ORIGINAL ARTICLE

Effects of racing on reticulocyte concentrations in Greyhounds

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Background: Greyhounds have several hematologic variables that are outside of the respective reference intervals of other dog breeds. In addition, increases in HCT, total protein and HGB concentration, and RBC and WBC counts occur immediately after exercise; these values return to resting values within a few hour after racing.
Objective: This study evaluated the effects of exercise on the concentration of reticulocytes in circulating blood in racing Greyhounds. We hypothesized that reticulocyte numbers are significantly increased immediately after a race, and return to baseline within one to 2 h postrace.
Methods: Fifty actively racing Greyhounds at the Wheeling Island Racetrack and Casino were included in the study. Samples were collected by jugular venipuncture one day prior to racing at the kennel (resting), immediately after racing, and one to 2 h after the race (recovery). Reticulocyte counts were determined with an IDEXX ProCyte Dx Hematology Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA). Due to a nonparametric distribution, the results were statistically compared using the Friedman test.
Results: Reticulocyte concentrations were significantly different among the 3 sample collection times ($P < .0001$). There was a significant increase in reticulocyte concentration immediately after racing ($P < .001$); one to 2 h after racing, the reticulocyte numbers decreased significantly ($P < .001$) to counts comparable to resting samples.
Conclusion: The increase in reticulocyte concentration is probably related to splenic contraction secondary to the release of catecholamines, although premature bone marrow release could also account for these changes. Thus, it is important to consider a Greyhound's activity and degree of excitement when interpreting selected hematologic data in a clinical setting.

Introduction

Greyhounds have slightly different hematology reference intervals (RI) when compared with other dog breeds.[1] These differences include lower WBC, neutrophil (NEU), and platelet (PLT) counts; higher HCT, HGB concentration, and RBC counts; and an atypical eosinophil (EOS) morphology.[1–4] Retired racing Greyhounds also have high-affinity HGB.[5] On the other hand, there are significant increases in HCT, total plasma protein (TP) and HGB, as well as RBC, WBC, NEU, and lymphocyte (LYM) counts after exercise.[6–10] In normal dogs, reticulocytes are often released from the bone marrow to the circulation in an immature stage of development. After homing to the spleen, they continue their maturation process for approximately 2 days.[11] This particular function has led to the term "reticulocyte training camp" for the spleen.[12] As a result, the proportion of reticulocytes within the RBC pool is higher in the spleen than in peripheral circulation.[13]

Increased reticulocyte counts are the hallmark of regenerative anemia. Occasionally, high reticulocyte counts are seen in healthy dogs with a normal HCT for a variety of reasons, including compensated hemolysis. However, to our knowledge, the physiologic

reticulocyte response during excitement or exercise, probably associated with splenic contraction or premature bone marrow release secondary to catecholamines, has not been reported in dogs. We observed 3 healthy blood donor Greyhounds (index patients) at The Ohio State University's College of Veterinary Medicine Animal Blood Bank that were excited during their office visit, and had a HCT above the RI (> 65%) for the breed and increased reticulocyte counts (> 60,000 × 10^9/L). In 2 of these dogs, HCT and reticulocyte count returned to the RI within 3 h of "hospital adjustment."

These observations suggested to us that the physiologic changes that occur during excitement, probably associated with catecholamine-induced splenic contraction, may affect the circulating concentration of reticulocytes in dogs, and particularly in Greyhounds. Interestingly, there is an increase in circulating numbers of hematopoietic progenitors and peripheral reticulocyte concentrations after short-term, all-out, exercise in human athletes.[14–16] Although, in our experience, Greyhounds have larger spleens than dogs of most other breeds, premature bone marrow release of reticulocytes may be responsible for increased reticulocyte concentrations in excited or exercising Greyhounds.

