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TOTAL AND DIFFERENTIAL LEUKOCYTE COUNTS, GLOBULINS AND ALBUMIN/GLOBULIN RATIO AFTER EXHAUSTIVE EXERCISE IN DOGS

P. DZHELEBOV, D. GUNDASHEVA, M. ANDONOVA & E. SLAVOV

Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

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The aim of the experiment was to study the effect of exhaustive aerobic exercise on total and differential leukocyte counts, globulins and albumin/globulin ratio in canine experimental model. We used 12 male, mongrel dogs divided into two groups – animals from experimental group were submitted to exhaustive exercise; animals from control group did no exercise. Total and differential leukocyte counts, globulins and albumin/globulin ratio were measured before exercise (BE), right after exercise (0 h) and on 2^{nd} hour, 4^{th} hour, 24^{th} hour, 48^{th} hour and 72^{nd} hour and on 7^{th} and 14^{th} day after exercise. Total leukocyte count increased insignificantly in experimental animals on 2^{nd} and 4^{th} hour after exhaustive exercise. Neutrophil count increased in a similar pattern. Lymphocyte count in experimental group increased on hour 72 after exercise ($P<0.05$). Total globulins decreased right after exercise ($P<0.05$), on 2^{nd} hour ($P<0.05$) and on 4^{th} hour ($P<0.001$) after exercise. A/G ratio increased right after exercise ($P<0.01$), on 2^{nd} hour ($P<0.05$) and on 4^{th} hour ($P<0.05$) after exercise. In conclusion, exhaustive exercise causes increase in total and differential leukocyte counts, decrease in globulins and increase in A/G ratio.

Key words: albumin/globulin ratio, dog, exercise, globulin, leukocyte count

INTRODUCTION

Defining the amount of healthy exercise is an important issue concerning not only humans, but also pets and sport dogs and horses. Depending on the type and amount of exercise changes in cardiovascular function and hematological and biochemical changes may be sometimes dramatic. Any kind of exercise alters metabolism and may have influence on the functioning of organism. Most effects of exercise are positive, but excessive exercise may have some negative effects – suppression of immunity (Robson *et al*., 2003), gastrointestinal bleeding (Halvorsen *et al*., 1990), exercise-induced haematuria, haemolysis, anaemia, traumas and electrolyte disorders (Schott *et al*., 2006). Haematological changes and changes in blood rheology occur especially during and after exhaustive exercise. Data about exercise in dogs concern mainly sled dog racing. Scientists state that exercise of long duration causes decrease in packed cell volume and hemoglobin concentration (Davis *et al*., 2008), while total leukocyte count increases. Increase in neutrophils count was also found in horses after endurance races (Robson *et al*., 2003; Serteyn *et al*., 2010). In humans increase in total leukocyte count and neutrophil count was found after running a marathon, while lymphocytes decreased (Kratz *et al*., 2002). The same authors report increase in total protein and albumin and decrease in globulins after marathon. In sled dogs Hinchcliff *et al*. (1997) have found decrease in serum total protein, albumin, and globulin concentrations. McKenzie *et al*. (2009) state that hypogammaglobulinemia observed in a large proportion of racing sled dogs might predispose them to infectious disease. While leukocytosis and neutrophilia are highly reported after long duration exercise in humans and different animal species, changes in serum concentration of total protein, albumin and globulin are sometimes controversial. Results are influenced by the type and amount of exercise, the animal species involved, environmental conditions, nutritional and hydration status.

The aim of the present study was to evaluate the changes in total and differential leukocyte counts, globulins and albumin/globulin ratio after exhaustive exercise in dogs.

MATERIAL AND METHODS

Experimental animals

We used twelve healthy male, mongrel dogs, 1.5–3.5 years of age. Adaptation period continued one month. The dogs were treated against parasites with Biheldon (Cheironpharma Europe, each tablet contains praziquantel 50 mg, pyrantel pamoate 150 mg) at a dose of 1 tablet/10 kg. Also they were treated against ectoparasites with antiparasite shampoo, Ectomin and Tapilan (Dorvet, Israel). An antirabies vaccine – Nobivac Rabies (Intervet International B. V.) was also applied. Animals were kept in individual cages (situated indoor, providing constant room temperature) and went for walks twice a day – half an hour in the morning and another walk in the evening. Thus conditions were similar to the way of pet breeding. Dogs were divided into two groups – experimental group and control group. Dogs of experimental group weighed 18.67±0.82 kg, and control animals – 20.08±3.29 kg. Only experimental animals were submitted to a prolonged strenuous exercise.

Exercise protocol

Adaptation. The aim of the study was to submit experimental dogs to a prolonged strenuous exercise until exhaustion. During adaptation period only one person took care of the dogs so that they can get used to him and accept him as “the leader of the pack”. In each day of the last week of adapting period, that person trained them to run on a leash after him riding a bicycle. Dogs were willing to run and easily performed the exercise, which had a very short duration of about 10 minutes and can not be considered as endurance training. Runs were performed off road to avoid trauma. During this week dogs got used to the environment so they were able to run without distracting their attention.

