Effect of exercise on physiological, blood and endocrine parameters in search and rescue-trained dogs

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ABSTRACT: Exercise induces a variety of physiological and laboratorial changes of different magnitude and direction, depending on the characteristics of the performed exercise (duration and intensity) and on the fitness and training level of the dog. The present research aims to describe the normal response to a session of search and rescue exercise in trained dogs in order to distinguish these changes from those derived from exhaustion or diseases. Nine healthy and trained dogs of both sexes (five females and four males), aged between 24 months and seven years (mean: 3.5 years) were studied. Exercise consisted in a normal session of searching and rescue training of 20 min of duration, carried out in an open terrain. During the exercise, heart rate (HR) was monitored continuously with a HR-meter. Furthermore, respiratory rate (RR) and rectal temperature (RT) were measured and venous blood samples were extracted at rest (R), immediately after exercise (E) and at 5, 15 and 30 min of a passive recuperation (5REC, 15REC and 30REC). The following laboratorial parameters were studied: red blood cells (RBC), hemoglobin concentration (HB), packed cell volume (PCV), RBC volumetric indices, white blood cells (WBC), creatinine (CREAT), total plasma protein (TPP), lactate (LA), glucose (GLU), triacylglycerols (TAG), creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), Na, K, Cl, cortisol (CORT) and insulin (INS). Clinical signs indicative of exhaustion or exercise intolerance were not observed in the dogs during the study. HR increased with E and remained over the reference range until 30REC. RR and RT also rose with E, with the highest RR at 5REC. RBC, HB and PCV were not affected by E, whereas WBC increased at E. TPP, GLU, AST and K were not affect by E neither by REC. E induced elevations in CK, LDH, LA and INS, reaching R values at 30REC, 30REC, 15REC and 5REC, respectively. Plasma Na decreased with E and recovered at 30REC. Plasma Cl decreased with E, without additional significant changes. Circulating CORT concentrations were reduced with E, with the highest reduction at 10REC. Modifications of RR, RT, WBC, CREAT and TAG persisted throughout the recovery period. In conclusion, significant modifications in physiological and laboratorial parameters were induced by the searching and rescue exercise, with values outside the reference range for healthy dogs. These data provide a data base for evaluating ill or injured dogs during this type of exercise. In addition, there was not evidence of dehydration, electrolyte imbalances, and stress or muscle disorders in the studied dogs.

Keywords: dog; exercise; heart rate; hematology; hormones; lactate; metabolism

It is well known that exercise induces a variety of physiological and laboratorial changes depending on the characteristics of exercise, i.e. duration and intensity, and on the fitness and training level of the athlete. The popularity of canine sports has led to an increased number of scientific reports addressing the systemic changes that happen during competitions, as well as the time needed to achieve resting values after exercise. The knowledge of these changes is essential in order to design specific and individual training protocols, for an early diagnosis of lack of performance, to assess

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the impact of different feeding or supplementation strategies and to minimize the risk of exercise-linked diseases, such as exertional rhabdomyolysis, exhaustion, dehydration, heat stroke and electrolyte imbalances within others. In this way, several researchers have been conducted in order to assess the modifications associated with exercise in racing Greyhounds (Lassen et al., 1986; Snow et al., 1988; Ilkiw et al., 1989; Rose and Bloomberg, 1989; Nold et al., 1991), sled dogs (Querengaesser et al., 1994; Hinchcliff et al., 1993, 1998; Burr et al., 1997; McKenzie et al., 2007), Labrador dogs during retrieving exercises (Matwichuk et al., 1999; Steiss et al., 2004) and in dogs of different breeds during Agility competitions (Rovira et al., 2007a,b).

Physiological changes in heart rate (HR), respiratory rate (RR) and rectal temperature (RT) appear as a result of exercise and depending on the climatic conditions. HR is considered an indicator of the relative cardiovascular workload and therefore, it is very useful to monitor the intensity of a training session as well as to detect subclinical diseases or pain (Foreman et al., 1990; Munoz et al., 1999b). Indeed, in horses, a delayed HR recovery after exercise is indicative of exhaustion due to the lack of fitness or the appropriate training level for the required exercise intensity (Munoz et al., 2006). Similarly, increased RR and RT are normal findings after exercise. However, higher than expected RR and RT values can appear in heat stroke, with severe complications for the athletic dog such as acute renal failure, rhabdomyolysis, coagulation disorders, hepatocellular and myocardial necrosis and disturbances of electrolyte and acid-base balances (Bjotvedt et al., 1984; Dickinson and Sullivan, 1994). Furthermore, hyperthermia developed during sustained muscular work exerts adverse effects on muscle metabolism that is clearly a limitation to performance (Kozlowski et al., 1985; Gonzalez-Alonso, 2007).

Additionally, exercise induces significant modifications in some laboratory parameters that should be differentiated from those derived from diseases or exhaustion. Acute polycythemia results from dynamic splenic contraction in response to increased oxygen demands and metabolic stress (Snow et al., 1988; Ilkiw et al., 1989) as well as from a mild dehydration caused by the loss of water vapor in expired air in order to thermoregulate (Rovira et al., 2007a,b). However, a marked polycythemia is consistent with a severe dehydration. This consequence of exertion not only compromise the performance of the canine athlete, but also can be life threatening and will require a rapid medical intervention (Dickinson and Sullivan, 1994). White blood cells (WBC) are also increased during exercise (Snow et al., 1988; Ilkiw et al., 1989; Rovira et al., 2007b). Athletes are at increased risk of developing both septic and non-septic inflammations partially due to the stress of training and release of hormones that induce immunosuppression as cortisol, CORT (Nieman and Pedersen, 1999). These inflammations will affect the WBC dynamic with changes that can be similar to those induced by exercise.