The role of the spleen on the concentration of circulating reticulocytes has been documented in non-Greyhound dogs. During anesthesia and splenic congestion, reticulocyte counts decreased to a greater degree than RBC counts, and reticulocytes remained lower than the RBC count 2 h following recovery from anesthesia; retention in the spleen to complete the maturation process was the main proposed mechanism.[17]

This study aimed at investigating the effects of exercise on the circulating reticulocyte concentration in racing Greyhounds. We hypothesized that absolute and relative reticulocyte counts will increase significantly from baseline immediately after a race, and will return to baseline values within one to 2 h postrace. As a result, we also expected to see an increase in MCV and RDW, and a decrease in MCHC.

Animals and Methods

Animals

Blood samples were obtained from 50 healthy, sexually intact, actively racing Greyhounds of both sexes (25 male and 25 female) at the Wheeling Island Racetrack and Casino in West Virginia. Signed consent was obtained from all dog owners, and the project was approved by the Racing Commission.

Blood collection and analysis

Whole blood samples were obtained at the following time intervals: one day prior to racing while at the kennel (resting), immediately after racing (ie, within 5 minutes), and at one to 2 h after racing (recovery). Blood was collected by jugular venipuncture using a 21-G butterfly needle attached to a BD Vacutainer collecting system (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Each sample was collected into a vacuum-sealed 1 mL VetCollect purple top tube containing EDTA (IDEXX Laboratories, Inc., Westbrook, ME). All samples were then refrigerated and analyzed on the same day of collection using an IDEXX ProCyte Dx Hematology Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA) according to the manufacturer's instructions; the instrument was installed and operated in the veterinary clinic at the racetrack. Samples collected at the kennel were processed within 3 h and those collected at the racetrack were processed within 2 h of collection.

Reticulocyte dot plots were visually inspected to determine cell distribution. Based on RNA content, reticulocytes are classified as low (L), medium (M), or high (H) fluorescence (or RNA content), where decreasing RNA content indicates maturation of reticulocytes from H to L reticulocytes, with the former located to the left in the dot plot.[14,18]

Statistical analysis

Using the statistical software GraphPad Prism (Graphpad Software, Inc., San Diego, CA, USA), the data were analyzed using descriptive statistics and evaluated for normality using the D'Agostino and Pearson omnibus test. A one-way ANOVA for repeated measures was used for data sets of Gaussian distribution. Data from each sampling period were compared with the other timepoints via the Tukey's Multiple Comparison Test. Nonparametrically distributed data sets were analyzed using the Friedman Test, followed by the Dunn's Multiple Comparison Test. Data from male and female dogs that ran different race lengths (ie, 548 yards vs 678 yards) were compared using the Student's *t*-test for parametrically distributed data, or the Mann–Whitney test for data with a nonparametric distribution. Statistical significance was set at $P < .05$.

Results

The median age of the tested Greyhounds was 2.2 ± 0.1 years and the median weight was

Table 1. Hematologic variables determined in Greyhounds before, immediately after, and one to 2 h after racing.

	Blood Collection Period			*P*-Value			
Parameter	Resting at Kennel (A)	Immediately Postrace (B)	One to 2 h Postrace (C)	ANOVA	A vs B	B vs C	A vs C
RETIC (10^3/μL)	13.85 ± 2.04	71.50 ± 5.40	17.40 ± 2.02	<.0001	<.001	<.001	NS
RETIC%	0.1550 ± 0.02	0.7050 ± 0.06	0.2150 ± 0.02	<.0001	<.001	<.001	NS
MCV (fL)*	69.26 ± 0.26	70.45 ± 0.39	68.81 ± 0.24	<.0001	<.001	<.001	<.05
RDW (%)	19.15 ± 0.15	20.55 ± 0.14	18.70 ± 0.17	<.0001	<.001	<.001	NS
MCHC (g/dL)	33.70 ± 0.072	32.80 ± 0.14	33.75 ± 0.06	<.0001	<.001	<.001	NS
HCT (%)	61.10 ± 0.58	73.70 ± 1.04	57.35 ± 0.72	<.0001	<.001	<.001	<.05
WBC (10^3/μL)	7.300 ± 0.25	7.790 ± 0.48	7.840 ± 0.30	.1532	NS	NS	NS
NEU (10^3/μL)	4.450 ± 0.16	5.330 ± 0.19	6.190 ± 0.22	<.0001	<.001	<.05	<.001
LYM (10^3/μL)	2.080 ± 0.17	1.460 ± 0.35	0.9600 ± 0.14	<.0001	NS	<.001	<.001
PLT (10^3/μL)	130.0 ± 4.73	142.5 ± 5.61	120.5 ± 4.58	.0002	NS	<.001	NS

