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Effects of aerobic long distance running training (up to 40 km · day $^{-1}$) of 1-year duration on blood and endocrine parameters of female beagle dogs

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Summary. The effects of long distance running training on blood parameters, hormone responses and bone growth were studied in young growing dogs. A genetically uniform group of female beagles matched with respect to age and body mass were used. The runner dogs ($n=10$) underwent gradually increased running exercise up to 40 km · day $^{-1}$ on a treadmill with 15° uphill gradient 5 days each week during a period of 1 year, while the littermate control dogs ($n=10$) were kept in their cages throughout the study. Low plasma lactate concentrations of the runners measured immediately after the running training indicated the aerobic metabolism of the dogs while running. Significant decreases of blood haemoglobin concentrations (11%), blood erythrocyte number (10%), and erythrocyte packed cell volume (12%) were found in the runner group. Throughout the experiment, the value of thyroxine was slightly lower (13%) in the runners but no changes were found in tri-iodothyronine, free thyroxine, or cortisol serum concentrations. Serum oestradiol concentration at 56 weeks was significantly lower (42%) in the runner group than in the control group but was not as low (27%) at 70 weeks. Somatomedin-C concentration had decreased significantly by 37% at the age of 56 weeks in the runner group but was again at the level of the control dogs at the end of experiment (at 70 weeks). Ulna and radius bone mass as a ratio to the body mass had significantly increased in the runners. It would seem from our study that long distance running has a positive effect on bone growth. However, inadequate energy intake may have brought about lowered body mass with altered endocrine homeostasis, especially affecting oestradiol and somatomedin-C.

Key words: Endurance training – Haematology – Serum hormones – Lactate – Dog

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Introduction

Long distance running exercise can be regarded as a complex physical stress to which the energy metabolism, reproductive system, and growth of the body must adapt. Exercise causes increased metabolic demand, which can be met in part by inductive effects of thyroid hormones and cortisol to carbohydrate, fat, and protein metabolism. However, disagreement exists over the effects of endurance exercise on the plasma concentrations of the thyroid hormones and cortisol in humans (Galbo 1986; Viru 1992). Plasma concentrations of oestradiol and progesterone, the important hormones in the regulation of the menstrual cycle and bone metabolism in women, have been found to increase (Jurkowski et al. 1978; Bonen et al. 1979), decrease (Lamon-Fava et al. 1989) or show no change (Bonen et al. 1979; Loucks and Horvath 1984) in response to exercise. Plasma somatomedin-C concentration, which plays a major role in muscle, bone and cartilage growth (Rogol 1989), would seem to be decreased by poor energy balance during running exercise (Smith et al. 1987).

The red blood cells also play an important role during physical exercise in enabling efficient oxygen transport to tissues. Endurance exercise in several sports, including long distance running, has been reported to be accompanied by decreased haemoglobin concentration (Carlson et al. 1986; Lampe et al. 1986). By measuring the haematological parameters, the possible roles of haemodilution, haemolysis and other mechanisms in this so called "sports anaemia" phenomenon can be studied.

In most human studies the interpretation of results is difficult due to numerous variables (age, sex, diet, body mass) affecting the endocrine system and haematological responses. Utilizing animal models in the study of the physiological effects of long distance running training, age, nutrient supply, and the environmental factors can be controlled better than in human experiments. However, despite the fact that animals have been extensively used to evaluate the effects of

exercise, most earlier studies have spanned only a period of about 3–4 months (Ready and Morgan 1984; Lassen et al. 1986; Sneddon et al. 1989), which is a relatively short time compared to normal exercise regimes of today's athletes, for example.

The purpose of this study was to investigate how haematological and hormonal systems of young beagle dogs adapt to long distance running. The running distance was gradually increased up to 40 km·day^{-1} during a period of 1 year. During the exercise period, the body mass and food consumption were controlled. Blood samples were taken for haematological screening and for the determination of serum thyroid hormones, cortisol, oestradiol, progesterone, somatomedin-C, creatinine, and plasma lactate concentrations. Urine creatinine excretion was determined. In addition, bone and muscle mass investigations were carried out. This study is part of a research project to study the effects of long distance running on articular cartilage (Arokoski et al. in press) and bone (Puustjärvi et al. 1992; Oettmeier et al. 1992). Because both tissues showed significant changes after running compared to the control tissues, it was thought to be necessary to evaluate how the physiological homeostasis of the body reacted towards long distance running exercise.