Many serum biochemical parameters also are modified by exercise. One of the most important biochemical parameters in Sport Medicine is lactate (LA), a metabolite derived from glycolytic pathways, which is an indicator for the onset of fatigue (Munoz et al., 1999a; Rovira et al., 2007b). Hyperlactacidemia has been implied in the development of rhabdomyolysis and exhaustion in dogs (Brzezinska, 1987; Breitschwerdt et al., 1992). Recently, there is a concern about the development of electrolytic imbalances in exercising dogs. Hyponatremia has been described in dogs competing both in prolonged and short races (Hinchcliff et al., 1997; McKenzie et al., 2007). Symptomatic hyponatremia in human athletes leads to several complications, ranging from mild symptoms, such as nausea, fatigue and confusion to more severe symptoms, including seizures, respiratory arrest, increased intracranial pressure, coma and death (Warburton et al., 2002).

Search and rescue dogs have a great social impact because of their invaluable aid in searching missing people in different kind of disasters. Although the most important skills to train in these dogs are obedience, bonding to the handler and detection of human scent, they also must have an appropriate fitness level because they have to cover large areas of terrain, sometimes in hard climatic or terrain conditions. Therefore, from a medical point of view is important to establish the normal laboratorial modifications induced by exercise. The research reported here analyses the exercise-induced changes in some physiological and laboratorial parameters during an exercise session of search and rescue in trained dogs. It is pursued to define the normal response to this type of exercise that will permit us to differentiate these changes from those associated with exhaustion or other diseases and will help in the diagnosis of lack of performance or exercise intolerance in these dogs.

MATERIAL AND METHODS

Dogs

Nine dogs of both sexes (five females and four males) aged between 24 months and seven years (mean age: 3.5 years) and of different large breeds (four Golden Retrievers, two German Sherpherd, one Pittbull, one Belgian Sherpherd and one Boxer) were studied. All of them were in active training for land searching and rescue activities at the moment of the experiment. The dogs were examined by one veterinarian (S.R.) the day prior to the study. All of them were in good health condition on the basis of results of physical examination, hematology, plasma biochemical analyses and ECG. Furthermore, all the animals were serologically tested for the most common diseases in Spain in dogs (*Leishmania* spp. and *Erhlichia canis*). The animals were fed with the same commercial diet formulated for performance dogs.

Training

All the animals belonged to a Regional Association of Search and Rescue Dogs formed by experienced fire fighters. The animals started the training when they were 10 weeks of age and consisted in combined exercises for development of obedience to the handler, socialization, agility exercises and training to detect and follow human scent. Agility exercises were performed 3–5 times at week for 10 to 30 min and human scent detection training was made 3–5 times at week for 20–60 min each session. Although the level of training was not determined prior to the study, all the dogs were at active training at the moment of the experiment and the handlers did not observe significant changes in physical performance during the last two months before the study.

Exercise

The exercise was performed always in the evening and at least after 3 h after feeding in order to avoid the influence of circadian rhythms and feeding on laboratorial parameters. Dogs were not allowed to drink water or other kind of rehydration solutions during the 30 min before the exercise and during the first 30 min of recovery. The exercise consisted in a 20 min of a normal session of searching and rescue training. During this exercise, the speed and the gait of the dogs varied and therefore, no attempt to control these variables was made. The exercise was carried out in an open terrain of approximately 600 m by 600 m. Mean ambient temperature during the exercises was 21.2 ± 4.2°C and relative humidity was 58.4 ± 10.2%.

Data acquisition and samples analysis

Heart rate (HR) was monitored continuously during the exercise using a commercial HR-meter (Polar Horse Trainer®) at 5 s intervals. After that, data were transferred to a computer for later analysis. HR values reported in the present study are the mean HR values attained during the whole exercise session. Respiratory rate (RR) and rectal temperature (RT) were measured according to the conventional methods.

Venous blood samples were extracted from the cephalic vein immediately before starting the exercise session, at rest (R), within the first 2 min after finishing the exercise (E) and at 5, 15 and 30 min of a passive recuperation (5REC, 15REC and 30REC, respectively). Samples were poured into tubes with EDTA-3K (hematology and insulin concentrations, INS), into tubes with lithium heparin (biochemistry) and into glass tubes without anticoagulants (serum cortisol concentrations, CORT). Heparinized blood samples were centrifuged within the first 5 min after blood withdrawal and plasma was harvested. Samples in glass tubes were allowed to clot, they were centrifuged and serum was obtained. All the samples were stored at 4°C during their transport to the laboratory and analysis was made within the first 12 h after blood extraction.

In EDTA-blood, the following hematological parameters were determined using a semiautomatic cell counter (Sysmex-F820): red blood cells (RBC), hemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBC). In heparinized plasma, the concentrations of creatinine (CREAT), total plasma proteins (TPP), lactate (LA), glucose (GLU), triacylglycerols (TAG), creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and Cl were measured using spectrophotometric techniques (Helios α, Thermospectronic) with reagents designed for this instrument (SpinReact). Plasma Na and K concentrations were determined

by flame photometry. Serum CORT concentrations were quantified by competitive immune-enzyme analysis (Immulite Cortisol, PILKCO-6, Diagnostic Products Corporation) and plasma INS concentrations were measured by a sandwich immune-enzyme analysis (Immulite Insulin 2500, Diagnostic Products Corporation). Additional information about these determinations is provided elsewhere (Rovira et al., 2007a,b).

