Fluid and Electrolyte Shifts During and After Agility Competitions in Dogs

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ABSTRACT. This research assesses the relative contribution of splenic contraction and fluid shifts out of the vascular compartment to the increases in packed cell volume associated with Agility exercises. It also aims to evaluate the changes in the concentrations of electrolytes and markers of hydration state. Fifteen dogs of both sexes were subjected to an Agility exercise of an approximate duration of 100 s. Blood samples were obtained within the first 30 s after competition and at 5, 15, and 30 min of recuperation. Resting values were established previously. The following parameters were determined: packed cell volume (PCV), plasma lactate (LA), total plasma protein (TPP), albumin (ALB), urea (BUN), creatinine (CREA), chloride (Cl), calcium (Ca), phosphorus (P), sodium (Na) and potassium (K). Changes in plasma volume (PV), total RBC volume (V_{RBC}) and blood volume (BV) were calculated immediately after exercise and at 30 minutes of recovery. It was found that during Agility competition, BV, V_{RBC} and PV increased 12, 21 and 4% respectively, indicating that the spleen contraction was the main determinant on the increase of BV. In comparison with resting values, BV decreased after recuperation (–5%), due to the recapture of erythrocytes by the splenic reserve (V_{RBC}, –12%). Additionally, Agility exercise induced increases in plasma Cl and LA, with significant reductions of ALB, Ca and P and absence of modifications in Na, K, BUN and CREA concentrations.

KEY WORDS: agility, canine, electrolyte, exercise, plasma volume.

Exercise induces changes in fluid, electrolyte and acid-base balance, depending on several factors, as exercise intensity and duration, training level, thermoregulation, and weather conditions. Animal species have developed different strategies to control core heat temperature. Horses and humans are the only species that cool primarily through evaporation of sweat, while dogs are more dependent on panting [2, 7, 10].

Short maximal exercise in dogs produces metabolic acidosis with respiratory compensation, and increases in plasma Na, K, protein concentrations and osmolality [9, 19]. Increased plasma Na concentration after racing could derive from the loss of water from the plasma, and occasional high Na concentrations have been observed in clinically normal Greyhounds [11, 19]. Contracting muscle fibres are the major source for plasma K during exercise, and hyperkalemia has been partially related to the efflux of K from active muscles [16]. In contrast, plasma Cl concentrations did not vary proportionally, assuming that some of it must have moved to other compartments, as red blood cells (RBC) [15]. Additionally, Greyhound dogs undergo significant increase in plasma protein concentration (TPP) during racing, implying fluid shifts out of the vascular compartment, after assuming that no proteins were added from the interstitial space to the bloodstream [19].

On the other hand, prolonged running in dogs has been associated with decreases in plasma cation, TPP, albumin (ALB), urea (BUN) and creatinine (CREA) concentrations [8]. The hyponatremia has been linked to a large water turnover, with conservation of Na by the kidneys, and high urinary osmolality. The reduction in TPP may be attributable to plasma volume (PV) expansion associated with prolonged and repetitive exercise training, loss of proteins from the vascular space, protein catabolism during exercise or a combination of these factors [7, 8]. Despite these fluid and electrolyte abnormalities, clinical consequences were not observed in these studies.

Although speed racing and sled races are the most known sports in dogs, the canine athlete can compete in a large range of sports. Recently, we have studied the haematological and biochemical requirements of dogs during Agility competitions [17]. We found that packed cell volume (PCV) increased during exercise, although if this rise was due to splenic contraction or/and to fluid shifts out of the vascular compartment could not be clarified. Toll *et al.* [19] developed an indirect procedure to determine the changes in PV in dogs, based on the modifications in PCV and TPP after exercise. This method will be used in the Agility dogs to estimate the changes in PV and the relative importance of the spleen during this type of exercise. We also know that the Agility competition represents a submaximal effort for dogs, with post-exercise heart rates near 135 bpm [17]. Despite this reduced exercise intensity, some electrolyte changes might occur during Agility competitions. These animals are subjected to moderate intensity training programmes, consume high energy diets and drink water and hypotonic solutions. Therefore, this research aims to assess the relative contribution of splenic contraction and fluid shifts out of the vascular compartment to the increases in PCV associated with Agility exercises, and to evaluate the changes in plasma electrolyte concentrations.

MATERIAL AND METHODS

Animals: Fifteen dogs of both sexes (9 females and 6 males), of different breeds, with body weights between 3.6 and 37.5 kg, and ages between 1.5 and 11.5 years, were studied. A veterinary control carried out a week before the competitions ensured all the animals were healthy and fit.

