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EFFECTS OF EXPERIMENTAL PROLONGED STRENUOUS EXERCISE ON HAEMATOLOGICAL PARAMETERS IN DOGS

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Summary

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Six experimental male, mongrel dogs (1.5–3.5 years of age), were submitted to a prolonged strenuous exercise until exhaustion, in order to study its effects on some haematological parameters. The time course of haemoglobin concentration (Hb), red blood cells counts (RBC counts), haematocrit (Hct) and red blood cells indices were measured: before exercise, right after exercise (0 hour), on 2nd hour, 4th hour, 24th hour, 48th hour, 72nd hour, 7th day and 14th day after exercise. Hb and RBC counts decreased right after exercise, on 4th hour, 48th hour, 72nd hour, 7th day and 14th day after exercise. Hct decreased significantly on 48th hour and 72nd hour after exercise. Mean corpuscular haemoglobin (MCH) decreased statistically significantly right after exercise and on 48th hour. Mean corpuscular haemoglobin concentration (MCHC) decreased only right after exercise, while mean corpuscular volume (MCV) changed right after exercise and on 2nd and 24th hour.

Key words: dog, erythrocyte indices, erythrocytes, exercise, haematocrit, haemoglobin

INTRODUCTION

Exercise has been a matter of great interest in the recent years and scientists have studied its effects in many aspects. Lots of experiments involving humans and animals have been conducted. More than three decades ago, it was established that anaemia in humans can be caused by exercise (Bonilla *et al*., 2005). Changes in haematological parameters and haemodynamics occur during exercise of any type (Brun *et al*., 1998). It is however considered that changes in blood rheology are most dramatic during and after acute endurance exercise. Numerous field studies concerning humans have shown that changes are quite controversial. Some scientists report haemoconcentration occurring right after marathon race (Maughan *et al*., 1985), while others find post-race haemodilution (Maron *et al*., 1977). In another study variable individual responses – from haemoconcentration to haemodilution, or no changes at all have been described (O'Toole *et al*., 1999). Results from such studies are often influenced by pre-race overhydration leading to expansion of plasma volume. Endurance running in horses leads to significant elevation in erythrocyte counts and haematocrit in the first stages of the race, followed by a slight decrease suggesting a loss of red cells mass during the prolonged exercise (Boucher *et al.*, 1981). Data about exercise in dogs concern mainly studies conducted in the sled dog sport field. It is reported that both training and exercise cause significant decrease in packed cell volume and haemoglobin concentration (Davis *et al.*, 2008), but immediately after a run, packed cell volume tends to increase (Querengaesser *et al.*, 1994). These changes have been observed in dogs that were trained and competed in very severe conditions.

The aim of our study was to evaluate the effects of prolonged strenuous exercise on some haematological parameters in dogs performing in the conditions of moderate climate in spring and autumn.

MATERIALS AND METHODS

Experimental animals

Twelve healthy male, mongrel dogs, 1.5 – 3.5 years of age were used. Adaptation period lasted 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. They were also 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 indoors, providing constant room temperature) and went for walks twice a day – half an hour in the morning and another walk in the evening. In this way, conditions were similar to the way of pet breeding. Dogs were divided into two groups – control group and experimental 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.

The experiment was approved by the local ethics committe.

Exercise protocol

The aim of the study was to submit experimental dogs to a prolonged strenuous exercise until complete exhaustion. During the 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 adaptation period, that person trained dogs 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 min and could 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. In the day of the experiment each dog was submitted to a prolonged strenuous running until complete 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. When fatigue emerged and they were unwilling to run they were encouraged by the leader verbally and by pulling the leash. Exercise was stopped when dogs could no longer run and simply lay down on the ground refusing to continue. At every five kilometers of running they had access to water to prevent dehydration. 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 were 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.

Blood samples

Blood samples were collected in sterile glass tubes by cephalic venepunction in the following dynamics – before exercise, right after exercise (0 hour) and on 2^{nd} hour, 4^{th} hour, 24^{th} hour, 48^{th} hour, 72^{nd} hour, 7^{th} day and 14^{th} day after exercise. We used 0.2 mL heparin (50 units/mL) for each sample as anticoagulant.