Data are median ± SE except for MCV (*, expressed as mean ± SE, parametric distribution).
RETIC indicates absolute reticulocyte concentration; RETIC%, percent reticulocytes; NEU, absolute neutrophil concentration; LYM, absolute lymphocyte concentration; PLT, platelet concentration

30.3 ± 0.4 kg. Compared with the females, the males had significantly higher resting WBC counts as well as higher one to 2 h postrace MCHC values, respectively ($P = .02$, $P = .01$, Table 1). There were no other significant hematologic differences between the sexes.

Of the 50 dogs in the study, 42 ran a distance of 548 yards and 8 ran 678 yards. The RDW was significantly higher immediately postrace in those dogs that ran 678 yards ($P = .04$); there were no other significant differences in any of the measured variables between the 2 race lengths.

Reticulocyte concentrations differed significantly among the 3 sample collection periods ($P < .0001$, Table 1, Figure 1). Reticulocyte concentration increased significantly from baseline immediately after the

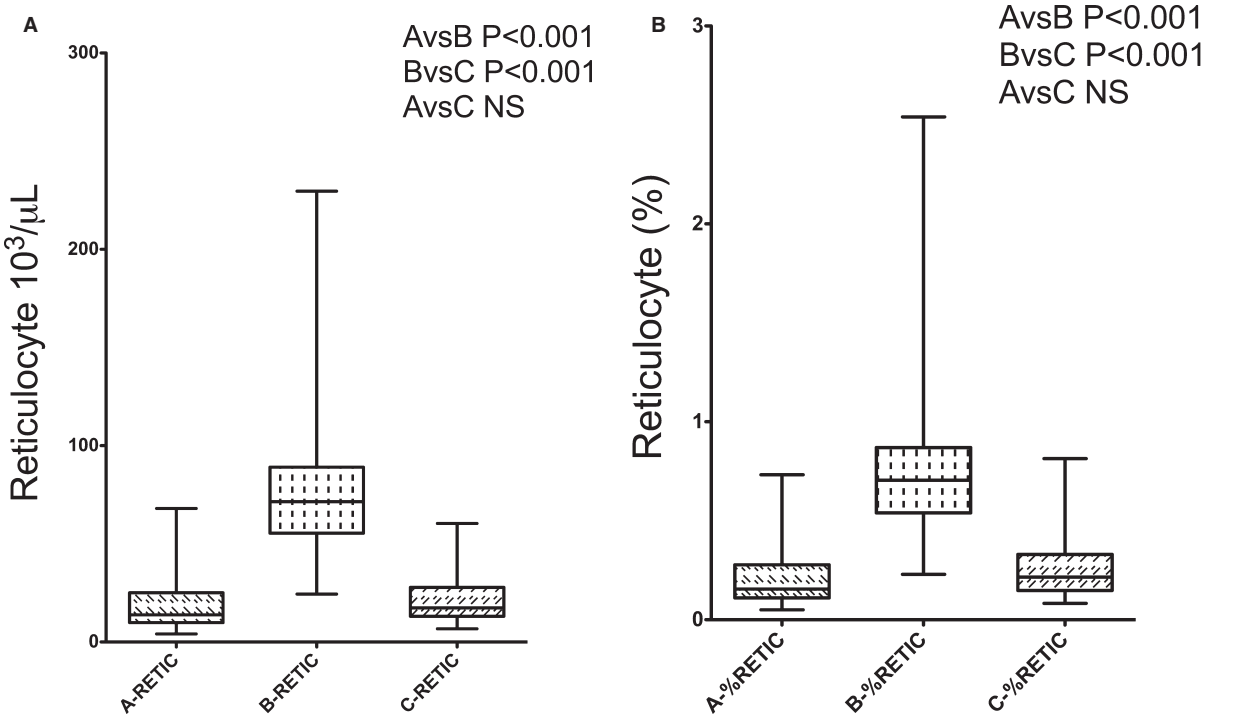

Figure 1. Box and whisker plots of changes in relative (**A**) and absolute (**B**) reticulocyte counts in Greyhounds before, immediately after, and one to 2 h after a race. Whiskers depict 2.5 and 97.5 percentiles.

race ($P < .001$), and then returned to baseline counts one to 2 h postrace ($P < .001$). Visual inspection of the reticulocyte dot plots revealed that in all dogs with increases in reticulocyte concentration, the reticulocytes were in the left half of the *X*-axis (ie, L and M, Figure 2).

The MCV and the RDW also increased significantly immediately after racing ($P < .001$, $P < .001$), and returned to baseline one to 2 h postrace. In addition, the MCHC decreased significantly immediately after the race compared with the baseline, and returned to baseline in one to 2 h postrace samples ($P < .001$, Table 1, Figure 3).

