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Exercise-induced haematological and blood lactate changes in whippets training for lure coursing

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Abstract

Introduction: Whippets are traditionally trained to compete in lure coursing. While in humans and horses, training is routinely monitored by special tests, this is not carried out in the training of whippets. The aim of this study was to check if laboratory tests designed for racehorses could be useful in monitoring whippets training for lure coursing. **Material and Methods:** Blood samples were taken from 14 whippets at several time points: before exercise (including warm-up), immediately after, 15 min after and 30 min after exercise sessions of straight 400 m runs (T) and coursing (C). Routine haematological values and lactate concentrations (LA) were measured. **Results:** White blood cell count, red blood cell count, haemoglobin concentration and haematocrit increased significantly in both types of exertion, and no differences between the types were observed. The LA measured immediately after the run were increased, but there was no significant difference between the types of session (T and C). After both types of activity, LA decreased within 30 min post run by 9–11 mmol/L. Lactate concentrations were significantly higher 30 min after the T sessions than after the C sessions. **Conclusion:** The results confirmed that typical exercise-induced changes occurred in whippets training for lure coursing; however, the scale of changes was different to that in horses. The sampling scheme used in racehorses can be applied to whippets and can be useful as a laboratory tool for monitoring their training.

Keywords: exercise physiology, haematology, lactic acid, lure coursing, sighthounds.

Introduction

Coursing was traditionally a canine activity involving the pursuit of other animals. Today, it has become a no-victim sport in race form and found popularity in many countries. The races take place on open, grassy terrain which is as flat as possible. The lure is placed on a line stretched close to the ground, arranged in a track. Unlike greyhound track runs, the coursing track does not follow a regular oval, but it consists of straight lines as well as turns. The distance ranges between 250 and 900 m depending on the rank of the competition and the breed of the competing dogs. Whippets are known as the fastest accelerating breed and one which can quickly change direction. They are also solo hunters eager to take the lure as quickly as possible (8). Thus, they constitute an ideal breed for lure coursing.

The competing dogs are trained to increase their speed, agility and endurance, in order to meet all judging criteria; however, the first two are the most important since the competitions demand anaerobic exertion. Training programmes usually draw on the trainers' experience and respond to general indications, but exercise tests with objective benchmark values that can be used for the evaluation of individual dogs are still lacking. Such tests have been widely described and used on human athletes of various disciplines. In the case of animals, horses have undeniably been best investigated in this respect (5, 6, 15, 16, 20, 29, 33). One of the parameters most frequently tested during speed exercise tests on humans and horses is the lactic acid (lactate) concentration (LA). Lactate is a metabolite produced from pyruvate in anaerobic fermentation during exercise. When energy is needed rapidly, glycogen is

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broken down anaerobically into lactate. When the rate of lactate production exceeds the rate of its removal, its concentration increases markedly, which can be detected by blood LA measurements (21). The duration of exercise that can be undertaken when using glycogen alone as the energy source is limited due to the muscle fatigue related to acidification.

Blood LA measurements are widely used to assess the level of fitness in sport horses (3, 5, 29). Speed exercise tests are based on the evaluation of the time it takes for LA to reach the level of 4 mmol/L (the anaerobic threshold) (17). Studies have shown that a lactate-guided conditioning programme can significantly enhance performance and that LA, along with basic haematology, can be used to determine fitness in different types of exercise; it follows that the optimised enhancement is attained partly through the most reliable determination of fitness, and therefore the right preparation of the test and the correct sampling are crucial (5, 29). Exercise tests have been performed and changes in blood count before and after exercise have been reported for greyhounds, but not for whippets (19, 22). Therefore, the aim of this study was to determine the changes in haematological parameters and blood LA in whippets at various exercise sessions.