Methods

Animal husbandry and feeding. Female beagle dogs of pure breed from the National Laboratory Animal Centre (Kuopio, Finland, 4 animals) and from the Shamrock Ltd. (Hereford, England, 16 animals) were used as experimental animals. All the dogs had been born within a period of 1 month. Each runner ($n=10$) dog had her age-matched sister as the control ($n=10$). All dogs were kept in individual stainless steel cages (bottom area 0.9×1.2 m and height 0.8 m) equipped with a stainless steel bed raised 40 cm above the floor. The animal husbandry was performed according to the outlines of the Institute of Laboratory Animal Resources, NIH (1985). The dogs were fed with commercial dog food (Hankkija, Kolppi, Finland). When the running distance was less than 20 km·day^{-1}, the dogs were fed twice a day at 11 a.m. and at 3 p.m. However, when the running distance was more than 20 km·day^{-1}, the daily feeding took place only between 3 and 4 p.m. The mass of the daily food portion for each dog was determined from the weekly measurements of body mass with the aim of maintaining the body mass equal in both groups. Daily food intake and food refusals were recorded. The dog food contained an energy equivalent of 13.5 MJ·kg^{-1} (proteins 38.0%, fat 37.8% and carbohydrates 24.2% in dry matter). Water was available ad libitum. The indoor dark period was between 9 p.m. and 7 a.m. and the lights were on between 7 a.m. and 9 p.m.

Running protocol. A specially built ten-track treadmill for the running training of the dogs was used (Arokoski et al. 1991). The running exercise programme started at the age of 15 weeks (Fig. 1). The dogs were first accustomed to running on the treadmill set at 15% uphill gradient. The speed of the treadmill belt was adjusted so that all 10 dogs could run successfully on the treadmill at the same time. The distance of the daily run, and of the treadmill belt speed, was gradually increased until the daily running distance was 40 km at the age of 55 weeks (final treadmill belt speed 5.5–6.8 km·h^{-1}). From this time onwards, the dogs ran 40 km·day^{-1} for an additional 15 weeks. When the dogs ran less than 20 km·day^{-1} the rest period between two daily running

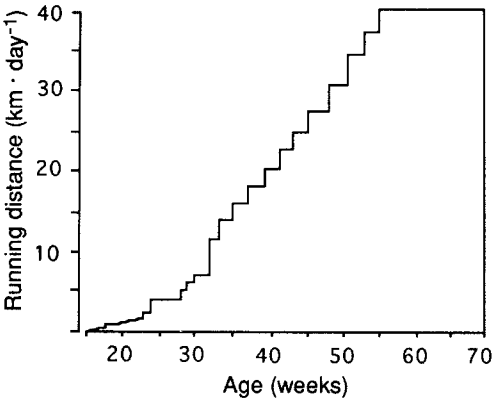

Fig. 1. Design of the long distance running experiment (40 km·day^{-1}) with beagle dogs. From the age of 15 weeks the dogs ran 5 days a week on a treadmill with 15° uphill gradient. The running distance was gradually increased to 40 km·day^{-1}. The dogs ran this distance on each working day (5 days a week) during the final 15 weeks (from age 55 to 70 weeks)

sessions was 1.5 h. The dogs ran 5 days·week^{-1} from Monday to Friday. Duration of a daily 40-km running session varied between 6 h 50 min and 7 h 10 min. The active running time was from 6 h 10 min to 6 h 25 min (three pauses with access to water were allowed, each 10–15 min). The design of the experiment was approved by the Animal Care and Use Committee of the University of Kuopio.

Cage activity measurements. The activity of the dogs in cages was determined using the rest shelf method (Harri et al. 1988) during the season when the daily running distance was 40 km·day^{-1}. The estimation was derived from temperature changes of the steel bed plate, when the animal rested on the plate. Temperature changes were recorded for different time intervals, i.e. 8 a.m.–4 p.m., 4 p.m.–8 a.m. and 6 p.m.–6 a.m. Since the running training took place from Monday to Friday, the measurements from 8 a.m. to 4 p.m. were made during the weekends (Saturday or Sunday).

Blood sampling and analytical procedures. Blood samples were obtained from the jugular vein on Tuesday or Friday. The haematological screening analysis (red and white blood cell counts), thyroid hormone, cortisol, oestradiol, progesterone, somatomedin-C, and creatinine samples were taken between 7.30 a.m. and 8.15 a.m. at rest (before the running exercise). Blood samples for lactate measurements were taken within 15 min after the daily running exercise (between 3 p.m. and 3.30 p.m.) (Proscurshim et al. 1989). The blood samples from the control dogs were taken simultaneously.

Samples for haematological analysis were taken at the age of 39, 56 and 70 weeks into Venoject VT-574TKZ tubes containing K_3-ethylenediaminetetra-acetic acid (TERUMO Europe N.V., Belgium). Haemoglobin concentration ([Hb]), red blood cell count (RBC), packed cell volume (PCV), erythrocyte mean cell volume (MCV), erythrocyte mean cell haemoglobin (MCH), erythrocyte mean cell haemoglobin concentration [MCH], platelet count (PLC), white blood cell count (WBC), and numbers of neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), and reticulocytes were determined.