There was a significant increase in HCT immediately after racing ($P < .001$); the HCT decreased from immediately after racing values to below baseline values at the one to 2 h postrace sampling ($P < .05$). There were no significant differences in WBC counts after racing. However, NEU counts significantly increased immediately postrace ($P < .001$) and were still high one to 2 h postrace ($P < .05$). There was no significant difference in LYM counts immediately after racing; however, a significant decrease in LYM counts occurred after one to 2 h postrace, when compared to the sample collected immediately after exercise ($P < .001$). Interestingly, there were no significant differences in PLT counts between baseline and either of the postrace samples; however, PLT counts were significantly lower at the one to 2 h postrace sampling compared with the immediate postrace sample ($P < .001$) (Figure 4).

Discussion

The HCT, MCV, RDW, reticulocyte concentration, and NEU counts increased, while the MCHC decreased in Greyhounds immediately after a race. These changes were primarily attributed to exercise-induced catecholamine release and subsequent splenic contraction. However, other contributing mechanisms include premature release of hematopoietic and endothelial precursors from the bone marrow, and decreased plasma volume immediately after a race, as previously reported in dogs and people.[19–21]

All of these variables returned to baseline within one to 2 h postrace, except HCT and NEU counts. In addition, LYM counts significantly decreased from the resting and immediate postrace and the one to 2 h postrace samples. The significant decrease in HCT one to 2 h postrace could be due to an extracellular fluid volume expansion as suggested in sled dogs;[22] alternatively, splenic relaxation after the race could sequester part of the circulating erythrocyte pool. This pattern and the degree of neutrophilia and lymphopenia are consistent with stress-induced