Statistics

Data are presented as mean ± SD. The normality of the variables was checked with a Shapiro-Wilk's test and the homocedasticity of the variance was checked with a Levene test. Those parameters that were not normally distributed or were heterocedastic (RR, CREAT, K, Cl and CK) were logarithmically transformed. The effect of exercise and recovery times in the studied variables was evaluated with an ANOVA for repeated samples. When significant differences were found, a post-hoc analysis (Tukey test) was made in order to investigate between which times significant differences existed. Correlations between the studied variables were assessed with a Pearson product-moment correlation. Statistical significance was set at $P < 0.05$ in all the cases. Statistical analysis was performed using the statistical software package Statistica® (Statistica for windows, v.6.0, Statsoft Inc.).

RESULTS

The eight studied dogs performed the exercise and recovered properly, without evidences of clinical signs indicative of fatigue, exhaustion or exercise intolerance. In order to better categorize the response to searching and rescue exercise, mean values after exercise are presented in relation to those values previously described for dogs competing in different exercises (Agility, sled dogs, Greyhounds and Labrador Retrievers) (Table 1).

Table 1. Physiological, hematology and biochemistry data after different types of exercise in dogs

Parameter	Search and rescue	Agility[a,b]	Sled dogs[c]	Greyhound[d]	Labrador Retrievers[e]
	Distance or time of exercise				
	20 min	100 s	1 100 miles	722 m (47.7 ± 1.7 s)	10 min
HR (beats/min)	132.8 ± 8.79	134.0 ± 17.2	–	245.0 ± 39.0	150.0 ± 20.0
RR (breaths/min)	196.0 ± 50.9	174.0 ± 46.4	–	136.0 ± 30	183.0 ± 34.0
RT (°C)	40.64 ± 0.46	39.20 ± 0.60	–	40.60 ± 0.3	41.80 ± 0.30
PCV (%)	53.16 ± 0.64	51.60 ± 5.59	–	64.00 ± 3.0	51.00 ± 3.00
WBC (10^3/μl)	13.73 ± 4.78	8.310 ± 1.40	–	7.800 ± 1.9	10.30 ± 1.90
TPP (g/dl)	6.680 ± 1.50	6.230 ± 1.05	5.800 ± 0.13	7.300 ± 0.6	–
CREAT (mg/dl)	1.580 ± 0.16	1.370 ± 0.26	0.600	–	–
GLU (mg/dl)	74.60 ± 8.02	88.10 ± 7.88	–	171.0	–
TAG (mg/dl)	82.90 ± 16.3	75.80 ± 17.7	–	–	–
LA (mmol/l)	5.030 ± 0.68	4.550 ± 2.31	1.265	28.90 ± 3.0	3.570 ± 2.20
Na (mmol/l)	142.8 ± 5.89	147.9 ± 12.8	147.3 ± 1.00	158.0 ± 5.0	154.0 ± 2.00
K (mmol/l)	4.060 ± 0.57	4.630 ± 0.84	4.500 ± 0.12	4.300 ± 0.5	5.000 ± 0.30
Cl (mmol/l)	109.0 ± 13.0	115.5 ± 15.4	113.7 ± 2.10	114.0 ± 3.0	123.0 ± 1.10
CK (IU/l)	108.0 ± 143	43.13 ± 36.9	472.9	218.0 ± 163	143.0 ± 65.0
AST (IU/l)	31.50 ± 6.25	23.00 ± 6.70	94.30	60.00 ± 19	–
LDH (IU/l)	312.0 ± 153	207.0 ± 93.8	94.90	83.00 ± 33	–
INS (mU/l)	16.20 ± 7.00	13.30 ± 11.0	–	–	–
CORT (mmol/l)	2.560 ± 1.57	4.530 ± 2.49	–	–	–

[a]Rovira et al. (2007a); [b]Rovira et al. (2007b); [c]Burr et al. (1997); [d]Ilkiw et al. (1987); [e]Matwichuk et al. (1999)

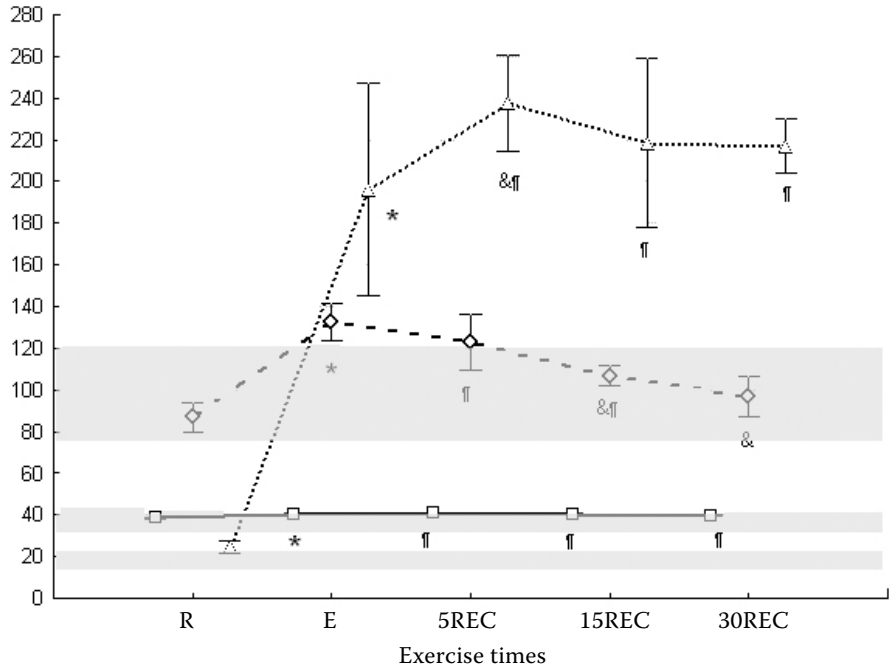


Figure 1. Mean ± SD of the physiological variables in nine healthy dogs during a session of searching and rescue exercise of 20 min of duration. Shaded areas show the reference intervals for adult dogs