Samples for lactate analysis were taken to Venoject VT-053FX fluoride tubes (6.75 mg sodium fluoride, 6.75 mg potassium oxalate) (TERUMO Europe N.V.) at the age of 56 and 70 weeks. The samples were centrifuged (1000×g, 5 min) at 21–22° C, and plasma was stored at −20° C until assayed. Plasma lactate concentration was determined using Kone Specific Clinical Chemical Analyser (Kone Ltd., Espoo, Finland).

Blood samples for hormone analysis were taken into Venoject VT-AS109Y gel tubes (TERUMO Europe N.V.). The samples were centrifuged ($1000 \times g$, 10 min) at 4° C, and the serum was stored at −70° C until assayed. All the samples for hormone analysis were assayed in duplicate and all analyses for a particular hormone of 1 dog were made at the same time. The intra-assay and interassay coefficients of variation were determined by duplicate measurements of the same samples in the same assay and in separate assays, respectively.

Blood samples for thyroid and cortisol hormone analyses were taken at seven specified times during the experiment. Analyses of serum thyroid hormones and cortisol were performed using radio-immunoassay (RIA) test reagents from Farmos Diagnostica (Turku, Finland). Spectria T_3, T_4, FT_4 and cortisol Coated Tubes RIA were utilized for the quantitative determination of T_3 (tri-iodothyronine), T_4 (thyrosine), FT_4 (free thyroxine) and cortisol in serum, respectively. The sensitivity of the methods was as follows: T_3, 0.2 nmol·l^{-1}; T_4, 5 nmol·l^{-1}; FT_4, 1 pmol·l^{-1}; and cortisol, 5 nmol·l^{-1}. The intra-assay coefficients of variation of the above methods were as follows: $T_3 < 6\%$, $T_4 < 7\%$, $FT_4 < 7\%$, and for cortisol <5%. The interassay coefficients of variation were: $T_3 < 8\%$, $T_4 < 8\%$, $FT_4 < 11\%$, and for cortisol <7%.

Serum oestradiol and progesterone assays were carried out using the radio-immunoassay kit from DPC (Diagnostic Products Corporation, Whitney, England). The samples were taken at ages 56 and 70 weeks, when all the dogs were at different phases of the estrus cycle. The method sensitivity was as follows: oestradiol, 10 pmol·l^{-1}; and progesterone, 160 pmol·l^{-1}. The intra-assay and interassay coefficients of variation for oestradiol were <7% and <10%, respectively. Those for the progesterone assays were <8.5% and <10%, respectively.

Serum somatomedin-C concentration was determined at ages 56 and 70 weeks by RIA test reagents from Incstar Corporation (Stillwater, Mich., USA). This method is designed to measure somatomedin-C in double antibody disequilibrium assay which includes an ODS-silica extraction procedure for serum samples. The method sensitivity is less than 2.0 nmol·l^{-1}. The intra- and interassay coefficients of variation for somatomedin-C were <9% and <10%, respectively.

Samples for serum creatinine and urine creatinine analyses were taken at ages 56 and 70 weeks. Blood creatinine samples were taken into Venoject VT-100PZ tubes (TERUMO Europe N.V.) and the samples were centrifuged ($1000 \times g$, 10 min) at 21–22° C, and serum was stored at −20° C until assayed. Urine specimens were collected over 24 h at the metabolic cages during the weekends (Saturday or Sunday) and urine samples were stored at −20° C until assayed. Serum and urine creatinine samples were analysed using Kone Specific Clinical Chemical analyser (Kone Ltd.).

The dogs were sacrificed at age 70 weeks. Radiographs were taken of the knee and shoulder joints. The thorax of the animals was immediately opened and the heart was removed and weighed. The ulnar and radius bones were feed from soft tissue and weighed. The semitendinosus muscle (high percentage of type I fibres; Rosenblatt et al. 1988) and tibialis cranialis muscle (high percentage of type II fibres; Newsholme et al. 1988) as well as thyroid and adrenal glands were weighed.

Statistical methods. The nonparametric Wilcoxon's matched-pairs signed-rank test was used for statistical analysis when the values of treated animals were compared with those of the control animals. Statistical significance between the group means was determined by two-tailed, nonparametric Mann-Whitney *U*-test.

Results

In general the dogs were healthy throughout the study period. Minor paw excoriations and injuries were detected as described earlier (Arokoski et al. 1991). At the beginning of the training, there was individual variation in the ability and the willingness of the animals to run on the treadmill. All runners completed the running programme successfully, however. The runner dogs were never fully exhausted during the running training. The running exercise caused no statistically significant differences in the activity behaviour of the dogs in the cages (Table 1).

