

INVITED REVIEW

## Clinical pathology of Greyhounds and other sighthounds

S. Zaldívar-López<sup>1,2</sup>, L.M. Marín<sup>1</sup>, M.C. Iazbik<sup>3</sup>, N. Westendorf-Stingle<sup>1,3</sup>, S. Hensley<sup>1</sup>, C.G. Couto<sup>1,3,4</sup>

<sup>1</sup>Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; <sup>2</sup>Center for Molecular and Human Genetics at The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA; <sup>3</sup>Veterinary Medical Center, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; and <sup>4</sup>Ohio State University Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

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### Correspondence

Guillermo Couto, Veterinary Medical Center, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp St., Columbus, OH 43210, USA

E-mail: guillermo.couto@cvm.osu.edu

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**Abstract:** Owing to the development of Greyhounds as racing sighthounds, these dogs have acquired unique physiologic adaptations that distinguish them from other breeds. Reference intervals for many analytes in retired racing Greyhounds (RRGs) differ from those of other breeds; most of the hematologic differences have also been described in other sighthounds. In this review, we provide a survey of the literature on clinical pathology of Greyhounds and other sighthounds and results of laboratory testing, including analysis of CBCs, biochemical profiles, coagulation tests, and blood gases, in RRGs at The Ohio State University. Major clinicopathologic differences in this breed include higher RBC mass, creatinine concentration, glomerular filtration rate, activities of hepatic enzymes, and concentration of cardiac troponin, as well as lower WBC, neutrophil, and platelet counts, thromboelastographic values, and concentrations of serum haptoglobin, total globulins, and T4.

- I. Introduction
- II. Literature Review
  - A. Hematology
    - 1. Erythrocytes
    - 2. Leukocytes
    - 3. Platelets
  - B. Hemostasis
  - C. Clinical chemistry
    - 1. Urea nitrogen and creatinine
    - 2. Hepatic enzymes
    - 3. Serum electrolytes and acid-base balance
    - 4. Serum proteins
    - 5. Thyroid hormones
    - 6. Cardiac troponin
- III. Urinary fractional excretion of electrolytes
- IV. Preliminary Reference Intervals for Greyhounds at The Ohio State University
- V. Conclusion
- VI. References

### Introduction

Since the early 1990s, more than 180,000 retired racing Greyhounds (RRG) have been placed in adoptive homes and this number rises each year. Therefore, practicing veterinarians are seeing an increasing num-

ber of Greyhounds for routine wellness examinations and medical and surgical ailments, and clinical pathology laboratories are receiving more samples from these dogs. Thus, it is imperative that veterinarians, including practitioners and clinical pathologists, become aware of the unique hematologic and biochemical characteristics of the breed (Table 1).

Owing to the development of Greyhounds as racing sighthounds, these dogs have acquired unique physiologic adaptations that distinguish them from other breeds. Greyhounds have larger muscle mass than most breeds<sup>1</sup>; higher HCTs<sup>2</sup>; lengthened carpal, tarsal, metacarpal, and metatarsal bones; and a keen sense of sight.<sup>1</sup> These adaptations, among others, have probably contributed to the unique hematologic and biochemical characteristics of Greyhounds compared with those of non-Greyhound breeds, and these characteristics have been well documented over the last 50 years.<sup>2-6</sup> Results of routine laboratory tests in RRGs frequently lie outside the reference intervals for other non-sighthound breeds; most of the distinctive hematologic features in Greyhounds have also been described in other sighthounds.<sup>7</sup> Thus, there is a need to establish reference intervals specific for Greyhound dogs, and these intervals may also apply to other sighthound breeds. In this review, we survey the literature

**Table 1.** Analytes and features characteristic of Greyhound dogs compared with those of other breeds.

Higher Values	Lower Values	Unique Features
PCV/HCT	WBC count	Non-staining
RBC count	Neutrophil count	eosinophil granules
Hemoglobin concentration	Platelet count	
MCV*	Fibrinogen	Higher frequency
MCHC	TEG values: K-time, angle, MA, and G	of DEA 1.1-negative dogs
Hemoglobin affinity for O <sub>2</sub>		
Creatinine	Potassium	
Glomerular filtration rate	Phosphate	
Alanine aminotransferase	Calcium, ionized	
Aspartate aminotransferase	Magnesium, ionized	
Sodium	Serum total protein	
Chloride	Total globulins	
Total CO <sub>2</sub>	α- and β-globulins	
Bicarbonate	IgA and IgM	
Cardiac troponin I	Haptoglobin	
	Total T4 and free T4	

\*Reported in only one study.<sup>3</sup>

on clinical pathology of Greyhounds and other sight-hounds and provide results of laboratory testing in RRGs conducted at The Ohio State University.