Δ = respiratory rate (breath/min), ◇ = heart rate (beats/min), □ = rectal temperature (°C)
* = differences between R and E, & = differences between E and REC, ¶ = differences between R and REC

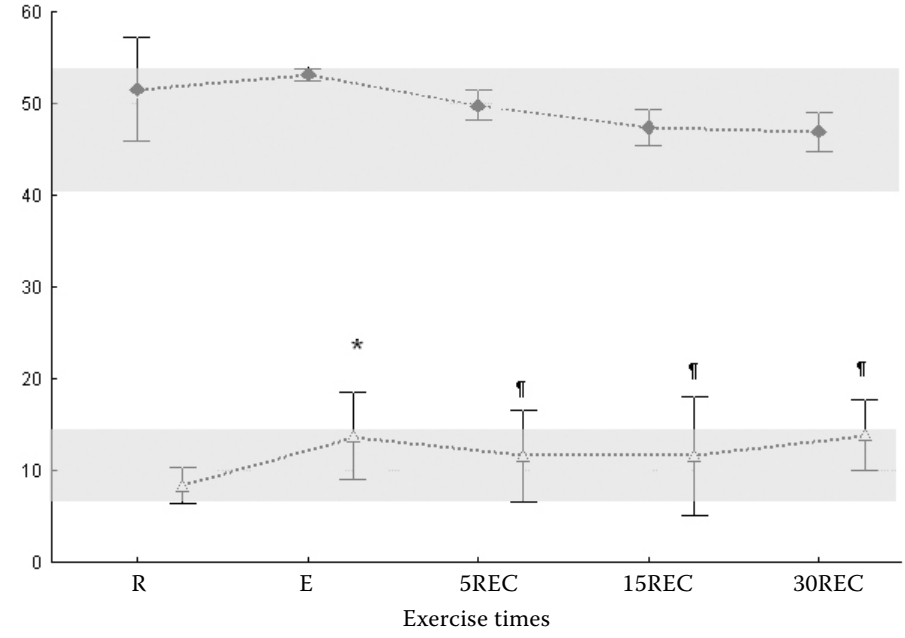
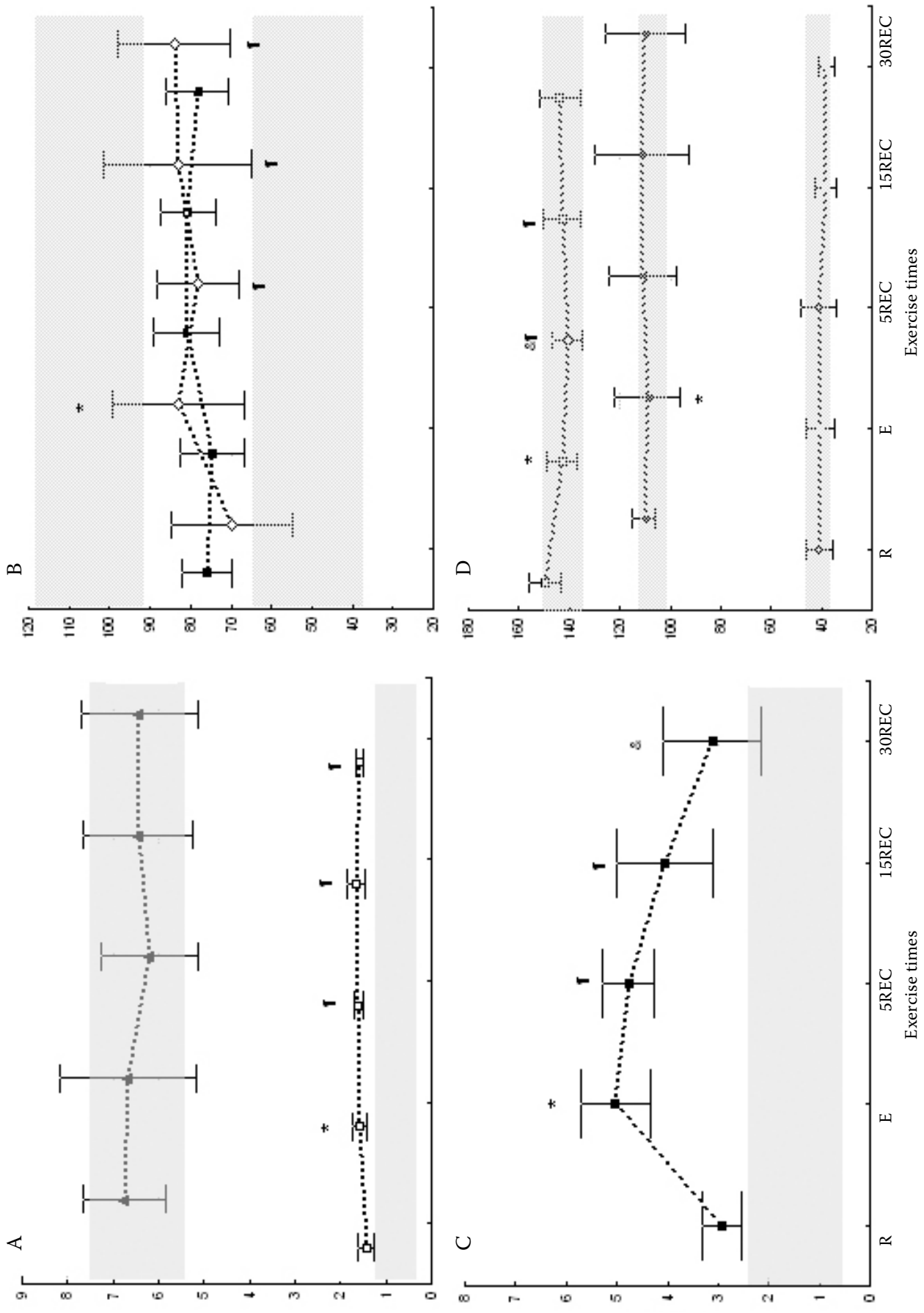