Haematological parameters

Haemoglobin concentration – concentration of hemoglobin (g/L) was measured by the cyanhaemoglobin method. Red blood cells($x10^{12}$/L) were counted using the Bűrker chamber. Morphology of erythrocytes was defined on blood smears (May-Grunwald-Giemsa staining). Haematocrit (L/L) was determined by centrifuging heparinized blood in a microhaematocrit tube at 10,000 rpm for five minutes. The following haematological indices were also calculated: mean corpuscular haemoglobin (MCH) – average weight of haemoglobin in a red blood cell (pg); mean corpuscular volume (MCV) – average volume of a red blood cell (fL) and mean corpuscular haemoglobin concentration (MCHC) – average concentration of hemoglobin in a red blood cell (g/L).

Statistical analysis

Results are presented as means±SD and submitted to standard F- and t-tests (StatMost, v. 2.5, DataMost Co). Differences were considered statistically significant at the $P<0.05$ level.

RESULTS

Two of the dogs completed 24 km of running, two of them ran 25 km and the other two covered a distance of 30 km. All dogs completed the exercise for about 2 hours (1h 58 min – 2h 09 min).

Changes in haematological parameters are shown in Table 1.

In control animals none of the parameters changed statistically significantly as compared to initial levels.

In experimental group, as compared to levels before exercise, haemoglobin concentration decreased right after exercise ($P<0.01$), on 4^{th} hour ($P<0.05$), 48^{th} hour ($P<0.01$), 72^{nd} hour ($P<0.01$), 7^{th} day ($P<0.05$) and 14^{th} day ($P<0.01$) after exercise. Red blood cells counts decreased on the same time intervals – right after exercise ($P<0.05$), on 4^{th} hour ($P<0.05$), 48^{th} hour ($P<0.05$), 72^{nd} hour ($P<0.01$), 7^{th} day ($P<0.05$) and 14^{th} day ($P<0.05$) after exercise. Haematocrit decreased on 48^{th} hour ($P<0.05$) and 72^{nd} hour ($P<0.05$) after exercise. Haematocrit also decreased on some other points of the study following the trendlines of depression of red blood cells counts and haemoglobin concentration, but differences were not statistically significant. MCH had a statistically significant decrease right after exercise ($P<0.05$) and on 48^{th} hour ($P<0.05$). MCHC decreased only right after exercise ($P<0.01$), while MCV increased right after exercise ($P<0.01$) and on 2^{nd} and 24^{th} hour ($P<0.05$).

Comparison between groups revealed statistically significant difference: MCV in experimental group was higher on 24^{th} hour ($P<0.05$) as compared to control.

Morphology of erythrocytes showed no changes.

DISCUSSION

Results from our study indicated decrease in red blood cells count and haemoglobin

Table 1. Red blood cells (RBC) counts, haemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) in experimental (n=6) and control (n=6) groups. Results are expressed as mean±SD

Parameter	Group	Initial level	Right after exercise	2 h	4 h	24 h	48 h	72 h	7 day	14 day
Hb	Experimental	165.48	143.33	145.48	140.08	150.29	134.93	140.05	145.57	142.23
(g/L)		±15.62	±21.79 ^{a**}	±22.65	±22.79 ^{a*}	±26.16	±19.86 ^{a**}	±17.46 ^{a**}	±23.21 ^{a*}	±21.85 ^{a**}
	Control	151.68	142.87	139.71	143.2	145.97	148.61	143.39	151.17	153.27
		±25.00	±31.73	±22.63	±15.04	±22.13	±13.98	±9.03	±16.58	±13.64
RBC	Experimental	7.52	7.08	7.01	6.76	6.85	6.65	6.63	6.78	6.70
count		±0.80	±0.96 ^{a*}	±0.88	±0.92 ^{a*}	±0.87	±0.67 ^{a*}	±0.78 ^{a**}	±0.90 ^{a*}	±0.89 ^{a*}
(10^{12}/L)	Control	7.35	7.18	7.15	7.12	7.15	7.22	6.99	7.30	7.37
		±1.32	±1.37	±1.11	±0.90	±1.00	±0.88	±0.82	±0.51	±0.59
Hct	Experimental	0.49	0.50	0.48	0.47	0.48	0.44	0.45	0.46	0.45
(L/L)		±0.03	±0.06	±0.05	±0.05	±0.06	±0.06 ^{a*}	±0.06 ^{a*}	±0.06	±0.07
	Control	0.47	0.47	0.47	0.46	0.46	0.47	0.46	0.47	0.48
		±0.06	±0.07	±0.05	±0.06	±0.06	±0.06	±0.04	±0.03	±0.04
MCH	Experimental	22.07	20.22	20.75	20.36	22.06	20.25	21.10	21.34	21.20
(g/L)		±1.80	±1.11 ^{a*}	±1.60	±1.47	±2.45	±1.61 ^{a*}	±0.69	±0.95	±1.10
	Control	20.71	19.79	19.58	20.20	20.41	20.67	20.64	20.77	20.88
		±1.34	±0.88	±1.53	±1.27	±1.01	±1.52	±1.33	±2.58	±2.29
MCV	Experimental	65.62	70.92	69.03	68.56	70.00	65.53	67.38	67.44	67.54
(fL)		±4.04	±5.68 ^{a**}	±4.47 ^{a*}	±5.30	±4.39 $^{a*, b*}$	±4.94	±2.42	±2.43	±6.17
	Control	64.67	66.22	65.41	65.14	63.99	64.56	65.56	64.92	65.42
		±4.79	±4.29	±3.75	±3.23	±3.44	±4.82	±2.65	±3.09	±2.23
MCHC	Experimental	336.63	286.92	301.63	302.08	314.73	309.61	311.81	317.46	315.80
(g/L)		±24.38	±31.26 ^{a**}	±31.39	±38.66	±22.25	±21.21	±20.57	±18.93	±29.09
	Control	320.68	300.19	299.59	310.12	319.24	321.23	314.89	319.14	328.31
		±15.60	±27.55	±19.10	±13.23	±10.11	±16.44	±17.94	±25.97	±43.03