Material and Methods

Dogs. Fourteen privately owned whippets living with their owners in domestic conditions were enrolled in the study. They were 12 males (2 neutered) and 2 intact females, aged from 11 months to 5.5 years with a mean (SD – standard deviation) age of 2.6 (1.3) years, and weighing from 11.0 to 19.2 kg with a mean (SD) weight of 14.2 (2.4) kg. All dogs were active lure-coursing competitors at different levels of training. The movement and general condition of each dog were assessed as they walked in a straight line on a leash in the owner's hand. Each owner filled in a questionnaire about the dog with questions regarding nutrition, dietary supplements, vaccination and health history. All tests were performed as part of routine health examinations and at the owners' requests, and thus, according to the European Directive EU/2010/63 (7) and Polish regulations regarding experiments on animals (12), there was no need for the approval of the Ethical Committee for the described procedures. These qualified as non-experimental clinical veterinary practices, excluded from the directive.

Exercise sessions. A speed-training session (training – T) and a run that mimics competitions (coursing – C) were investigated. Both sessions, T and C, were conducted outdoors on a grassy meadow in the morning hours. The distance in both cases was 400 m; however, during the T session the lure was moving in a straight line, while in the C session the track was designed with turns as well as straights, in a similar manner to competition courses. The dogs ran in pairs selected by the trainer based on previous experience and performance. Each dog was dressed in a coursing blanket and had a muzzle. The owner started the run on their own initiative. Before the run, each dog was put through an individual warm-up by its owner, consisting mainly of run-ups after a frisbee or ball, stretching and tricks.

Blood analyses. Blood samples were collected at rest and after exercise at time points selected in reference to exercise tests performed on racehorses. The number of blood samples collected from each dog depended on the dog's size, what was possible without impacting the dog's welfare, and the owner's preference. Samples for haematology were obtained at rest and immediately after the runs in both session types. Samples for blood LA testing were taken as follows: in the T session immediately after, 15 min after and 30 min after the run, and in the C session immediately after and 30 min after. Blood LA was also measured immediately after the warm-up before the C session. Blood was collected from the cephalic or saphenous vein into a 2 mL tube with ethylenediaminetetraacetic acid (EDTA) and a plain tube using a 0.7 mm (22G) needle. Lactic acid concentration was measured in blood from the plain tubes immediately after collection using hand-held analysers (Accusport; Roche, Mannheim, Germany). All samples were stored in a cooled container and within 4 h the researchers' laboratory took the following haematological measurements from blood from the EDTA tubes: red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), white blood cell count (WBC), and platelet count (PLT). The plain tubes were centrifuged and the serum was used to evaluate the biochemical profile. For haematological and biochemical measurements, published reference intervals (RI) were used (31) while the RI for LA ranged from 1.2 to 3.1 mmol/L (27).

Statistical analysis. The normality of the data distribution was confirmed using the Shapiro–Wilk test. Numerical variables were presented as the arithmetic mean, SD, and range, or as the arithmetic mean, 95% confidence interval (CI 95%) and measurements of individual dogs in figures. Haematological measurements and LA were compared in the 14 whippets between time points and types of exertion using the paired Student's *t*-test with Bonferroni correction for multiple comparisons. Comparisons between three time points were made using the repeated-measure analysis of variance with Dunnett's post-hoc test. All tests were two-tailed. A significance level (α) was set at 0.05. The analysis was performed in TIBCO Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

Results

Haematology. Significant increases were noted in WBC, RBC, HGB and HCT after both C and T compared to the baseline; however, they remained within the RI. There were no significant differences between the two types of exertion (Table 1). The PLT count increased significantly after the T session but not after the C session. As a result, PLT was significantly higher

after the T session than after the C session (Table 1). The WBC increased significantly after both sessions, with no differences between the session types (Table 1).

Biochemistry. The biochemistry results, tested at rest, were within the RI. Relevant data is presented in supplementary Table 1 associated with this article.

Lactate concentrations. In the T session, the LA measured immediately after the run significantly exceeded the upper reference limit, doing so even sixfold, and then decreased to values slightly below and above the upper RI. The decrease was significant between both 0 and 15 min post run concentrations ($P < 0.001$) and 15 and 30 min post run concentrations ($P < 0.001$) (Fig. 1). The results in blood collected 15 min after the end of the exercise were on average twofold higher than the reference interval, indicating that this time had been too short for recovery after the LA threshold was exceeded. Therefore, we decided to abandon measuring LA 15 min after the run in the C session. Instead, we measured LA immediately after the warm-up and the concentrations were below the upper RI in all dogs but one (Fig. 2). Immediately after the C session, LA concentrations increased significantly to more than 10 mmol/L on average and reverted to normal after 30 min of recovery.