## Literature Review

### Hematology

Many clinicopathologic differences between Greyhounds and other breeds have been investigated. However, most of the research has focused on differences in hematologic values in the breed, and hematologic reference intervals for the Greyhounds recently have been published.<sup>8</sup>

#### *Erythrocytes*

Previous studies have reported that Greyhounds have higher HCT, hemoglobin (HGB) concentration, MCV, and MCHC when compared with values in non-Greyhound dogs.<sup>2-4,9</sup> Most studies also reported higher RBC counts<sup>2,4</sup>; in one study, the mean RBC count for Greyhounds, although similar to counts reported in other studies, was lower than the mean count for non-Greyhounds owing to higher values among the latter.<sup>3</sup> Traditionally, high HCT, HGB concentration, and RBC count have been considered an adaptation to athleticism, under selective breeding for superior track performance, resulting in dogs with higher total

oxygen-carrying capacity<sup>10</sup>; however, many studies are underway to further investigate the underlying factors that influence hematologic features in Greyhounds.

Investigations of the potential roles of age, sex, and training on hematologic values in young pretraining Greyhounds, and the age at which hematologic differences become apparent in the breed, found that HCT, HGB concentration, and RBC count correlated positively with age.<sup>11</sup> Greyhounds that were 9–10 months old had higher values and a tendency toward higher MCVs when compared with non-breed-specific reference intervals. The hematologic differences were less marked in Greyhounds that were 5–6 months old. There was no significant difference in HCT between Greyhounds in the 9–10 month old and 12–13 month old groups, suggesting that adult hematologic values in Greyhounds are probably reached by 9–10 months of age, similar to what has been described for other breeds.<sup>12,13</sup> At the same time, these findings suggest that training and racing are not primarily responsible for differences in hematologic values between adult Greyhounds and other breeds.

Selective breeding of Greyhounds might be the cause of reported altered HGB function and properties owing to the imperative need for appropriate oxygen supply at the tissue level under extreme conditions, ie, during a race.<sup>3,10</sup> Greyhounds had lower hemoglobin P<sub>50</sub> values (the partial pressure of oxygen at which 50% of the hemoglobin is saturated) than those of non-Greyhound dogs,<sup>3</sup> meaning that the oxyhemoglobin dissociation curve was left-shifted and implying that HGB has a higher affinity for oxygen in Greyhounds than in non-Greyhound dogs. Interestingly, RBC 2,3-diphosphoglycerate (2,3-DPG) concentration, which influences the position of this curve, was not different between Greyhounds and non-Greyhounds. High HGB concentration and PCV in Greyhounds was suggested to be a compensatory change secondary to decreased oxygen delivery to the tissues (low P<sub>50</sub>), as is seen in people with high-affinity hemoglobinopathies.<sup>3</sup> We recently conducted additional studies on high-affinity HGB and its function in RRGs, using blood gas analysis with cooximetry and confirmed low P<sub>50</sub> and higher oxygen content and oxygen-binding capacity of HGB in Greyhounds.<sup>10</sup> In a different study, we found that HGB in Greyhounds has a few unique amino acid mutations that are relevant to the oxygen affinity properties, and alter the position of the globin chains.<sup>14</sup> Overall, HGB sequence in Greyhounds is closer to the human sequence than the equine or bovine sequences; thus, it may be used as a model for HGB of high-oxygen affinity in people.<sup>14</sup>

The mean lifespan of Greyhound RBCs has been reported to be significantly shorter than that of non-Greyhound RBCs, with mean values of  $53.6 \pm 6.5$  days and  $104.3 \pm 2.2$  days, respectively.<sup>4</sup> Possible explanations for the shorter RBC lifespan include differences in membrane structure and hastened removal from circulation due to decreased membrane fluidity or increased membrane affinity for IgG, a trigger mechanism for the removal of senescent RBCs.<sup>4</sup> Another proposed mechanism is preferential splenic sequestration of labeled cells, as Greyhounds are reported to have larger spleens than most other breeds have, but this hypothesis has not been tested. Recently, another study using the same technique of erythrocyte biotinylation demonstrated no differences in RBC survival among Greyhounds and non-Greyhounds.<sup>15</sup> Additional studies will be needed to clarify this controversy.