Figure 2. Mean ± SD of packed cell volume (PCV) and white blood cells (WBC) in nine healthy dogs during an exercise session of searching and rescue of 20 min of duration. Shaded areas show the reference intervals for adult dogs

◆ = packed cell volume (%), Δ = white blood cells (10^3/μl))
* = differences between R and E, & = differences between E and REC, ¶ = differences between R and REC


A
B
C
D
R
E
5REC
15REC
30REC
Exercise times

← Figure 3. Mean ± SD of indicators of hydration – **A**: ▲ = total plasma protein (mg/dl), □ = creatinine (mg/dl); metabolism – **B**: ■ = glucose (mg/dl), ◇ = triacylglycerols (mg/dl); lactate – **C** (mmol/l) and electrolytes – **D**: □ = sodium (mmol/l), ▲ = chloride (mmol/l), ◇ = potassium (mmol/l) in nine healthy dogs during an exercise session of searching and rescue of 20 min of duration. Shaded areas show the reference intervals for adult dogs

* = differences between R and E, & = differences between E and REC, ¶ = differences between R and REC

HR increased 1.5-fold over the resting values, decreasing significantly from E at 15REC, although mean values remained elevated in relation to R until 30REC. RR and TR also rise with E (4.7-fold and 1.1-fold, respectively). RR values underwent a further increase at 5REC. At the end of the study, no recov-

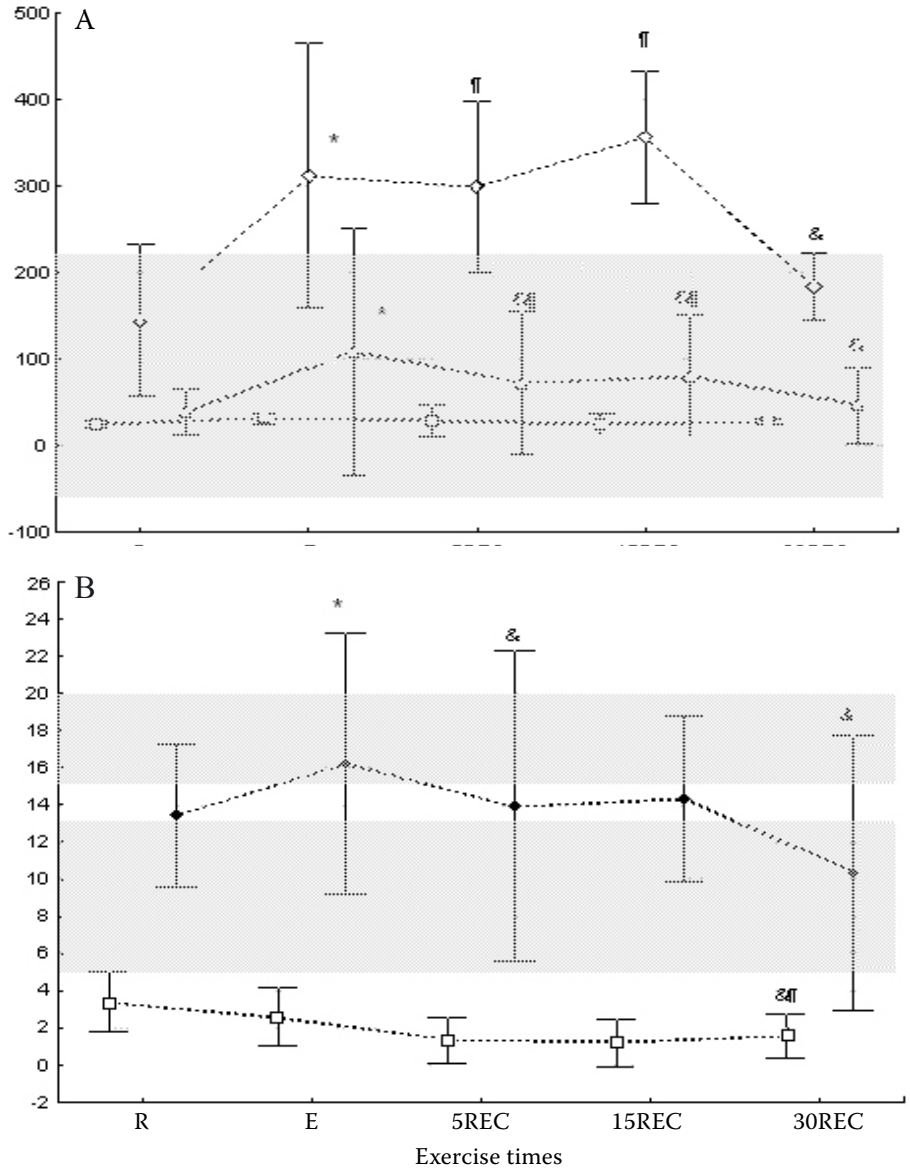


Figure 4. Mean ± SD of plasma muscle enzymes – **A**: ◇ = LDH (IU/l), Δ = CK (UI/l), □ = AST (IU/l) and hormones – **B**: ◆ = insulin (mU/l), □ = cortisol (mmol/l) in nine healthy dogs during an exercise session of searching and rescue of 20 min of duration. Shaded areas show the reference intervals for adult dogs

* = differences between R and E, & = differences between E and REC, ¶ = differences between R and REC