When comparing both types of exercise, the LA measured immediately after the run were significantly higher than the baseline and did not differ significantly between the C and T sessions ($P = 0.249$) (Fig. 2). The values decreased significantly within 30 min after both types of activity by approximately 9–11 mmol/L on average ($P < 0.001$ for both types of exercise) (Table 2). However, 30 min after the run, LA were significantly higher after the T session compared to the concentrations after the C session ($P = 0.002$) (Fig. 3).

Table 1. Change in haematological measurements after two types of session – training (T) and coursing (C)

Blood parameter	Reference interval	Baseline	After T session	Baseline *vs* T P-value[a]	After C session	Baseline *vs* C P-value[a]	T *vs* C P-value
WBC (g/L)	6.0–16.5	5.4 ± 0.9 (4.1–7.4)	7.8 ± 2.1 (5.1–11.4)	0.002*	7.27 ± 0.94 (5.8–8.6)	<0.001*	0.999
RBC (T/L)	5.5–8.5	7.5 ± 0.9 (4.8–8.3)	8.5 ± 0.5 (7.6–9.7)	0.001*	8.18 ± 0.51 (7.2–9.0)	0.039*	0.149
HGB (mmol/L)	7.5–11.3	11.5 ± 1.4 (7.4–13.0)	12.9 ± 0.7 (11.9–14.6)	0.010*	13.1 ± 0.8 (12.1–14.4)	0.007*	0.482
HCT (L/L)	0.37–0.55	0.54 ± 0.06 (0.35–0.61)	0.62 ± 0.03 (0.57–0.70)	0.001*	0.6 ± 0.03 (0.6–0.7)	0.009*	0.696
PLT (g/L)	200–580	216.4 ± 26.2 (174–281)	243.9 ± 32.8 (193–294)	0.028*	211.4 ± 16.2 (188–242)	0.999	0.002*

[a] – paired Student's *t*-test P with Bonferroni correction for three comparisons; * – Significant at $\alpha = 0.05$; WBC – white blood cell count; RBC – red blood cell count; HGB – haemoglobin concentration; HCT – haematocrit; PLT – platelet

Table 2. Changes in mean lactate concentrations (mmol/L) after two types of session – training (T) and coursing (C)

Type of effort	Lactate concentration (mmol/L)		Mean difference ± SD (CI 95%)	Immediately after *vs* 30 min after, P-value
	Immediately after session	30 min after session		
T session	13.6 ± 3.2 (7.7–18.2)	3.1 ± 0.9 (1.3–5.2)	10.5 ± 2.7 (8.5, 12.6)	<0.001
C session	10.7 ± 3.1 (4.8–15.0)	1.8 ± 1.1 (0.1–3.8)	8.9 ± 3.3 (6.4, 11.5)	<0.001
Mean difference ± SD (CI 95%)	2.9 ± 5.4 (−1.2, 7.1)	1.4 ± 1.1 (0.5, 2.2)		
T *vs* C, P-value	0.2	0.002*		

SD – standard deviation; CI 95% – 95% confidence interval; [a] – paired Student's *t*-test P with Bonferroni correction for four comparisons; * – significant at $\alpha = 0.05$