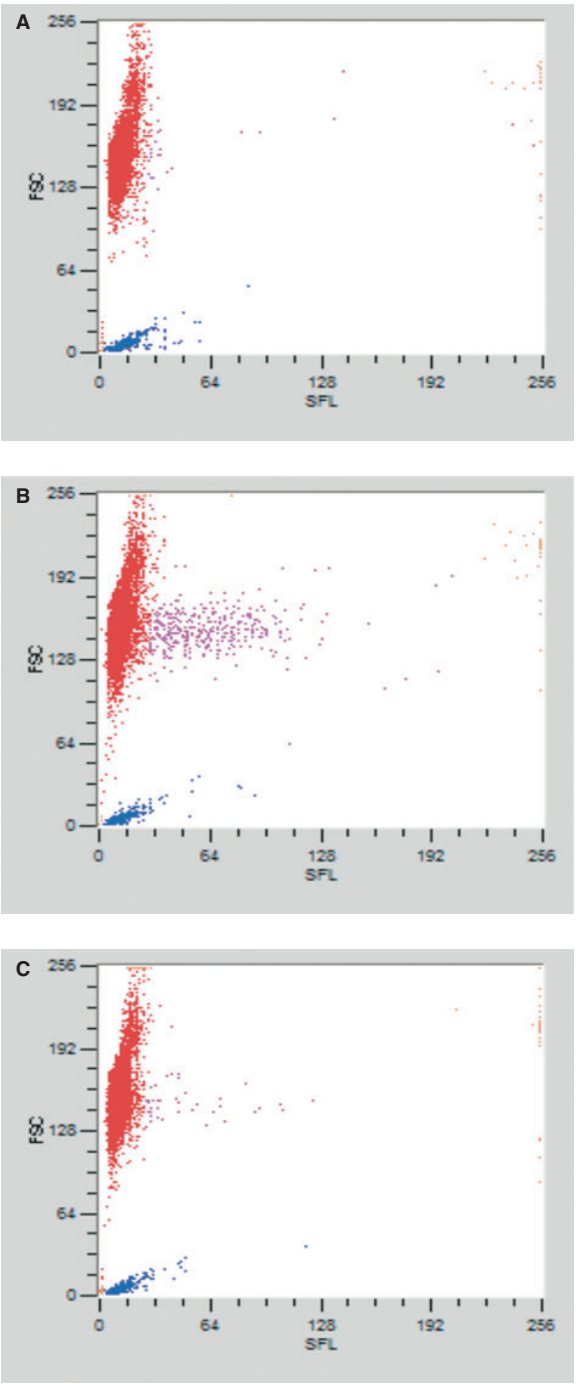

Figure 2. Reticulocyte dot plots for Greyhound #35, where the *x*-axis depicts RNA content as an index of maturation, and the *y*-axis the size of reticulocytes. (**A**) Before exercise. (**B**) Immediately after a 548-yard race. Note a purple cloud of larger reticulocytes with slightly more RNA extending to the right of the *x*-axis and in the middle of the *y*-axis. (**C**) One hour after a 548-yard race.