Macrocytosis has been reported in Greyhounds.<sup>2,3,6</sup> If their RBCs had a shorter lifespan, perhaps they would have higher numbers of circulating reticulocytes with a higher MCV. However, a mean reticulocyte percentage of 0.2% for Greyhounds suggests that macrocytosis is not the result of reticulocytosis, and could represent a physiologic breed-specific feature.<sup>3</sup> It has been reported that Greyhound RBCs are larger and possibly have higher HGB concentrations, leading to increases in both MCV and MCHC.<sup>2,3</sup> Other studies and data generated in our laboratory, using a Cell-Dyn 3500 hematology analyzer (Abbott Diagnostics, Abbott Park, IL, USA) as a reference instrument and a LaserCyte (IDEXX Laboratories, Westbrook, ME, USA) as a portable bench-top analyzer, have demonstrated MCVs and reticulocyte numbers in Greyhounds and other sighthounds that are within nonbreed-specific reference intervals for dogs.<sup>7</sup>

Interestingly, the distribution of dog erythrocyte antigen (DEA) types is different in Greyhounds from that in other breeds. We recently reported that only 13.3% of RRGs expressed DEA 1.1, whereas 60.6% of all other breeds combined were DEA 1.1-positive; 2.9% of RRGs expressed DEA 1.2, which was not detected in other breeds. Among Greyhounds, 63.4% were considered universal donors compared with 18.2% of other breeds.<sup>16</sup> In another study, 45.5% of Galgos Españoles (Spanish Greyhounds) expressed DEA 1.1.<sup>17</sup>

#### *Leukocytes*

Greyhounds have been reported to have lower mean WBC counts than other breeds,<sup>2,9,11</sup> and reference intervals recently established for total WBC, neutrophil, and lymphocyte counts have been published.<sup>8</sup> The Greyhound eosinophil has been widely studied. In

the early 1960s, it was reported that many Greyhounds had “vacuolated” eosinophils, termed “grey” eosinophils, which were devoid of visibly stained cytoplasmic granules.<sup>18</sup> In most Greyhounds, eosinophils lack the typical orange granules detected with Romanowsky stains, such as Wright–Giemsa or rapid stains,<sup>18</sup> and may be mistaken for toxic neutrophils on a routine blood smear stained with Diff-Quik, leading to an unnecessary search for a source of inflammation.<sup>19</sup> We recently evaluated the morphologic, ultrastructural, and cytochemical characteristics of Greyhound eosinophils. On Wright-stained blood smears, vacuolated eosinophils were found in > 50% of the Greyhounds.<sup>19</sup> Granules in vacuolated eosinophils were ultrastructurally similar to typically staining granules in eosinophils from other breeds. None of the affected Greyhounds had clinical signs of diseases, suggesting that vacuolated eosinophils represent a change in tinctorial properties rather than a functional abnormality.<sup>19</sup> Possible causes for the nonstaining granules include an alteration in basic proteins that confer the pink-orange hue in eosinophil granules or a decrease in the pH of components of eosinophilic granules, resulting in less binding of the eosin in Romanowsky stains.

#### *Platelets*

Sullivan was the first to report lower platelet concentrations in Greyhounds than in dogs of other breeds,<sup>3</sup> a finding that has been supported by subsequent studies.<sup>9,11,20,21</sup> Sullivan proposed the stem-cell competition model of hematopoiesis as a possible mechanism for lower platelet counts, suggesting that bipotential stem cells within bone marrow are programmed to become either megakaryocytes or erythrocyte precursors. These stem cells are responsive to hormonal stimuli for production of one lineage over the other, thus leading to an increase in one cell line and concurrent decrease in the other.<sup>63</sup> Another hypothesis for lower platelet counts in Greyhounds relates to the left shift of the oxygen–hemoglobin dissociation curve, leading to mild hypoxia, and potentially resulting in increased production of erythropoietin, increased erythropoiesis, and a consequential decrease in megakaryocytopoiesis.<sup>3</sup> A negative correlation between HCT and platelet count in Greyhounds, fitting the bipotential stem cell theory, also has been reported.<sup>11</sup> In a study of the effects of increased RBC mass (HCT 85%) on hemostasis in a population transgenic mice that overexpressed the human erythropoietin gene, the transgenic mice had lower platelet counts than the control mice. As RBCs comprised 85% of the blood volume, the platelet count was calculated for the plasma volume fraction, and transgenic mice were no longer

thrombocytopenic, with counts similar to those of wild-type mice; this suggests that the number of platelets per unit of whole blood is not necessarily equivalent to the number of platelets per unit of plasma in patients with high HCT.<sup>22</sup>