Based on x-ray pictures, the growth plates in the distal femur, the proximal tibia and the proximal humerus were closed (calcified) in all animals at age 70 weeks. The mass and the length of ulnar and radius bones were slightly, but not significantly, increased (Table 2). Ulna and radius bone mass as a ratio to body mass was significantly increased in the runners. The mass of semitendinosus and tibialis cranialis muscles showed no differences between the groups (Table 2). The heart mass of the runners was significantly higher than in the controls (Table 2). The masses of

Table 1. The activity of beagle dogs in the cages during a 24-h cycle at the season when the animals ran 40 km·day^{-1}

Measurement time	Rest period			
	Controls (%) ($n=6$)		Runners (%) ($n=10$)	
	mean	SEM	mean	SEM
8 a.m.–4 p.m.	21.3	3.7[a]	13.5	5.2
4 p.m.–8 a.m.	55.7	5.2	45.5	8.7
6 p.m.–6 a.m.	60.4	5.8	48.5	5.8

[a] Values are expressed as proportion (%) of the rest (including sleep) period of the whole measurement time; *n*, number of dogs. No statistically significant differences were observed between the groups using two-tailed Mann-Whitney *U*-test

Table 2. Physiological parameters of beagle dogs at the end of running 40 km·day^{-1} for 15 weeks

Parameter	Controls		Runners	
	mean	SEM	mean	SEM
Ulna bone				
mass (g)	14.4	0.4[a]	15.5	0.6
bone mass (g)/body mass (kg)	1.16	0.03	1.26	0.04*
length (mm)	139	3	145	2
Radius bone				
mass (g)	12.8	0.4	14.4	0.6
bone mass (g)/body mass (kg)	1.03	0.02	1.17	0.04*
length (mm)	114	1.9	116	2.5
Muscle mass (g)				
MTC	9.6	0.6	9.3	0.3
MS	41.3	1.4	39.0	1.7
Heart mass (g)	117	5	130	4*
Thyroid gland, mass (g)	0.84	0.04	0.93	0.06
Adrenal gland, mass (g)	1.5	0.1	1.6	0.1

[a] Values are for 9–10 animals per group. MTC, tibialis cranialis muscle; MS, semitendinosus muscle. Significantly different from corresponding control values using Wilcoxon's matched-pairs signed-rank test: * $P<0.05$

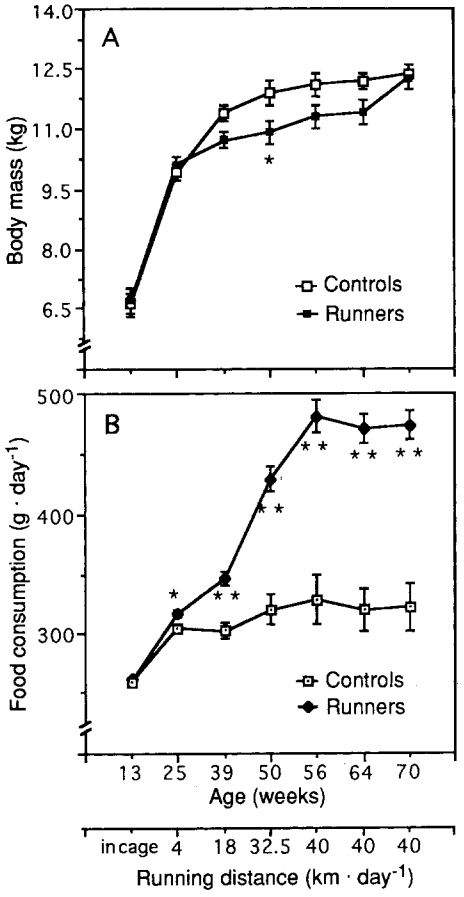

Fig. 2. Body mass (**A**) and food consumption (**B**) during the long distance running of beagle dogs. Values are means and SEM for ten animals per group. Significantly different from corresponding control values using Wilcoxon's matched-pairs signed-rank test: ** $P<0.01$, * $P<0.05$

the thyroid and adrenal glands were equal in both groups (Table 2).

The body mass of the growing beagles nearly doubled during the experiment (Fig. 2A). The control dogs had a slightly higher body mass than the runner dogs between weeks 39 and 64. At age 50 weeks the difference was statistically significant. At 70 weeks the body mass was again the same in both groups. The food intake of the runners increased nearly linearly as the running distance increased (Fig. 2B). At 70 weeks, the energy requirement was 4.3 (SEM 0.3) and 6.4 (SEM 0.2) MJ · day^{-1} for the control and the runner dogs, respectively.

The running training decreased [Hb], RBC and PCV during the experiment (Table 3). The average decrease of Hb was 11%. The MCV, MCH and [MCH] decreased significantly in the middle of the experiment (running distance 20 km · day^{-1}). The number of PLC, leucocytes and reticulocytes did not alter on account of running (Table 3).