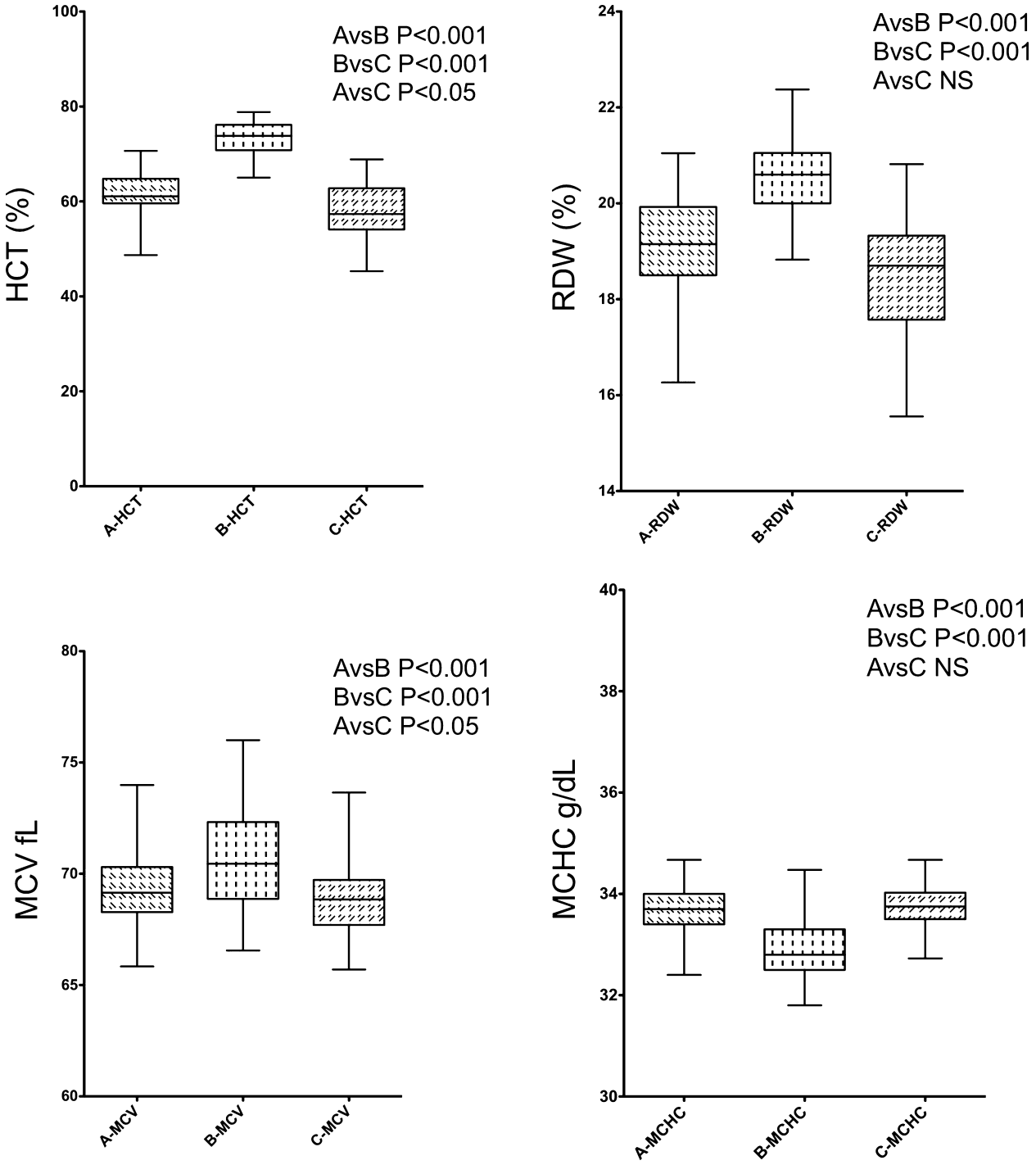

Figure 3. Box and whisker plots of changes in HCT, RDW, MCV, and MCHC in Greyhounds before, immediately after, and one to 2 h after a race. Whiskers depict 2.5 and 97.5 percentiles, the horizontal line is the median.

cortisol release; however, the timepoint of the response was more rapid than that reported in the literature.[23] In experimental models, it takes a minimum of 4 h after exogenous glucocorticoid administration to see these changes.[23,24] Alternatively, NEU demargination from the peripheral marginal pool due to catecholamine release may have accounted for some of the observed changes. The lymphocytosis

