireli M. Comparison of behavioral and physiological responses of Kangal dogs in different livestock flocks. *Ankara Üniversitesi Veterinerlik Fakültesi Dergisi*. 2015; 62: 261-267 (article in Turkish with an abstract in English).

22. Koçkaya M, Özşensoy Y, İnsal B. Comparisons of some physiological and stress behavioral parameters of Kangal shepherd dogs with and without ankyloglossia in different environmental temperatures. *Turkish Journal of Veterinary and Animal Sciences*. 2019; 43: 314-322

23. Onbaşı H. Kangal'a sahip çıkalım. *Tempo Dergisi*. 2004; 45: 882 (in Turkish).

24. Oscai LB, Williams T. Effect of Exercise on Blood Volume. *J. Appl. Physiol*. 2002; 24(5): 622-624.

25. Özbal Y. The relationship between the changes in physical fitness and in total blood volume in subjects having regular and measured training. *The J. Sport Med. Physical Fitness*. 1999; 14(2): 73-77.

26. Özçelik O, Karataş F. Şiddeti düzenli olarak artan işe karşı yapılan egzersizin obezlerde serum malondialdehid ve vitamin a,e,c düzeyleri üzerine olan etkisi. *Fırat Üniversitesi Sağlık Bilimleri Tıp Dergisi*. 2008; 22(6):337-341.

27. Öztürk M, Güzelhan Y, Sayar K, Tüzün U. Yaygın gelişimsel bozukluğu olan çocuklarda plazma malondialdehit ve glutatyon düzeylerinin araştırılması. *Psikofarmakoloji Bülteni*. 2001; 11: 155-159.

28. Palmer FM, Nieman DC, Henson DA, Mcanulty SR, Mcanulty L, Swick, NS, Utter AC, Vinci DM, Marrowet JD. Influence of vitamin C supplementation on oxidative and salivary iga changes following an ultramarathon. *European Journal of Applied Physiology*. 2003; 89: 100-107.

29. Rovira S, Munoz A, Benito M. Effect of exercise on physiological, blood and endocrine parameters in search and rescue-trained dogs. *Veterinari Medicina*. 2008; 53(6): 333–346.

30. Sabbağ Ç, Sürücüoğlu MS. likopen: insan sağlığında vazgeçilmez bir bileşen. *Gıda Teknolojileri Elektronik Dergisi*. 2011; 6(3): 27-41.

31. Tabor B. Heatstroke in dogs. *Today's Veterinary Practice*. 2014; 50-56.

32. Urso ML, Clarkson PM. Oxidative stress, exercise and antioxidant. *Supplementation Toxicology*. 2003; 189(1-2): 41-54.

33. Vandewalle H, Lacombe C, Lelievre JC, Poirot C. Blood viscosity after a 1 hour submaximal exercise with and without drinking; *Int. J. Sports Med*. 1998; 9: 104- 107.

34. Wiik P, Opstad A, Boyum A. Granulocyte chemiluminescence response to serum opsonized particles ex vivo during long-term strenuous exercise, energy and sleep depletion in humans. *Eur. J. Appl. Physio*. 1996; 73: 251-258.