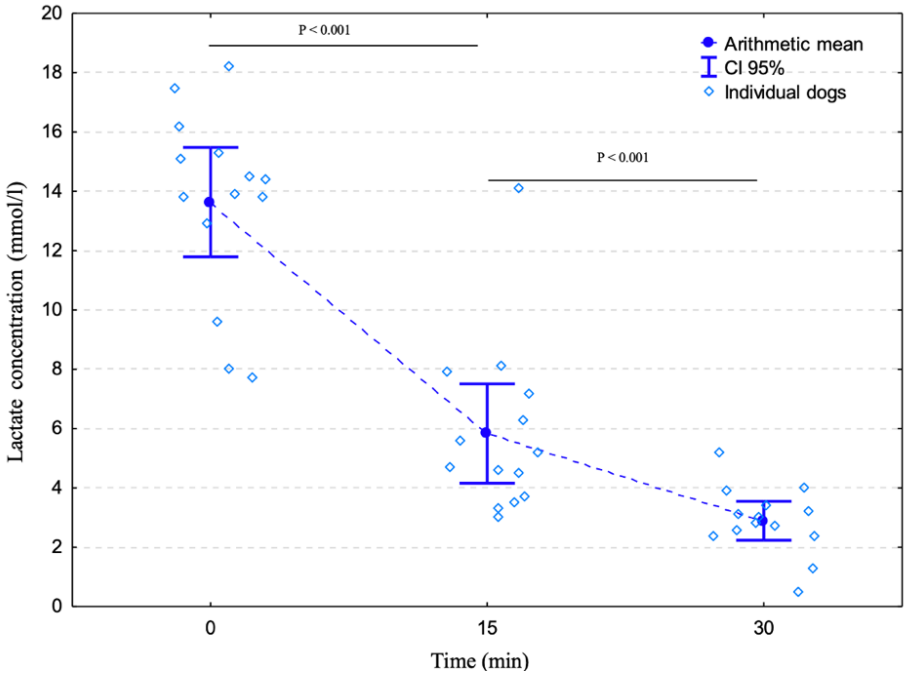


Fig. 1. Change in lactate concentration (mmol/L) after the training (T) session measured immediately after (0), 15 min after and 30 min after the exertion

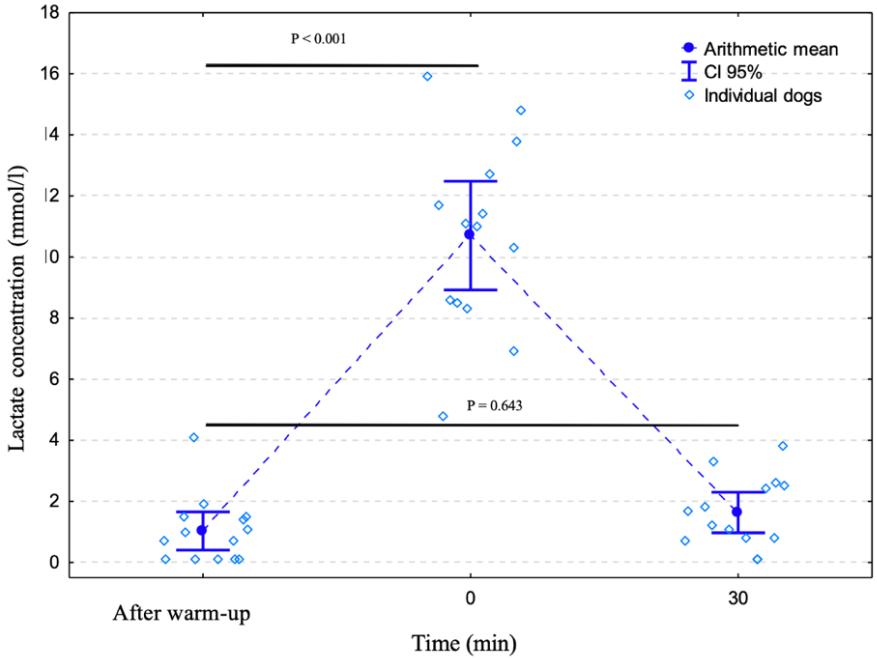


Fig. 2. Change in lactate concentration (mmol/L) after the exercise (C) session measured after the warm up, immediately after, and 30 min after the exertion