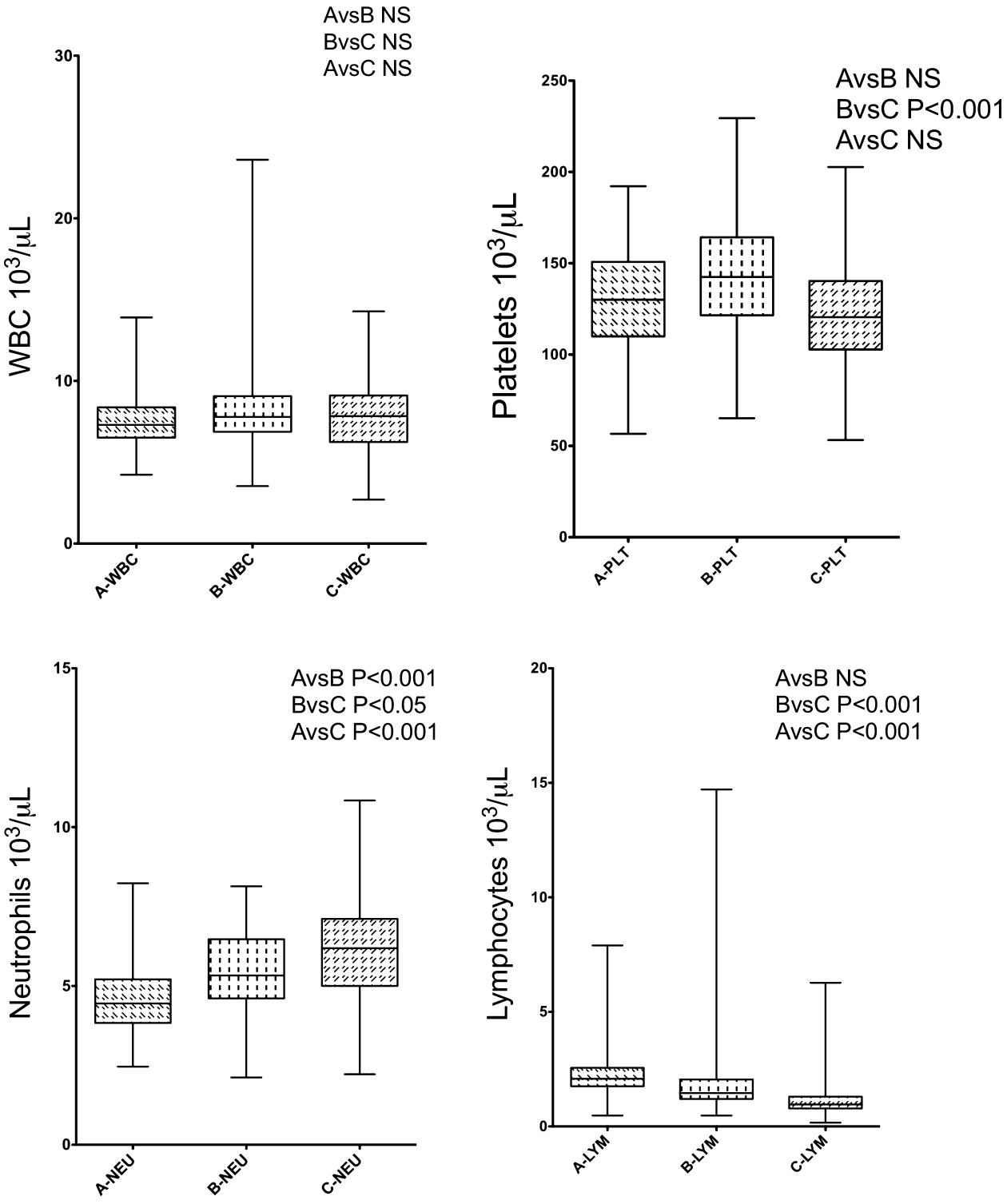

Figure 4. Box and whisker plots of changes in WBC, platelet, neutrophil, and lymphocyte counts in Greyhounds before, immediately after, and one to 2 h after a race. Whiskers depict 2.5 and 97.5 percentiles, the horizontal line is the median.

sometimes seen in dogs in response to catecholamines was absent;[23] however, this is more common in cats and horses.[25]

There was a significant difference in the resting WBC counts and in the recovery MCHC values between sexes. A significantly higher immediate

postrace RDW was observed in dogs after racing 678 yards compared with those that ran 548 yards. These differences may reflect endogenous or exogenous hormonal influences on the bone marrow.[26] A similar study in a larger population may provide more insight on this difference.