Diet: Dogs were fed high energy commercial diets, containing a mean of 31.33% digestible protein, 1.79% Ca and 1.2% P. No electrolyte supplements were administered during training or competitions. Food and water intakes were restricted before the Agility exercise for at least 2 hr.

Agility test (AT): The animals were subjected to a simulated Agility competition, composed of 2 courses of 180 to 200 m, without a rest period between both courses. Each course had a total of 20 obstacles, including open and closed tunnels, weave poles, dog walk, A-frame, see-saw, bar jumps and tire jumps.

Blood samples: Blood samples were withdrawn from the cephalic vein, within the first 30 s after finishing the exercise and at 5, 15 and 30 min of a passive recuperation. Resting values were previously established from samples collected one week before the test in a familiar environment for the dogs, in order to reduce stress-induced splenocontraction. Samples were poured into tubes with EDTA-3K and lithium heparine for haematological and biochemical determinations, respectively. The samples stored in lithium heparine were centrifuged within the first 5 min after blood withdrawal, and the supernatant was harvested. Both blood and plasma samples were stored at 4°C during their transportation to the laboratory.

Laboratorial determinations: PCV was obtained with a semiautomatic cell counter (Sysmex-F820®). Lactate (LA), TPP, ALB, BUN, CREA, Cl, Ca and P concentrations were determined spectrophotometrically (Heλios α®, Thermospectronic). Finally, plasma Na and K were measured by flame photometry.

Calculation of PV changes during AT: Changes in PV, total RBC volume (V_{RBC}) and blood volume (BV) were calculated according to the method described by Toll *et al.* [21] for Greyhounds. This method assesses the percent changes in PV using changes in TPP. It was assumed that the total amount of protein in the vascular compartment did not vary with the exercise [14, 19]. These calculations were performed at two times, immediately after exercise and at 30 min of recovery.

At any time (t), indices of PV, V_{RBC} and BV relative to resting values were obtained from PCV and changes in TPP according to the following equations, where $PV_{(t)}$ represents PV in relation to rest values (PV_{rest}):

$PV_{(t)} = (PV_{rest})$(fractional change in $PV_{(t)}$)$+PV_{rest}$,
Fractional change in $PV_{(t)} = (TPP_{rest} - TPP_{(t)})/TPP_{(t)}$,
$PV_{rest} = 1 - PCV_{rest}$,

and where $V_{RBC(t)}$ was the index of V_{RBC} at time t relative to V_{RBC} at rest:

$V_{RBC(t)} = (PV_{(t)})(PCV_{(t)})/(1-PCV_{(t)})$,

$BV_{(t)}$ was defined as the index of BV at time t relative to BV at rest:

$BV_{(t)} = V_{RBC(t)} + PV_{(t)}$

Statistical data processing: Data were expressed as mean ± SD. The differences between resting, post-exercise and recuperation values were investigated with a two-way ANOVA analysis for dependent variables. In this statistical model, the weight of the dog was considered a covariable, and the animal was fixed as a random effect. The correlations between the variables were evaluated by a Pearson product-moment correlation. Statistical significance was fixed at $p < 0.05$.

RESULTS

Weight, height and sex of the dogs did not influence any of the measured parameters, and hence, the data of all the animals were processed together in each sampling time. The mean time to complete the AT was of 100.7 ± 10.98 s, ranging between 80 and 120 s.

AT induced a rise of 7.7% in PCV, reaching means of 51.59 ± 5.59% and initiating the recovery during the first 5 min after the exercise. PCV at this time of the recuperation was significantly different from post-exercise values. The variations in V_{RBC}, PV and BV after exercise and at 30 min of recovery are shown in Fig. 1. Immediately after AT, dogs showed an increase in BV (+12%), which was more dependent on the rise of V_{RBC} (+21%) than on PV (+4%). Compared to resting values, BV was 5% lower at 30 min of recuperation, probably associated with the reduction of V_{RBC} (−12%). PV was 2% higher at this time than at rest.

Plasma Na and K concentrations did not show significant changes during the AT. On the contrary, plasma Cl concentration showed a significant increase (+8.5%) immediately after exercise (Fig. 2). During the recuperation, plasma Cl remained elevated, with maximum concentrations at 30
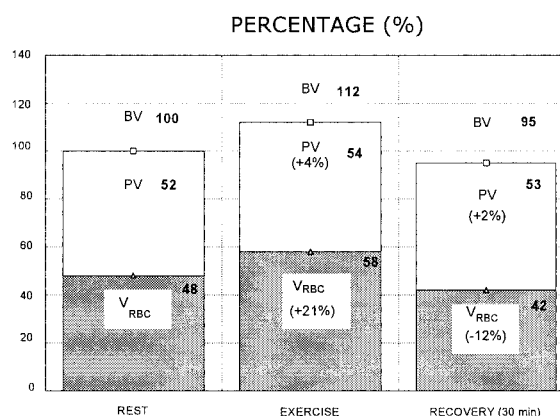