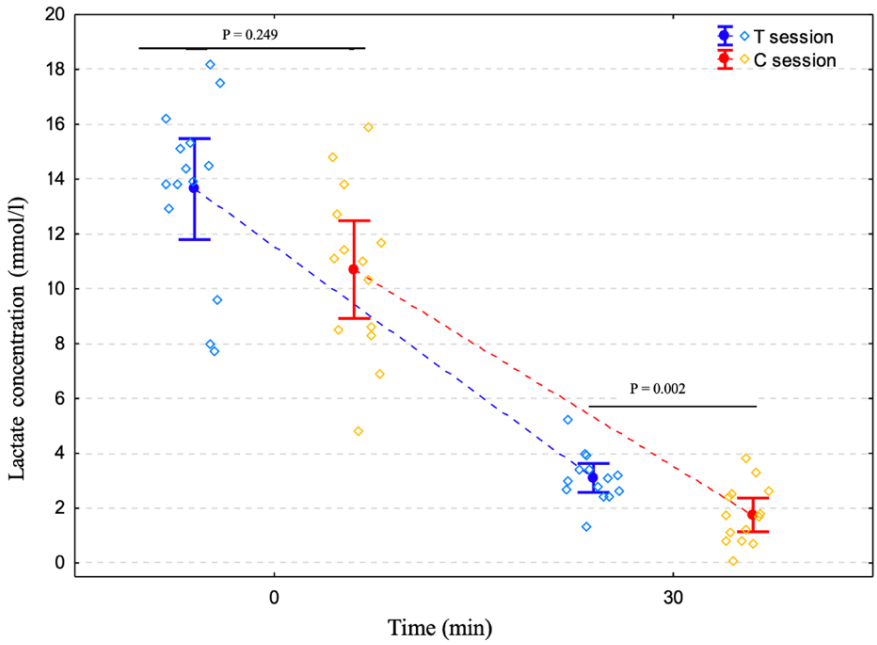


Fig. 3. Change in lactate concentration (mmol/L) after two types of exertion (a training (T) session and a coursing (C) session) presented as the arithmetic mean (dot), 95% confidence interval (whiskers) and measurements of individual dogs (diamonds)

Discussion

Our study shows that basic haematological measurements and LA significantly increase after lure coursing in whippets, but that LA reverts to normal within 30 min of the end of a run. The detected changes are in general consistent with those previously reported in humans and horses (16, 23). The resting values of the haematological parameters in the dogs examined in our study were at the upper limit or in excess of the published RI (31). This is in line with earlier haematology results typical for sighthounds (19, 28, 30, 32). Several explanations of this phenomenon have been given in the literature regarding hounds, but whippets have not been discussed extensively. It has been indicated that the natural adaptation to running leads to higher oxygen demands in muscles during exercise and thus more effective oxygen transport in the blood is required. However, other findings suggest that training and racing are not primarily responsible for

differences in haematological values between adult greyhounds and other breeds (25, 32). Investigations by Shiel *et al.* (25) of the potential impact of age and sex on blood component proportions in young greyhounds before the start of regular training revealed that HCT, HGB, and RBC correlated positively with age, and haematological values typical for adults were reached by 9–10 months of age. In the case of the whippets examined in our study, the youngest one was 11 months old, so according to the results of Shiel *et al.* (25), the blood of all dogs should have already yielded values typical of adult dogs.

The differences in routinely measured erythrogram parameters in greyhounds correspond to lower haemoglobin P_{50} values (the oxygen tension at which haemoglobin is 50% saturated), meaning that haemoglobin has a higher affinity for oxygen than it does in dogs of other breeds. Moreover, at approximately 54 days (18), the RBC lifespan in greyhounds is significantly shorter than it is in other breeds. This transience has been suggested to result from differences in membrane structure and preferential splenic sequestration effected by the reportedly larger spleens of greyhounds (32). These features contribute to the excellent predisposition to exercise in greyhounds, which has been expected also in whippets.

In our study erythrogram parameters increased significantly after exertion, regardless of its type, which is consistent with previous reports regarding racing greyhounds (22). In the investigated greyhounds, RBC, HGB and HCT increased after 400 m runs by 11%, 7% and 10%, respectively (22). In the whippets examined in our study, the increases were similar, but slightly higher after the straight run (the T session): 13%, 11% and 14% for RBC, HGB and HCT, respectively. After the C session, the results had a slightly different pattern to that in the greyhounds referred to previously: 9%, 13% and 12% increased, respectively. The differences between the types of exertion examined in our study were not significant at the group level, but are worth mentioning as an indicator for blood analyses in individual dogs. The exercise-induced erythrogram increases are well known in sportsmen and sport horses and result from the splenic contraction leading to the release of stored erythrocytes into the circulation (33) and are manifested by high RBC, HGB and HCT values. The rises might also be supported by exercise-induced fluid shifts as well as splenic contraction. The parameters listed above reverted to the baseline within one hour of the end of exercise (33). Comparing the increases in the values of haematological parameters after exercise in horses with the values obtained in the whippets examined in our study, it is clear that their percentages were not as high as in, for example, polo horses (33), where RBC increased by 49%, HCT by 51% and HGB by 45%, but still the changes we observed are visible and statistically significant.