a*P<0.05, a**P<0.01 within experimental group vs. initial level; b*P<0.05 between groups.

right after exercise up to the end of experimental period. Changes in haematocrit and erythrocyte indices were similar but less dramatic and not so long lasting. Similar effects of exercise on haematological parameters have been described in numerous studies that have been conducted in the recent years. Altogether it is considered that exercise has a positive effect on health, which has been called in question by some scientists, especially having in mind exhaustive exercise. During prolonged hard muscular exercise the circulatory system is placed under stress due to the requirements for a high blood flow to the working muscles to sustain a high metabolic rate (Maughan *et al*., 1985). It has been proved that exercise affects haemorheology, which has a possible connection between beneficial haemodynamics and metabolism (Brun *et al*., 1998). In most studies short-term effects of exercise on haematological parameters are controversial (O'Toole *et al*., 1999), which is probably due to individual differences or sometimes to pre-exercise overhydration. Middle-term and long-term effects in all studies are the same – increase in blood fluidity, explained by plasma volume expansion (autohaemodilution) that lowers both plasma viscosity and haematocrit and is result of hormonal and metabolic alterations (Brun *et al*., 1998). This status has been described as "sports anaemia" and is actually no true anaemia so it should be preferably called "sports pseudoanaemia". The term "sports anaemia" has been used by exercise scientist investigating dogs for several decades till now (Richard *et al*., 1998). There is a number of theories explaining the mechanism of this anaemia. One theory points intravascular haemolysis as the main reason contributing to anemia (Smith *et al*., 1995). Haemolysis occurs consequently to oxidative and osmotic stress. Haemolysis can also be mechanical – red blood cells can be impaired by repeated foot impact ("foot-strike haemolysis") or by contraction of muscles (Weaver & Rajaram, 1992). Intravascular haemolysis during and after exercise is accompanied by haemoglobinuria, which has been described more than a century ago.

Nowadays some authors suggest that endurance training in rats reduces acute exhaustive exercise-induced oxidative stress in erythrocytes (Oztasan *et al*., 2004). Nutritionists have proposed the use of antioxidants (Machefer *et al*., 2007), but "sports anaemia" still exists. Another theory attempting to explain "sports anaemia" concerns exercise-induced iron deficiency. Possible mechanisms include iron losses in sweat, gastrointestinal bleeding and haematuria, and chronic increase in hepcidin levels, which have been described after acute exercise (Roecker *et al*., 2005). The effects of acute exercise on iron parameters are controversial (Duca *et al*., 2006). Elevated blood levels of sodium after exercise have been found in horses (Goundasheva & Katsarova, 2008). Such changes have also been described in humans and could be a possible reason for ha emodilution (Maron *et al*., 1977). Another research describes elevation of aldosterone and antidiuretic hormone during and after exhaustive run of rats in heat, which could be adaptional response to maintain or increase plasma volume (Francesconi & Milton, 1983).

Regardless of factors contributing to this "pseudoanaemia", we can conclude that the observed middle- and long-term decrease in erythrocyte counts and haemoglobin concentration, even after a single bout of acute exhaustive exercise, was a physiological phenomenon contributing to increased blood fluidity, which is needed to maintain high blood flow to the working muscles through lesser tension of the circulatory system.

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