Fig. 1.   Relationships between total blood volume (BV), plasma volume (PV), and erythrocyte volume (V_{RBC}) in Agility dogs at rest, immediately after Agility exercise and at 30 min of recuperation. Numbers in parentheses are the percentage from rest in PV and V_{RBC}.
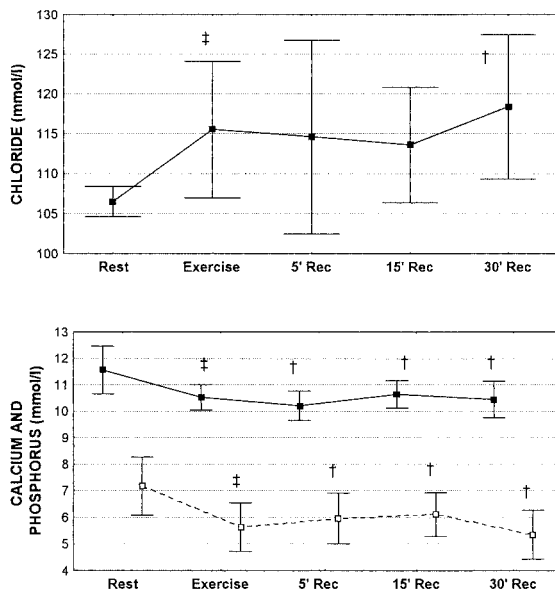
Fig. 2. Plasma chloride (■), calcium (■) and phosphorus (□) concentrations during an Agility exercise and during the first 30 min of recuperation in 15 dogs (‡: significant differences between rest and exercise; †: significant differences between rest and recuperation); p<0.05.
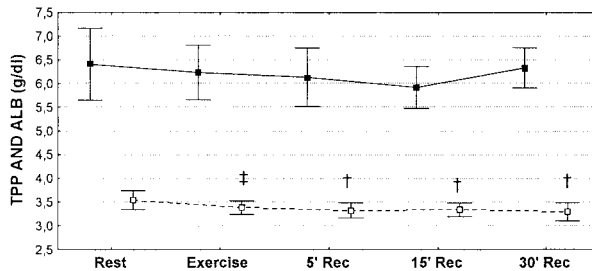

Fig. 3. Total plasma protein, TPP (■) and albumin, ALB (□) concentrations after an Agility exercise and during the first 30 min of recuperation in 15 dogs (‡: significant differences between rest and exercise; †: significant differences between rest and recuperation); p<0.05.

min. Plasma Ca and P concentrations presented a similar evolution during the study, with a sudden decrease after exercise. This reduction was more intense in plasma P (–21.5%) than plasma Ca (–9.93%) (Fig. 2). Both electrolytes remained significantly lower than resting concentrations along the 30 min of recuperation.

The AT causes significant increases of LA, with mean post-exercise concentrations of 4.558 ± 0.359 mmol/l. During the recuperation period, plasma LA underwent a progressive decrease, without significant differences between rest (2.364 ± 0.71 mmol/l) and 15 (2.891 ± 1.91 mmol/l) and 30 min of recuperation (2.369 ± 0.79 mmol/l). The mean concentrations of TPP and ALB are presented in Fig. 3. TPP remained unchanged, while ALB showed a significant reduction immediately after exercise that persisted over the recuperation period. In fact, plasma ALB at 30 min of recuperation was still significantly lower than resting values. No significant changes were detected in plasma BUN and CREA concentrations during exercise and recuperation.

The correlations between values obtained from biochemical and electrolytic assays are shown in Table 1.

DISCUSSION

This research analyzes the modifications in hydration and electrolyte markers in dogs during AT. BV, V_{RBC} and PV were increased by 12%, 21 and 4%, respectively. From these results, splenic intervention appears to be responsible for the expansion of BV during AT. Furthermore, V_{RBC} decreased during recuperation even below the resting value (–12%), suggesting the effect of catecholamines on RBC withdrawal from the splenic pool. This result is also supported by the high resting plasma LA concentrations, as catecholamines activate muscle glycolytic pathways [9]. In addition, AT caused increases in Cl and LA, decreases in ALB, Ca and P and absence of changes in Na, K, BUN and CREA concentrations.