Exhaustive exercise. In the day of the experiment each dog was submitted to a prolonged submaximal exercise until exhaustion. Dogs ran on a leash following the “leader” riding bicycle at an average speed of about 12 km/h or faster depending on dog` s abilities. Dogs ran galloping and trotting. Heart rate during exercise exceeded resting heart rate by 77%. When fatigue emerged and they were unwilling to run they were encouraged by the leader verbally and by pulling gently the leash. Exercise was stopped when dogs demonstrated inability to perform and refused to continue.

Explanatory remarks. Exhaustive exercise began with a 5 min warm up running. To prevent dehydration in every five kilometers of running dogs had access to water. Experiment was conducted under the conditions of moderate climate in spring and autumn, and ambient temperature did not exceed 20 ^{o}C. We preferred this exercise protocol to running on a treadmill, because treadmill running is less difficult and dogs get easily bored. On the contrary when running off road they are eager to follow their "leader" as long as they can run. Dogs had no endurance training before conducting the experiment so they reached exhaustion easier through this strenuous and prolonged exercise.

Authors declare that the experiment complies with the current laws of Republic of Bulgaria. Experiment was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Trakia University.

Blood samples

Blood samples were collected in sterile glass tubes by punction of *vena cephalica* in the following dynamics – before exercise, right after exercise (0 hour) and on 2nd hour, 4th hour, 24th hour, 48th hour, 72nd hour, 7th day and 14th day after exercise. We used 0.2 mL heparin (50 units/mL) for each sample as anticoagulant. Blood for protein analysis was collected in tubes and was allowed to cloth and then serum was separated. Blood samples for BE level and on the following days were taken at 8 a.m.

Blood analysis

Total leukocyte counts ($\times 10^9$/L) were counted using the Bürker chamber. The differential leukocyte counts ($\times 10^9$/L) were determined on blood smears (May-Grünwald-Giemsa staining). Globulin concentration (g/L) was calculated by subtracting albumin from total protein, directly measured by colorimetric method (Giesse diagnostics). The albumin globulin ratio was calculated using the values for albumin and globulin concentrations.

Statistical analysis

Results are presented as means ± SD. Data was submitted to standard F-test and t-test (StatMost, version 2.5, provided by DataMost corporation). Differences were considered statistically significant at the $P<0.05$ level.

RESULTS

Results for all parameters measured are presented in Table 1. Total leukocyte count increased in experimental animals on 2nd and 4th hour after exhaustive exercise, but increase was not statistically significant. Leukocyte count did not change in control animals and comparison between two groups in dynamics also had no significant differences.

Neutrophil count had a statistically significant increase ($P<0.05$) in experimental group on hour 2 and hour 4 after exercise, compared to control group at the same points of dynamics. Neutrophil count in control group did not change during experimental period.

Lymphocyte count in experimental group increased as compared to BE level on hour 72 after exercise ($P<0.05$). Lymphocyte count did not change in control animals and comparison between two groups in dynamics also had no significant differences.

Eosinophil count did not change in experimental group. Comparison between groups also showed no significant difference.

Table 1. Total and differential leukocyte counts (x10^9/L), globulin (g/L) and albumin/globulin ratio in experimental (n=6) and control group (n=6). Results are presented as mean ± SD

Parameter	dynamics	Before exercise	0h	2h	4h	24h	48h	72h	7d	14d
Total leukocyte counts (x10^9/L)	Experimental group	8.6± 0.98	8.25± 2.52	9.75± 2.86	9.6± 2.62	8.67± 2.73	8.4± 1.51	9.05± 1.99	8.08± 1.35	8.15± 1.25
	Control group	7.5± 1.42	8.2± 1.47	8.1 ±2.15	7.6± 1.89	8.4± 1.36	7.65± 1.96	7.4± 1.75	7.75± 2.07	7.9± 2.19
Neutrophil counts (x10^9/L)	Experimental group	5.07± 0.72	5± 1.44	6.72± 2.01**b1**	6.27± 1.66**b1**	5.07± 2.65	4.67± 0.72	4.9± 0.96	4.4± 0.72	4.3± 0.69
	Control group	4.48± 1.3	4.82± 0.94	4.65± 0.71	4.32± 1.22	4.73± 0.54	4.35± 0.48	4.58± 1.55	4.53± 1.24	4.38± 1.2
Lymphocyte counts (x10^9/L)	Experimental group	2.75± 1.19	2.37± 1.58	2.27± 0.91	2.92± 1.41	2.75± 0.95	2.93± 1.16	3.1± 1.1**a1**	2.75± 0.71	2.97± 0.51
	Control group	2.05± 0.58	2.32± 1.4	2.5± 1.47	2.37± 1.01	2.68± 1.26	2.37± 1.29	2.45± 1.38	2.3± 1.07	2.5± 0.54
Eosinophil counts (x10^9/L)	Experimental group	0.65± 0.51	0.78± 0.64	0.7± 0.39	0.77± 0.49	0.78± 0.55	0.65± 0.39	0.9± 0.72	0.82± 0.51	0.85 ±0.55
	Control group	0.82± 0.43	0.92± 0.37	0.8± 0.46	0.72± 0.31	0.85± 0.54	0.82± 0.45	0.72± 0.34	0.78± 0.48	0.85± 0.56
Globulin (g/L)	Experimental group	41± 7.87	35.67± 9.33**a1**	32.67± 4.41**a1**	31± 6.39**a3**	35.5± 9.27	37.67± 9.52	38.67± 6.41	33.83± 10.42	33.17± 10.57
	Control group	35.5± 6.83	35± 11.58	37.83± 7.31	36.67± 9.18	33.83± 5.6	33.17± 5.34	35.17± 5.88	32.33± 4.18	30.67± 7.94
A/G ratio	Experimental group	0.84± 0.24	1.15± 0.4**a2**	1.1± 0.22**a1**	1.23± 0.49**a1**	1.1± 0.46	0.94± 0.45	0.94± 0.36	1.19± 0.62	1.17± 0.6
	Control group	1.1± 0.27	1.21± 0.63	0.95± 0.27	1.1± 0.38	1.1± 0.24	1.14± 0.26	1.04± 0.21	1.16± 0.3	1.29± 0.32