As previously described, Greyhounds have a significant increase in the circulating RBC mass immediately after, and probably during, exercise. This may be due to the need to increase oxygen tissue delivery,[1,5–9] or to buffer the low pH that occurs during exercise,[1,5] among other mechanisms. It has been documented for over a century that exercise induces splenic contraction in people, horses, and dogs.[19–21] The expected splenic contraction probably resulted in the increased HCT in our study.[19–21] Already back in 1927, a marked decrease in splenic volume associated with exercise, blood loss, or anxiety in dogs was described.[20,21]

Average increases in reticulocyte numbers immediately after all-out exercise in human rowers of up to 25–30% have also been described.[14] Likewise, a median baseline reticulocyte percentage of 0.9%, and a modest, but significant, increase of 0.05% after a short, all-out, exercise period in human athletes have been reported.[16] This is similar, although of lesser magnitude, to the findings in our study, where the median baseline reticulocyte percentage of 0.15% increased by 4.7-fold to 0.7% immediately after racing, and returned to baseline one to 2 h postrace.

There are several possible explanations for the appearance of high numbers of circulating reticulocytes immediately after racing. First, during periods of erythropoietic stress, as occurs with exercise-induced hypoxia, large immature reticulocytes are released early from the bone marrow. These "stress reticulocytes" express surface receptors for adhesive proteins, such as fibronectin, which is lost as the reticulocytes mature.[14,27,28] One proposed hypothesis for the increase in reticulocyte concentration after all-out exercise in people was an activation of angiogenesis by tissue hypoxia, stimulating a release of young reticulocytes from the bone marrow, as indicated by an increase in AC133 cells, hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF).[14] This represents another potential contributing mechanism or an alternative theory for the reticulocyte changes seen in our study. Possibly, once released from the bone marrow, these stress reticulocytes are filtered from circulation mostly by the spleen and to a minor extent by the liver where they complete their maturation process.[27–29]

It is reasonable to expect that splenic contraction associated with catecholamine release could lead to increases in circulating reticulocytes in dogs, and particularly in racing Greyhounds, given their larger splenic size. However, as discussed above, premature bone marrow release secondary to hypoxia, and decreases in circulating plasma volume could also play a role in these hematologic changes.[14–16] Further investigations are needed to discern the source of the reticulocyte pool observed in the present study. Evaluation of the postrace reticulocyte dot plots revealed that all dogs had increased L and M reticulocytes, which are probably not immature reticulocytes, further supporting the splenic origin of these circulating red cell precursors. Ultrasonographic evaluation of splenic volume at the same time points when we collected blood in this study may allow for inferences regarding proportional changes in splenic size and hematologic changes.

The changes observed in MCV, MCHC, and RDW are probably directly related to the reticulocytosis. As reticulocytes are larger than mature erythrocytes and have lower concentrations of intracellular HGB, the observed increased MCV and decreased MCHC during the periods of reticulocytosis in this study are expected. The increased RDW, an objective measure of anisocytosis, is best explained by the presence of increased numbers of the larger reticulocytes mixed with normal-sized erythrocytes.

Based on our finding of both increases in HCT and peripheral reticulocyte concentration immediately after all-out exercise in Greyhounds, exercise-induced splenic contraction is the most likely cause, although premature bone marrow release cannot be excluded at this time. Unexpectedly, the platelet counts did not increase after the race. Given the size of the Greyhound spleen, we anticipated postrace thrombocytosis. However, the absence of this change may be due to the fact that Greyhound platelets are more likely to aggregate,[1] representing a preanalytical factor affecting the platelet count. Increased platelet adhesion/aggregation secondary to inflammatory mediators may also have contributed to a lack of net increases in the platelet counts.[30]

As a result of catecholamine release, various degrees of splenic contraction can also occur during times of excitement, such as visiting the veterinary hospital, resulting in similar hematologic variances as described in the present study. Thus, it is important to consider these physiologic variables when interpreting select Greyhound CBC data in a clinical setting.

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