Throughout the running experiment, the values of serum T_4 concentration were slightly but not significantly lower in the runner group (Table 4). The running did not affect T_3 or FT_4 concentrations. The mean values of FT_4 concentration decreased after age 13 weeks in both groups.

Although the mean serum cortisol concentration was lower in the running group in the beginning of the experiment, there were no significant differences in serum cortisol concentrations between the control and the runner groups (Table 5). The age of the animal did not affect the cortisol concentration.

Serum oestradiol concentration at 56 weeks was significantly lower (42%) in the runner group than in the control group, but was not as low (27%) at 70 weeks (Table 6). There was no statistical difference between oestradiol values measured at 56 and 70 weeks either

Table 3. Effect of long distance running training on haematological parameters of beagle dogs. ($n=10$ animals per group)

Parameter	20 km · day^{-1} (39)[a]				40 km · day^{-1} (56)[a]				40 km · day^{-1} (70)[a]			
	Controls		Runners		Controls		Runners		Controls		Runners	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
[Hb] (g·l^{-1})	155	2	135	3**	157	3	138	4*	151	4	137	3
RBC (10^{12}·l^{-1})	6.81	0.14	5.85	0.16**	6.74	0.13	6.19	0.15*	6.79	0.16	6.24	0.12*
PCV (%)	0.46	0.01	0.39	0.01**	0.45	0.01	0.40	0.01*	0.45	0.01	0.40	0.01*
MCV (fl)	67.2	0.6	67.1	0.3	66.5	0.5	64.5	0.5**	65.8	0.6	64.6	0.5
MCH (pg)	22.8	0.2	23.1	0.2	23.3	0.2	22.2	0.2**	22.3	0.2	21.9	0.1
[MCH] (g·l^{-1})	339	2	344	1	350	1	345	2*	339	2	339	2
PLC (10^9·l^{-1})	319	18	330	24	390	32	377	23	361	27	357	17
WBC (10^9·l^{-1})	10.3	0.6	10.8	0.4	10.2	0.4	11.7	0.9	9.4	0.6	8.8	0.3
NEUT (%)	0.55	0.02	0.57	0.03	0.65	0.03	0.68	0.02	0.58	0.02	0.57	0.02
LYMPH (%)	0.40	0.02	0.36	0.02	0.29	0.03	0.26	0.02	0.31	0.02	0.32	0.02
MONO (%)	0.05	0.01	0.04	0.01	0.03	0.01	0.04	0.01	0.09	0.01	0.09	0.01
EOS (%)	0.02	0.00	0.04	0.01	0.04	0.01	0.04	0.01	0.02	0.00	0.02	0.01

[a] Age of dogs in weeks; [Hb], haemoglobin concentration; RBC, red blood cell count; PCV, packed cell volume; MCV, erythrocyte mean cell volume; MCH, erythrocyte mean cell haemoglobin; [MCH], erythrocyte mean cell haemoglobin concentration; PLC, platelet count; WBC, white blood cell count; NEUT, neutrophils; LYMPH, lymphocytes; MONO, monocytes; EOS, eosinophils. Significant differences between the runner and control groups using Wilcoxon's matched-pairs signed-rank test: ** $P<0.01$, * $P<0.05$

Table 4. Effect of long distance running training on serum thyroid gland hormone concentrations of beagle dogs ($n=8$–10 animals per group)

Running distance ($km \cdot day^{-1}$)	Age (weeks)	T_4 ($nmol \cdot l^{-1}$)				T_3 ($nmol \cdot l^{-1}$)				FT_4 ($pmol \cdot l^{-1}$)			
		Controls		Runners		Controls		Runners		Controls		Runners	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
In cage	13	41.4	3.4	40.3	2.4	1.10	0.07	1.09	0.09	20.2	1.7	20.9	1.4
4.0	25	38.0	4.5	34.5	2.2	1.05	0.09	1.15	0.11	16.1	2.6	16.4	1.4
18.0	39	31.3	2.2	29.3	1.9	0.96	0.05	0.97	0.15	13.8	2.0	15.5	1.8
32.5	50	43.0	5.6	31.7	3.0	1.16	0.03	1.09	0.14	16.3	2.4	15.3	1.5
40.0	56	39.6	4.1	31.5	2.6	1.12	0.08	1.16	0.10	17.1	2.4	16.7	2.4
40.0	64	34.9	4.7	29.9	1.6	1.06	0.04	1.04	0.08	15.8	2.6	15.9	2.3
40.0	70	35.7	4.8	31.5	2.3	1.23	0.09	1.07	0.09	16.4	2.1	16.2	1.8