Statistically significant differences are presented as follows: **a**[1]p<0.05 within experimental group as compared to before exercise; **a**[2]p<0.01 within experimental group as compared to before exercise; **a**[3]p<0.001 within experimental group as compared to before exercise; **b**[1]p<0.05 experimental vs. control group, compared at the same point of dynamics.

Total globulins decreased in experimental animals right after exercise ($P<0.05$), on 2nd hour ($A<0.05$) and on 4th hour ($P<0.001$) after exercise. Comparison between groups had no significant difference.

A/G ratio increased in experimental animals right after exercise ($P<0.01$), on 2nd hour ($P<0.05$) and on 4th hour ($P<0.05$) after exercise. Comparison between groups had no significant difference. None of the parameters changed in control group within the experimental period.

DISCUSSION

Our study found an insignificant increase in total leukocyte count and a statistically significant increase in neutrophil count. Leukocytosis and neutrophilia are highly reported after endurance exercise in humans, horses and dogs (Davidson *et al*., 1987; Kratz *et al*., 2002; Davis *et al*., 2008). It is generally proposed that these changes are the result of demargination of white blood cells caused by the increased blood flow during exercise (Kratz *et al*., 2002). Increase in cortisol levels during exercise could be another factor contributing to increase in neutrophil counts, as stress-related endogenous release of cortisol is well-known to induce demargination of neutrophils (Davis *et al*., 2008).

Some authors state that increase of neutrophils count, but not of lymphocytes, is typical for inflammation and actually leukocytosis is the result of inflammatory response (Kratz *et al*., 2002). In our study we found increase in both neutrophils and lymphocytes, which point demargination as a reason for the increase in leukocytes counts. On the other hand the increase in neutrophils counts in our study is the most prominent change in differential white blood cells counts, so we can conclude that both demargination and inflammation contribute to the changes in total and differential leukocyte counts.

Our study found also significant decrease in serum globulins and increase in albumin/globulin ratio. The increase in albumin/globulin ratio is either due to decrease in globulins or increase in albumin levels, and sometimes changes in both fractions can contribute to it. In humans postmarathon increase in total protein, albumin and globulin is considered to be the result of dehydration, which usually occurs during prolonged exercise (Maughan *et al*., 1985). Some studies have found that albumin and globulin levels may change independently from total protein. In our previous studies we have found decrease in total protein and an increase in albumin levels after exhaustive exercise in dogs (Dzhelebov *et al*., 2009). The increase in albumin is probably due to increased flow of lymph rich in albumins. The exercise did not result in dehydration and even more a hemodilution was found. The latter can be considered to cause the decrease in globulins found in the present study. As the immunoglobulins represent the largest part of globulins, underproduction of antibodies should always be taken in mind when the globulin level is low. Increase in A/G ratio is also indicative for underproduction of immunoglobulins. Underproduction of immunoglobulins is possible after endurance exercise (McKenzie *et al*, 2009). The decrease in globulins and eventually the increase in albumins lead to increase in albumin/globulin ratio. Having in mind that these changes were found only within a 4-hour interval after the exercise, haemodilution seems to be a more reasonable explanation for the dynamics in globulin concentration.

In conclusion, exhaustive exercise causes increase in total and differential leukocyte counts, decrease in globulins and increase in A/G ratio.

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Correspondence:

Dr. P. Dzhelebov,
Department of General and Clinical Pathology,
Faculty of Veterinary Medicine
Trakia University,
6000 Stara Zagora, Bulgaria
e-mail: petkovet@abv.bg