Short-term exercise causes a decrease in PV in horses and humans, due to increases in arterial pressure and capillary hydrostatic pressure that make water, electrolyte and proteins of low molecular weight to be excluded from the vascular compartment [12,18]. Maintenance of PV level in the Agility dogs contrasted with reduced PV in horses, humans

Table 1. Linear correlations between plasma electrolyte concentrations and markers of hydration state after an Agility exercise and during the first 30 min of recuperation in 15 dogs. Black font indicates significant correlation; p<0.05

	PCV	LA	TPP	ALB	CREA	BUN	Na	K	Cl	Ca
LA	**0.450**									
TPP	0.210	0.230								
ALB	**0.380**	–0.060	–0.030							
CREA	0.020	–0.060	–0.140	**0.490**						
BUN	–0.020	–0.130	0.100	**0.340**	0.610					
Na	–0.030	–0.040	0.200	0.110	–0.050	0.060				
K	0.130	–0.030	**0.390**	0.250	–0.020	0.060	**0.740**			
Cl	–0.080	**0.320**	0.020	**0.480**	–0.200	**–0.380**	–0.100	–0.210		
Ca	0.090	–0.130	0.060	**0.810**	**0.490**	**0.420**	0.200	**0.360**	**0.580**	
P	0.220	0.120	–0.060	**–0.270**	**–0.360**	–0.250	–0.010	–0.090	**0.350**	**0.560**

and Greyhounds, which are given more intense exercise [4, 14, 19]. McKeever *et al.* [14] found a decrease in PV proportional to the exercise intensity in horses performing an incremental exercise test in treadmill. Similarly, Toll *et al.* [19] documented a 21% decrease in PV in Greyhounds 5 min after racing. It could be suggested, therefore, there was a balance between 2 processes during the AT: firstly, the introduction of fluid from the interstitial space and lymphatics into the vascular compartment and secondly, the movement of fluid from the vascular compartment to the muscles because of the increased osmolality from LA accumulation and increased arterial pressure.

It is interesting to remark that V_{RBC} increased 21% after AT in comparison to resting values. It appears that the dogs during AT were able to mobilize a large number of RBC from the spleen, and this factor could have been important in increasing blood oxygen carrying capacity. In fact, AT depended more on aerobic metabolism, as shown by the low plasma LA concentrations after exercise. The recapture of RBC released from the spleen seems to happen during the first min of recuperation and hence, V_{RBC} decreased by 12%. Furthermore, the lack of significant change in mean corpuscular volume (MCV) indicates that the modifications of V_{RBC} are linked to the number of RBC not to the size of the cells [17].

Despite the subtle changes in PV during AT, it seems plausible to think that they could influence somewhat plasma concentrations of electrolytes and markers of hydration state. Agility dogs showed a significant rise in plasma Cl and LA and mildly reduced ALB, Ca and P concentrations. The rise in plasma Cl concentration after AT could have derived from the simultaneous influences of metabolic acidosis from LA formation and adaptations in PV. The plasma LA accumulation indicated a certain degree of involvement of the anaerobic pathways in muscle energy resynthesis during Agility exercise. Decreased Ca and P concentrations might have been linked to the change in ALB concentration and glomerular filtration rate. ALB shows a high positive correlation with Ca but weak negative correlation with P. In sled dogs, mild expansion of PV and real decrease in intravascular ALB are reported [8]. Reduction in intravascular protein concentration has been attributed to loss in feces, urine or excessive catabolism. Recently, Gary *et al.* [6] reported that not more than 15% of the dogs developed microalbuminuria after a 20-min exercise of flat treadmill running. Nevertheless, the importance of these routes in the ALB loss during exercise has not yet been quantified in the canine athlete. The lack of significant changes in plasma BUN and CREA concentrations after Agility exercise was in agreement with the slight increase in PV.

It was found that 20% of the Agility dogs presented resting hyponatremia (a plasma Na concentration <140 mmol/*l*) [5]. Probably, this finding was due to the uptake of hypotonic drinks during training and/or to modifications in renal responses to training. It has been demonstrated that sled dogs can develop hyponatremia during exercise and training associated with an increased water turnover [7, 8, 13], and

the same might happen in Agility dogs during physical conditioning. The lack of significant changes in plasma Na concentrations during AT indicated that renal Na conservation was matched with the modifications in PV. Similarly, the absence of changes in plasma K concentrations during AT suggested that the changes in PV were not reflected in K concentrations and/or it was counteracted by muscle K release and metabolic acidosis after plasma LA accumulation.

According to these results, Agility dogs unlike horses and human athletes [1,3] did not experience important electrolyte disturbance during short submaximal exercise. Therefore, electrolyte alteration is not considered an important factor in exercising-related diseases.

In conclusion, Agility exercise induced an important splenic contraction and a subsequent release of RBC, which could account for the high aerobic potential of these animals. There was a very little change in PV during this type of exercise, and no important electrolyte disarrangement was found. However, more research is needed in understanding the mechanism for reduced plasma Ca and P concentrations during AT.

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