T_4, thyroxine (3,5,3′,5′-tetraiodothyronine); T_3, 3,5,3′-tri-iodothyronine; FT_4, free thyroxine. For statistical analysis, Wilcoxon's matched-pairs signed-rank test was used to compare the runner and control values at different ages. No significant differences were observed

Table 5. Effect of long distance running training on serum cortisol concentration of beagle dogs. ($n=8$–10 animals per group)

Running distance ($km \cdot day^{-1}$)	Age (weeks)	Cortisol ($nmol \cdot l^{-1}$)			
		Controls		Runners	
		mean	SEM	mean	SEM
In cage	13	5.8	1.2	7.0	1.5
4.0	25	6.0	1.6	2.7	0.9
18.0	39	6.0	1.9	3.3	1.3
32.5	50	8.4	2.8	9.8	4.0
40.0	56	5.0	1.5	6.3	1.0
40.0	64	3.6	1.0	10.8	2.9
40.0	70	6.8	1.7	5.4	1.6

For statistical analysis, Wilcoxon's matched-pairs signed-rank test was used to compare the runner and control values. No statistically significant differences were observed

in the control or in the runner group. Serum progesterone concentrations were high [peak serum concentrations ($>32\ nmol \cdot l^{-1}$) in the luteal phase] in 3 of the control dogs [63.2 (SEM 12.2) $nmol \cdot l^{-1}$] and 2 of the runner dogs [54.0 (SEM 16.3) $nmol \cdot l^{-1}$].

Serum somatomedin-C concentration decreased significantly, by about 37% at age 56 weeks in the runners, but was again at the level of the control dogs by the end of the experiment (Table 6).

At age 56 weeks serum and urine creatinine concentrations decreased significantly in the runners by about 13% and 28%, respectively, but were again at the level of the control dogs at the end of the experiment (Table 6). There were no significant differences between the runner and control dogs in the excretion of creatinine into urine as calculated per 24 h or per 24 h per unit of body mass (kg) (Table 6).

The runner dogs had a significantly lower plasma lactate concentration after running than the control dogs, on an average 36% at weeks 56–70 (Table 6).

Discussion

To reduce the biological variation between individual animals (Robinson and Ziegler 1968), a genetically uniform group of beagles, matched with respect to sex and age, was used for investigation. The results from the cage activity measurements agreed with the find-

Table 6. Effect of long distance running training on serum oestradiol, progesterone, somatomedin-C, creatinine, plasma lactate, and urine creatinine concentrations of beagle dogs. ($n=9$–10 animals per group)

Parameter	40 $km \cdot day^{-1}$ (56)[a]				40 $km \cdot day^{-1}$ (70)[a]			
	Controls		Runners		Controls		Runners	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Serum oestradiol ($nmol \cdot l^{-1}$)	0.036	0.009	0.021	0.003*	0.030	0.007	0.022	0.004
Serum progesterone ($nmol \cdot l^{-1}$)	12.1	5.7	11.1	6.8	9.0	8.7	4.0	3.7
Serum somatomedin-C ($nmol \cdot l^{-1}$)	8.3	1.7	5.2	0.5*	7.1	0.9	6.9	1.0
Serum creatinine ($\mu mol \cdot l^{-1}$)	86.1	4.2	74.5	4.1*	83.5	2.4	80.3	6.3
Plasma lactate ($mmol \cdot l^{-1}$)	2.00	0.34	1.29	0.11*	1.3	0.12	0.83	0.06**
Urine creatinine ($mmol \cdot l^{-1}$)	14.9	1.6	10.7	1.1*	12.1	1.0	11.7	1.1
Urine creatinine in 24 h (mmol)	3.8	0.3	3.5	0.5	6.7	0.7	6.8	0.4
Urine creatinine in 24 h (mmol · body mass in kg^{-1})	0.32	0.02	0.31	0.04	0.54	0.06	0.56	0.02

[a] Age of dog in weeks. Significantly different from corresponding control values using Wilcoxon's matched-pairs signed-rank test: ** $P<0.01$; * $P<0.05$

ings of Hughes et al. (1988) and Campbell et al. (1988), who have shown that neither moderate running (35 min three times each week for 6 weeks) nor continuous cage housing and the size of the cage had a significant effect on the running activity of the dogs.

To inhibit the harmful effect of loss of body mass on hormone responses (Wentz 1980; Cella et al. 1989; Reimers et al. 1990), we tried to keep the body mass at the same level in both groups. With this aim the dogs were weighed once a week to determine whether the food intake was adequate. At ages 39–64 weeks the runner dogs did not gain sufficient body mass to maintain a body mass equal to that of the control dogs. There were no concomitant differences in thyroid and cortisol hormone concentrations, however. But at age 56 weeks, significantly lower serum oestradiol and somatomedin-C concentrations were observed in the runners. Because the serum creatinine concentration and its excretion in the urine has been found to be proportional to muscle mass (Forbes and Bruining 1976; Lucas et al. 1986), lower serum and urine creatinine concentrations at this age probably reflected lower muscle mass. This difference was no longer observed at 70 weeks. In agreement with earlier studies (Belcastro and Wenger 1982; Kovanen 1989), endurance running training did not induce muscle hypertrophy. Although, apparently, an enhanced fat metabolism took place during prolonged muscle work (Wasserman et al. 1989), there was no difference between the two groups in body fat content as judged from the mass of omentum majus at the end of the experiment (Arokoski et al. 1991).