Table 2. Correlation's coefficients between physiological, blood and endocrine parameters in search and rescue dogs during an exercise session of 20 min of duration (significant correlations indicated in bold)

	HR	RR	RT	PCV	WBC	TPP	CREAT	GLU	TAG	LA	Na	K	Cl	CK	AST	LDH	INS
RR	0.79																
RT	**0.68**	**0.84**															
PCV	0.06	0.15	0.01														
WBC	0.58	0.39	0.13	0.56													
TPP	−0.01	−0.03	−0.20	0.23	0.25												
CREAT	0.22	**0.61**	0.59	0.07	−0.15	−0.38											
GLU	−0.10	0.39	0.32	0.40	−0.08	−0.11	0.32										
TAG	0.41	0.07	0.00	0.26	0.38	−0.43	0.24	−0.12									
LA	**0.68**	**0.65**	0.51	**0.69**	0.25	−0.29	0.51	−0.41	0.46								
Na	−0.33	0.15	0.05	−0.34	−0.21	−0.39	0.49	−0.16	**0.67**	0.02							
K	0.02	−0.16	−0.26	0.54	−0.30	0.14	0.19	−0.33	−0.10	0.36	0.01						
Cl	−0.18	0.17	0.43	−0.06	0.45	−0.23	−0.15	−0.45	0.16	0.19	−0.20	−0.53					
CK	0.45	0.16	0.20	−0.05	0.28	−0.25	0.00	**−0.63**	0.07	−0.30	0.07	**−0.83**	0.67				
AST	0.05	0.47	**0.77**	−0.03	0.32	−0.11	0.04	−0.05	0.07	0.33	−0.10	−0.38	**0.80**	0.34			
LDH	**0.65**	0.40	0.68	−0.22	0.01	−0.20	**0.61**	−0.13	0.41	0.38	0.58	−0.32	0.26	0.31	0.47		
INS	0.20	0.21	0.20	0.17	0.25	−0.22	0.17	**−0.74**	0.20	0.28	0.00	0.14	**0.65**	0.34	0.07	0.07	
CORT	−0.55	**−0.79**	**−0.83**	−0.09	−0.37	0.29	−0.50	−0.34	0.06	−0.37	0.18	0.43	−0.46	−0.42	−0.37	−0.37	−0.21

ery of RR and TR was found in these dogs. Changes in these physiological variables during the exercise are presented in Figure 1.

RBC parameters were unaffected by E and by the posterior recovery period, except MCV, which presented a significant increase (1.0-fold after E), reaching the R values at 15REC. WBC increased at E (1.4-fold) and remained elevated throughout the whole recovery period (Figure 2). Some biochemical parameters were not significantly modified by exercise or recovery (TPP, GLU, AST, and K). Both CREAT and TAG rose significantly with E (1.1 and 1.2-fold, respectively) and the mean values at the end of the recovery period were significantly higher than the R values. E also induced significant elevations in CK, LDH, LA and INS (2.8, 2.2, 1.7 and 1.3-fold), but R values were achieved at 30REC, 30REC, 15REC and 5REC, respectively. Plasma Na concentrations decreased with E (1.0-fold), reaching statistically similar values than R at 30 REC. Plasma Cl concentrations were reduced with E (1.0-fold), rising significantly at 5REC, and after that, no significant differences were found in relation to the R values. Finally, although serum CORT concentrations decreased with E, the highest reduction was found at 15REC (3.2-fold), without significant differences between R and 30REC. The changes induced by the exercise session in the biochemical parameters are presented in Figure 3 and 4. Table 2 shows the coefficients of correlations between the studied parameters.

DISCUSSION

The present research focuses on the physiological and laboratorial changes that trained dogs undergo during a normal session of exercise for searching and rescue activities. To evaluate sporting dogs for exercise intolerance or exhaustion, control values for each type of exercise are needed because response varies with the type, duration and intensity of the performed exercise. Exercise induced moderate increase in RR, CK, LDH and LA and mild increase in HR, RT, MCV, WBC, CREAT, TAG and INS. By contrast, Na, Cl and CORT concentrations decreased as a result of exercise. Modifications of RR, RT, MCV, WBC, CREAT, and TAG persisted after 30 min of recuperation.

As HR is the main determinant of cardiac output and oxygen uptake, its increase during exercise is an index of cardiovascular workload. The mean HR of our dogs was lower than that described for racing Greyhounds (245 ± 39 beats/min; Ilkiw et al., 1989), Agility dogs (220 beats/min; Rovira et al., 2007a) and Labrador Retrievers (150 ± 20 beats per min; Matwichuk et al., 1999). However, Steiss et al. (2004) described in Labrador Retrievers similar HRs (119–143 beats/min) than those values found in the present study. Differences between studies probably result from the different oxygen demands in muscles during exercise upon the regulation of the sympathetic system, as suggested in horses (Munoz et al., 1999a). In Thoroughbred and endurance horses, HR recovery is considered a valuable index of fitness and in endurance horses is a sensitive indicator of metabolic, stress or fatigue levels (Foreman et al., 1990; Munoz et al., 2006). Additionally, delayed HR recovery has been associated with exercise intensities that exceed the physical ability of the athlete, lameness or pain (Foreman and Lawrence, 1991; Pollitt, 1993). In our dogs, HR was not statistically different from R at 15REC, suggesting a proper adaptation to the imposed exercise.