Platelets are not commonly counted in an exercise test. Greyhounds' baseline PLT seemed to be below the commonly accepted RI in previous research (1, 19, 30); however, it was not confirmed in the whippets examined in our study. Although the changes noted in our study were statistically significant, the results may have been materially affected by the mechanisms involved in the variations in PLT counts. Hence, we postulate that PLTs are not useful in the evaluation of exercise-induced changes in whippets just as they are not in horses (24).

The WBC increased significantly after the T and C sessions regardless of the type of exertion. In greyhounds, an increase in WBC by 36% was observed after dogs chased the lure over a distance of 400 m (22). The increases observed in our study were by 42% after T sessions and 34% after C sessions. In performance horses, leukocytosis is frequently observed after various types of exercise (4, 15) and its level depends on the nature of the exertion. In endurance horses competing over 162 km, the increase in WBC was 68% (4), while in thoroughbreds racing over 1,600 m the difference was 26% (13). Two mechanisms have been widely accepted for this phenomenon: adrenaline release and cortisol release stimulated by sustained exertion. Adrenaline stimulates the release of both mono- and polymorphonuclear cells from the marginal pool and the release of leucocytes such as RBC from the spleen. Additionally, the release from bone marrow and efferent lymphatics occurs and contributes to leukocytosis (14). The short duration of the dogs' exercise in our study causes us to suspect that adrenaline is responsible for leukocytosis. In humans, cortisol is involved in acute leukocytosis in low-volume, high-load exercise, but this is not the case in high-volume, medium-load exercise (26). This corresponds to the types of exercise the whippets examined in our study were given, where the intensity was high over a short period of time. It is also worth mentioning that baseline leukocyte studies with different breeds of sighthounds showed values below the RI in greyhounds and whippets (25, 32), but we did not observe this phenomenon. It is likely to correspond mainly to the lower neutrophil count. In a high percentage of greyhounds, "grey" or "vacuolated" eosinophils are found and some analysers are suspected of not correctly detecting them (11).

Coursing competitions demand exertion for attaining speed, which is anaerobic exertion, and therefore we hypothesised that training tailored to such competitions would produce similar exercise-induced changes to those known to occur in racehorses (13, 20, 16). Blood LA relative to exercise intensity is a relevant marker for exercise performance. The anaerobic threshold falls at a particular point in the range of exercise intensity and denotes the point below which there is an increased contribution of energy associated with metabolic acidosis and consequently respiratory compensation (10). The aerobic–anaerobic LA threshold is supposed to identify the maximal intensity of exercise at which blood lactate production and clearance are in balance. In humans it has been established at 4 mmol/L. Exercise intensity above this level will require a greater contribution by anaerobic metabolism in order to obtain