The low lactate concentration after running indicated that aerobic metabolism of the dogs was maintained during running. This finding agrees with the results of Ready and Morgan (1984) who have shown that submaximal 12 weeks' interval training reduced blood lactate concentrations in dogs. A greater oxidative capacity, reduced lactate production in skeletal muscle, or greater clearance of lactate from the blood, would explain the lower lactate concentrations in the trained animals and humans (Donovan and Brooks 1983; MacRae et al. 1992). Moreover, the runner dogs had a low pulse rate, which never reached maximal levels during running training, and the resting pulse rate was also lower than that of the control dogs (Arokoski et al. 1991). This might have been due to an increased cardiac output during the running exercise.

The amount and volume of erythrocytes ([Hb], RBC, PCV) was significantly reduced during the long distance running. This result is in accordance with the observation of Adkins and Kronfeld (1982), who have studied Alaskan huskies after a 1900 km race over 16 days. Athletes, especially trained endurance runners, often have lower [Hb] and PCV values than sedentary persons (Casoni et al. 1985; Carlson and Mawdsley 1986). An increase of the total blood plasma volume has also been shown to be associated with endurance training both in animals and in humans (Mackintosh et al. 1983; McKeever et al. 1985; Fellmann 1992). This may produce sufficient haemodilution to convert a normal [Hb] to anaemic levels. An increase in plasma volume with a lower PCV without RBC change, or an actual reduction in RBC, would cause a relative or a true anaemia, respectively. The decrease in [Hb] and PCV values was here associated with the decrease of RBC. Thus, decreased [Hb] of the runner dogs can be indicative of true anaemia.

There are two possible mechanisms for the decrease of RBC. Firstly, an increased red cell breakdown during endurance training has been suggested to occur due to accelerated mechanical destruction of erythrocytes (Miller et al. 1988). The characteristic haematologic profile of the footstrike haemolysis of runners has been found to include macrocytosis and increased reticulocytosis (Eichner 1985), which, however, was not seen in the present study. Secondly, a decreased erythrocyte count due to iron deficiency (Hunding et al. 1981; Weiser and O'Grady 1983; Nagel et al. 1992) might explain a decreased [Hb], because MCV, MCH and [MCH] were slightly but significantly decreased in the middle of the experiment (when the running distance was 20 $km \cdot day^{-1}$), although the erythrocytes remained normocytic and normochromic. It has been recognized that long distance runners may have a pronounced risk of iron deficiency compared with other athletes (Clement and Asmundson 1982). This has been shown to be particularly evident in female long distance runners (Lampe et al. 1986; Eichner 1992).

There was no change in total PLC or in WBC, or individual leukocyte subset counts (NEUT, LYMPH, MONO, EOS), suggesting that long distance running does not have an effect on the defense or immune system parameters. This contrasts with many previous reports. An increase has been found in the PLC, as well as leukocytosis being observed, following both short and long distance running exercises in studies by Lassen et al. 1986, Davidson et al. 1987, Oshida et al. 1988, Ilkiw et al. 1989, and Nagel et al. 1992. On the other hand, Wells et al. (1982) and Nieman et al. (1989) have reported that the leukocytosis which followed a long-lasting endurance run returned to normal in 21–24 h. Robbins and Kumar (1987) have suggested that the increase in WBC was due to increased blood flow which washed these cells from blood vessel walls and caused an apparent increase of WBC, although the actual number of intravascular WBC was not changed. When the blood specimen is collected in the resting state, as in this study, these cells may remain adherent to the blood vessel walls and increased values were not observed.

Controversy exists concerning the responses of thyroid function to chronic physical training. With regard to T_4 changes during or after exercise both increases (Kirkeby et al. 1977; Boyden et al. 1984; Schürmeyer et al. 1984; Sander and Röcker 1988; Hesse et al. 1989) and decreases (O'Connell et al. 1979; Hohtari et al. 1987) or no change (Premachandra et al. 1981) have been reported in humans. Our results are in good accord with the observations of Kirkeby et al. (1977) and Sander and Röcker (1988), who have reported that, 1 day after training, the concentration of total T_4 was un-

changed or slightly reduced. Reduced concentrations of pituitary thyroid stimulating hormone due to central feedback mechanism might explain this phenomenon. On the other hand, it has been thought that depressed T_4 concentrations in long distance runners might primarily be due to lowered thyroxine-binding globulin (TBG) levels (Hohtari et al. 1987). While it has been found that synthesis of TBG in liver is dependent on oestrogens (Dowling et al. 1960), lowered serum concentrations of oestradiol have been shown to be in part responsible for the decreased concentrations of total T_4 in running women (Hohtari et al. 1987). Since no significant T_3 changes were measured after long distance running, peripheral conversion of T_4 to T_3 was not likely to regulate T_4 blood concentrations.