The intense tachypnea observed after exercise that persisted throughout the whole recovery period probably came from the combined effects of increased oxygen requirements, respiratory compensation for the metabolic acidosis and thermoregulation. The increased HR in this study at E in comparison to R suggested enhanced oxygen requirements. However, the further rise in RR in spite of the decrease in HR during the recovery period indicated that more factors that oxygen demands are involved in the exercise-induced tachypnea. Accumulation of LA within the body causes modifications in acid-base status with acidosis of metabolic origin and respiratory compensation with hyperventilation (Munoz et al., 1999b). In fact, a significant positive correlation between plasma LA accumulation and RR was found in this research (r^2 = 0.650). In addition, it should be taken into account that dogs thermoregulate primarily through evaporative water loss from expired air and secondarily though convection and radiation (Hinchcliff et al., 1997). For this reason, the increased RR in the present study was attributable to the evaporative cooling of the respiratory tract, as demonstrated by the significant positive correlation between RR and RT (r^2 = 0.840). In spite of the activation of the thermolysis mechanisms, RT persisted elevated throughout the whole recovery period. The increased RT was an expected

finding, because muscle work leads to the generation of great amount of body heat. In mixed-breed dogs running in a treadmill for about 45 min and in Greyhounds racing for 48 s, RT of 40.1 ± 0.5 and 40.6 ± 0.3°C have been reported, respectively (Hastings et al., 1982; Ilkiw et al., 1989). Healthy Labrador Retrievers showed RT of 41.8 ± 0.3°C after 10 min of retrieving exercises (Matwichuk et al., 1999). Our dogs presented the highest mean RT at 5REC (40.82 ± 0.39°C), being 41.2°C the highest individual value recorded. It has been described that extremely high temperatures, above 42°C may result in abdominal distress, convulsions, unconsciousness and cellular damage (Bjotvedt et al., 1984). None of the studied dogs developed clinical signs indicative of thermoregulatory failure and hyperthermia.

Exercise-linked changes in RBC parameters in sport dogs vary according to the type of the exercise performed. In this way, an increase in RBC, PCV, HB and TPP is a normal response to speed racing (Lassen et al., 1986; Snow et al., 1988; Ilkiw et al., 1989) and agility exercises (Rovira et al., 2007a,b). By contrast, these parameters did not change or even decreased during prolonged submaximal exercise (Hinchcliff et al., 1993; Burr et al., 1997; McKenzie et al., 2007). The modifications in these parameters during exercise are influenced by splenic contraction and secondary hemoconcentration to dehydration. The absence of significant changes in RBC, PCV, HB and TPP in our dogs was interpreted as hemoconcentration is not a concern for searching and rescue dogs during exercise sessions of 20 min of duration. However, it does not seem accurate to extrapolate these results to longer exercises or when exercise is carried out in hot and humid conditions, especially if the amount of water lost from the body is not adequately offset by fluid intake. Nevertheless, despite the lack of significant changes in the erythrocyte parameters, it cannot be affirmed that splenocontraction did not happen. Blood samples were extracted immediately before exercise, after arriving to the field and therefore, the effect of the emotional stress could not be avoided. Therefore, it is suggested that splenocontraction appeared before exercise without significant additional recruitment during the 20 min of exercise.

TPP and CREAT concentrations were included in the present research as indicators of hydration status and rate of glomerular filtration, respectively. As stated before, as TPP concentrations did not undergo significant changes during the study, hydration status did not seem to be affected by 20 min of continued exercise. It has been established that both short maximal and submaximal exercises cause significant increases in TPP because a shift of fluids from the vascular compartment to the extracellular fluid spaces (Ilkiw et al., 1989; Rovira et al., 2007a). On the contrary, TPP decreased during prolonged exercise in dogs because of increased plasma volume, exercise-induced immunosuppression, catabolism of plasma proteins for energy and protein loss via renal and gastrointestinal tracts (Hinchcliff et al., 1998; Gary et al., 2004; McKenzie et al., 2007). On the other hand, plasma CREAT concentrations increased significantly with E and remained elevated throughout the recovery period. Therefore, if an unchanged glomerular filtration rate is assumed because the absence of other laboratory evidences of dehydration, increased CREAT concentrations might have been associated with an enhanced CREAT production by muscle catabolism during exercise. In fact, the production of CREAT has been found to be proportional to muscle mass (Arokoski et al., 1993).

Leukocytosis appears to be a normal physiological response to exercise in dogs. It has been reported following short-distance running (Lassen et al., 1986; Ilkiw et al., 1987), although agility exercises did not seem to affect WBC kinetics (Rovira et al., 2007b). The leukocytic response to exercise is believed to be caused by mobilization of WBC from the marginal pool in response to catecholamines and in horses has been also associated with the release of blood rich in lymphocytes from the spleen (Arokoski et al., 1993; Munoz et al., 1999a). In addition, a mild inflammatory response to exercise without clinical signs has been described in horses and human beings, with increased concentrations of pro-inflammatory cytokines and leukocytosis (Donovan et al., 2007; Wardyn et al., 2008). In knowledge of the authors, the changes in the concentrations of these cytokines with exercise and their relationship with leukocytosis has not been evaluated yet in dogs.

Although hypoglycemia has been suspected as a cause of collapse in exercising dogs (Steiss et al., 2004), plasma GLU concentrations remained unchanged throughout the study and within the reference range. The lack of significant changes in plasma GLU concentrations has been already reported in agility dogs during shorter exercises (80–120 s) and it was interpreted as a balance between hepatic mobilization caused by increased

catecholamine concentrations and GLU uptake for muscle metabolism. A negative significant correlation was found between circulating GLU and INS concentrations in this research (r^2 = –0.740). Previous studies have reported an enhanced lipid metabolism in exercising horses (Munoz et al., 2002) and dogs (Rovira et al., 2007b), as indicated by the increased circulating free fatty acid and TAG concentrations. This fact appears to be more marked after prolonged submaximal exercises, because muscle metabolism is mainly maintained by aerobic pathways. In the present study, the highest concentrations of TAG appeared after 30 min of recovery, probably reflecting an esterification of free fatty acids to TAG. According to these results, it seems that lipid metabolism is important in muscle energy resynthesis in dogs performing searching and rescue activities, probably because their prolonged duration.