energy. The lactate threshold concept is used to assess fitness in athletes. It has been widely tested in humans participating in different kinds of disciplines, mainly to determine the training level (10). In horses, this parameter is used to examine horses used in different disciplines, most commonly racing thoroughbreds, but also show-jumping and dressage horses. Special tests, named standardised exercise tests, were designed to match the nature of each discipline (6). In canine sports, such standardised methods for testing exercise capacity have not been evaluated. Nevertheless, some research has been done in dogs: in Labrador retrievers undergoing an incremental treadmill test, the LA threshold of 4 mmol/L was not exceeded; however, in some dogs a relevant difference between the resting and post-exercise results was visible (9). Agility testing induced a significant LA increase immediately after the exercise, especially in advanced competitors (2). While monitoring LA changes in sled dogs entering training, it was noted that LA measured 5 min after training decreased over a nine-week training programme, indicating progress (3). Between week 0 and week 9, the decrease in post-exercise LA reached 47%. However, the type of exercise differed from the one performed by sighthounds, because sled racing is an example of endurance sport. Greyhounds sprinting over a 100 m distance while chasing a lure showed very promising results in terms of efficiency. The RBC and blood LA remained elevated 10 min after the run, indicating the high intensity of the exertion. Moreover, the LA concentration after the exercise exceeded the 4 mmol/L threshold, suggesting a predominant anaerobic metabolism during the exercise (19). In the whippets examined in our study, LA ranged between 5 and 16 mmol/L immediately after exercise. Given that the dogs' training levels varied according to their owners' judgement, it is likely that the LA corresponded to the training level. However, verification of this hypothesis requires further studies. Most importantly, the exertion was tolerable. This was demonstrated by the LA falling below 4 mmol/L in all dogs after 30 min of recovery following the crossing of the lactic acid threshold during the runs. Post-exercise LA did not differ between types of exercise. The T session, which was examined first, showed that although LA decreased after 15 min, the threshold was still exceeded at that time, and the subsequent decrease (after 30 min) was more pronounced. We suppose that, similarly to how it is for sport horses, this is a better time point to evaluate the recovery in whippets.

Whippets are small and sometimes anxious, so the number of blood samples and the amount of blood collected must be limited. Therefore, we decided not to take blood more than twice after exercise. We think that collecting blood immediately after and 30 min after exercise is optimal. In the case of measurements taken 30 min post exercise, differences were visible as higher haematological parameter values after the T session than the C session. This indicates that running in a straight line is more exhausting for dogs than coursing competition simulation. It may be related to the specificity of the effort. The T session was an uninterrupted sprint in a straight line with maximal speed, while the C session imitated the chase after the lure, and although the latter was a fast run, it also consisted of changes in pace, *e.g.* in corners. Overall, the lactate threshold was exceeded in all dogs, but dropped below 4.1 mmol/L after 30 min, showing a good level of dog training. The results also indicate that the straight run (T session) was more demanding of the dogs than the competition (C session), which is in line with the assumptions of the training plan. We expected this result, as the training consisted of a fast and steady straight run, while in competitions dogs do not run steadily, but slow down on turns or when they lose the lure. We can also say that the warm-up given by the owners was at an appropriate level, because the lactate threshold was not exceeded and thus there was no muscle fatigue before the actual exercise. If owners are not sure whether they provide a proper warm-up, measuring LA can be helpful. For the dogs included in our study, both types of training exertion were taxing but appropriate, as the lactate threshold was exceeded immediately after running and returned to below 4 mmol/L 30 min later. Thus, LA measurement can be recommended as a laboratory method to confirm if the programme designed by the trainer is optimal for the dogs.

The main limitation of our study was the non-uniformity of the conditions provided by the keepers of the dogs tested. Each whippet lived with its owner and led a different lifestyle to the others including its diet and daily exercise routine. In the case of greyhounds, the group is more unified as the dogs are kept in the manner in which racehorses are kept. Another constraint was the limited possibility of blood collection due to the size of the dogs. Thus, we had to limit the number of blood samples taken during one session to three.

This study is the first to describe exercise-induced changes in basic blood parameters in whippets. Three main conclusions may be drawn: the first is that the schedule of LA and haematological measurements used in racehorses is applicable to whippets training for lure coursing, and the second is that exercise-induced changes were significantly different between the types of exertion at the group level only in terms of LA concentration 30 min afterwards, but that some other differences are likely and should be taken into consideration when analysing individual dogs. The last conclusion is that since collecting blood from whippets is difficult and stressful, testing LA immediately after exercise and after a 30-minute recovery period is to be recommended.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: All tests were carried out as part of routine health examination at the owners' request and thus, according to the European Directive EU/2010/63 and Polish regulations regarding experiments on animals (Act of 15 January 2015 on the protection of animals used for scientific or educational purposes), there was no need for the approval of the Ethical Committee for the described procedures, qualified as non-experimental clinical veterinary practices and thereby excluded from the scope of the directive.

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