Kirkeby et al. (1977) and Sander and Röcker (1988) have found an increase in FT_4 during or after prolonged exercise, whereas Schmid et al. (1982) and Hohtari et al. (1987) have found a decrease in FT_4 concentrations in humans. It would seem that the increase of FT_4 concentrations observed during exercise returns to the pre-exercise level during rest (Kirkeby et al. 1977; Sander and Röcker 1988). Because the concentration of the active form of FT_4 was unchanged, the risk of thyroidal hypo/hyperfunction after long distance running seems to be negligible.

Long distance running seemed not to have any effect on serum cortisol concentration. The blood samples for cortisol analysis were taken in the morning in an apparently stress-free situation, which might have yielded cortisol values lower than those drawn immediately after running. According to studies in humans and animals, the increase of the blood cortisol concentration measured immediately after exercise has returned to the control level, or even below, during recovery (Maron et al. 1975; Kuoppasalmi et al. 1981; Keizer et al. 1989; Radosevich et al. 1989). This may also have been the situation in this study. The circadian rhythm cycles have been found to develop in late adulthood in beagles (Palazzolo and Quadri 1987). In pups and young adult beagles, a number of cortisol secretion peaks per day can explain the fluctuation of the cortisol values (Johnston and Mather 1978).

In humans, prolonged running exercise has been shown to decrease serum oestradiol concentrations, which is associated with menstrual irregularities (Loucks and Horvath 1984; Lamon-Fava et al. 1989) and delayed menarche in cases when the sport activity was started before puberty (Frisch et al. 1981; Warren 1980). Menstrual disturbance has also been observed in women who had a loss of body mass during the exercise period (Bullen et al. 1985) or had abnormal thyroid function (Boyden et al. 1984). Although it has been found that there is considerable variation in oestrus cycles among female beagles (Concannon et al. 1989), the low concentration of oestradiol might be affected by the reduced body mass. However, according to clinical signs, the running did not affect the heat symptoms.

The ulna and radius bone mass as a ratio to body mass was significantly increased in the runners, suggesting that mechanical loading can stimulate bone remodelling, which would agree with a previous report of Fedler et al. (1991) on beagles. Several studies also on humans would indicate that athletes have higher bone mass, measured as bone mineral content, than sedentary controls (Nilson and Westlin 1971; Williams et al. 1984; Marcus et al. 1992). However, in the same runner dogs a slight decrease in trabecular bone mineral density has been seen in the radius (Puustjärvi et al. 1992). In humans, clinical studies have shown that running exercise with low oestrogen concentrations may contribute to reduced mineral content of bone tissue (Drinkwater et al. 1984; Marcus et al. 1985). In adult beagles bone loss by oestrogen-depletion has been shown not to be a constant feature (Shen et al. 1992).

Neither growth hormone nor thyroid hormone were likely to have caused a reduction in somatomedin-C concentration. The growth hormone secretion has been found to rise with increased intensity and length of the exercise (Galbo 1986). Exercise was shown not to generate hypothyroidism. Blood somatomedin-C concentration has been found to be affected in the main by the content of binding proteins (Jahreis et al. 1989), by energy balance (Clemmons et al. 1981; Cella et al. 1989), and by the time of the sample collection in relation to exercise (Smith et al. 1987). In dogs, there has been found to be a significant correlation between body mass and somatomedin-C concentrations (Eigenmann et al. 1988). In this study, concomitantly with the gain of body mass in the runner group, indicating an adequate state of nutrition, the concentration of serum somatomedin-C increased as well. The growth plates were closed in all of the animals by the end of the experiment, so the animals had completed long bone growth at about age 1 year. Because reduction in somatomedin-C can lead to a decrease of linear growth in growing children (Rogol 1989), care should be taken to guarantee adequate nutrient intake during prolonged exercise.

In summary, long distance aerobic running decreases Hb values in dogs. This phenomenon in humans has been called "sports anaemia". It can be regarded as a side-effect of endurance running training. Long distance running would seem to have a positive effect on bone growth. However, inadequate energy intake may bring about lowered body and muscle mass with altered endocrinological homeostasis, especially affecting to oestradiol and somatomedin-C concentrations.

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