LA is a metabolite derived from the anaerobic pathways, and its accumulation in blood/plasma is an indicator of fitness and training level and oxidative capacity (Munoz et al., 2002; Rovira et al., 2007b). LA concentration that exceeds the concentration of clinically normal dogs performing exercise of the same duration and intensity may be an evidence of reduced performance, lack of fitness or underlying disorder of metabolism (Matwichuk et al., 1999; Rovira et al., 2007b). In this study, high plasma LA concentrations were found at rest, probably indicating a certain degree of psychological stress and anticipatory response to the exercise, with release of catecholamines and activation of glycolytic pathways. Post-exercise plasma LA concentrations in the present study was higher (5.027 mmol/l) than those described in foxhounds after maximal exercise (2.710 mmol/l) (Musch et al., 1985), Labrador Retrievers after field trials (2.180–3.150 mmol/l) (Steiss et al., 2004) but similar to the data reported for dogs after Agility trials (4.560 mmol/l) (Rovira et al., 2007b). All these values were substantially different from those presented for Greyhounds, with plasma LA concentrations near 30 mmol/l (Ilkiw et al., 1989). These results were expected, as plasma LA accumulation after exercise is indicative of the exercise intensity, with the more strenuous exercise causing greater LA concentrations, even though the degree of this increase in response to a standardized exercise can be modified by training. In our study, plasma LA concentrations recovered at 30 REC. Persistent elevated plasma LA concentrations after exercise have been described in dogs with muscle defects of oxidative metabolism, including pyruvate dehydrogenase deficiency (Abramson et al., 2004) and defects in the mitochondrial respiratory chain (Breitschwerdt et al., 1992).

The mild but statistically significant reduction in plasma Na concentration is in concordance with the results of previous studies in exercising dogs (Hinchcliff et al., 1997; Rovira et al., 2007a). In Alaskan sled dogs, exercise-associated hyponatremia has been attributed to insufficient dietary intake and increased renal Na loss, although prolonged exercise increases plasma aldosterone concentrations and therefore, a reduction in urinary Na excretion should appear (Hinchcliff et al., 1997; McKenzie et al., 2007). No significant changes were found in plasma K concentrations, despite that a rise was expected due to the efflux of K ions from the myoplasma into the extracellular fluid from muscle contraction and to the metabolic acidosis secondary to hyperlactacidemia (Munoz et al., 2003). In the present research, the correlation between LA and K did not achieve statistical significance (r^2 = 0.360).

Plasma CK, AST and LDH activities were included in this research in order to detect (sub)clinical muscle damage. Plasma CK activity in dogs has a specificity of 98% for skeletal muscle diseases whereas AST and LDH are less specific (Chanoit et al., 2001). Exertional rhabdomyolysis is a well recognized clinical entity in dogs and it has been described after short races in Greyhounds (Wodecki and Heinrich, 1993), and after prolonged exercise in sled dogs (Hinchcliff et al., 2004). However, it should be taken into account that plasma muscle enzyme activities change with exercise, probably as a consequence of increased muscle metabolism and transient changes in muscle permeability, without significant fibrillar disruption (Munoz et al., 2002). In the research reported here, plasma CK and LDH activities rose at E, although in both cases resting values were reached at 30REC, indicating that persistent muscle damage did not developed in the dogs.

INS and CORT are important glucoregulatory hormones which might undergo significant changes during exercise in order to control energy flux into muscle cells. The INS response to acute exercise consists in a suppression of INS release during exercise, and during this period of low-INS levels, muscle GLU uptake is facilitated by a GLU transporter (GLU-4) (Poso and Hyyppa, 1999). On the

contrary, in the present study, 20 min of E induced a significant increase in INS concentrations in the dogs, which probably was linked to the non-significant fall in plasma GLU concentrations, as noted by the negative correlation between both variables (r^2 = –0.740). In opinion of the authors, this unexpected rise in plasma INS levels could partially derive from the duration of exercise. Miles et al. (1992) described that plasma glucagon/INS ratio falls slightly because increased INS and reduced glucagon at the onset of prolonged exercise, with a progressive increase in this ratio in dogs subjected to a 100 min-duration workload. INS is a very important hormone during the recovery from exercise when glycogen repletion is most active (Poso and Hyyppa, 1999). The rapid recovery of INS concentrations in the present study might indicate that the performed exercise did not cause marked muscle glycogen depletion, although no muscle biopsies were extracted in order to confirm this hypothesis. In relation to CORT, it is accepted that exercise leads to increased concentrations, depending more on the duration of the workload than on work intensity (Poso and Hyyppa, 1999). In this way, short submaximal Agility exercises in dogs did not have any significant effect on serum CORT (Rovira et al., 2007b). By contrast, search and rescue dogs presented a significant fall in the circulating concentrations of this hormone. On the other hand, and in agreement with data provided for horses (Nagata et al., 1999), maximum serum CORT concentrations were detected at 30REC.

In conclusion, the present study demonstrated that significant changes appear in some laboratorial parameters in healthy dogs during an exercise of searching and rescue of 20 min of duration, with values outside of the reference range for healthy dogs. These results highlight the necessity for using a standardized exercise protocol and appropriate control values when evaluating dogs with exercise intolerance and further, it provides a data base with which to compare physiological, blood and endocrine values of dogs that become ill or injured during this type of exercise. Importantly, there was not evidence of dehydration, electrolyte disturbances, stress, or muscle disorders in the dogs sampled.

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