Other proposed mechanisms for lower platelet counts in Greyhounds include splenic or pulmonary sequestration or a chronic low-grade immune-mediated process leading to decreased platelet lifespan.<sup>3</sup> The latter mechanism was ruled out in a study that investigated the presence of platelet surface-associated IgG (PSA-IgG) in Greyhounds.<sup>21</sup> PSA-IgG was not detected in any of the samples, suggesting that the lower platelet count in the breed was not attributable to immune-mediated mechanisms.<sup>21</sup> As platelet counts are inversely correlated with iron stores,<sup>23</sup> another possibility is that Greyhounds have higher body iron content, owing to their high HCTs, and therefore have lower platelet counts. In our experience, platelets tend to clump more in Greyhounds than in other breeds, similar to feline platelets. In summary, in-depth investigation of thrombocytopenia is not warranted in healthy Greyhounds with platelet counts of  $< 100,000/\mu\text{L}$ , as the low count probably represents a breed-related trait.

## Hemostasis

The main function of the hemostatic system is to maintain blood flow within the cardiovascular system. The term “Greyhound bleeder” has been used to describe dogs that tend to bleed spontaneously following minor trauma or after a simple surgical procedure.<sup>24</sup> Severe postoperative bleeding 1–4 days after limb amputation for osteosarcoma or trauma has also been reported in Greyhounds, often resulting in the need for blood component therapy during the postoperative period.<sup>24</sup> Historically, Greyhounds with spontaneous bleeding have had normal platelet counts for the breed as well as normal von Willebrand factor (VWF) concentration and one-stage prothrombin (OSPT), and activated partial thromboplastin times (aPTTs) at the time of postoperative hemorrhage, making common bleeding disorders, such as thrombocytopenia and deficiencies in coagulation factors or VWF, unlikely causes of the bleeding.

We recently investigated platelet function in Greyhounds using the PFA-100 System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) to evaluate primary hemostasis in healthy Greyhounds and in those with bleeding disorders.<sup>20</sup> Platelet count, plasma VWF:Ag concentration, and VWF activity measured using a collagen-binding assay were correlated with

the results of the PFA-100 assays. Surprisingly, the lower platelet counts in Greyhounds were not associated with prolongation of the closure time (CT), the time required for a platelet plug to form in the capillary aperture and halt blood flow, and Greyhounds had a shorter mean CT than non-Greyhounds had; however, CT ranges were similar to reported values for other breeds.<sup>20</sup> The shorter CTs in Greyhounds are probably explained by the higher PCV, and thus viscosity, in the breed,<sup>2,9</sup> as higher PCV and whole blood viscosity lead to peripheral platelet distribution and consequent increased interaction with the blood vessel surface. Shorter CTs in the breed may be an adaptive platelet response to accommodate higher shear in Greyhounds,<sup>20</sup> as Greyhounds also have significantly higher arterial blood pressure and aortic velocity than non-Greyhounds have.<sup>25–27</sup>

The most common canine hereditary hemostatic defect is von Willebrand disease.<sup>29</sup> Although some VWF-deficient Greyhounds have been identified in breed surveys<sup>28,29</sup> and in coagulation screens, the disease does not appear to be a common coagulopathy in Greyhounds. Of the Greyhounds tested at The Ohio State University and at the Cornell Comparative Hemostasis Laboratory,  $< 10\%$  (22/216) had activities of VWF below 30% (unpublished data).

Thromboelastography (TEG) permits evaluation of blood coagulation through assessment of the speed and strength of clot formation. TEG is dependent on the function of primary and secondary hemostasis and fibrinolysis, all of which can be affected by certain illnesses, environmental conditions, and pharmacologic agents.<sup>30</sup> We recently reported that Greyhounds have slower clot kinetics and weaker clot strength compared with these variables in non-Greyhounds,<sup>31</sup> lending support to increased bleeding tendencies in Greyhounds following minor trauma or surgical procedures.<sup>31</sup> The observed differences may be related to blood viscosity, as the higher HCTs in Greyhounds result in less plasma per unit volume of blood<sup>32</sup>; these hemostatic findings are similar to those reported in polycythemic mice.<sup>22</sup> It is also known that RBCs interfere with TEG variables; for example, it has been shown *in vitro* that an increase in RBC mass reduces clot strength and kinetics, even when TEG analysis is performed on plasma samples without platelets.<sup>33</sup> In our TEG study, we found no significant differences in fibrinogen concentration between Greyhounds and non-Greyhounds, suggesting that the slower clotting kinetics and weaker clot strength cannot be attributed to hypofibrinogenemia in the former.<sup>31</sup>

In investigations of postoperative bleeding in RRGs, we found that 26% (23/88) of Greyhounds

had moderate-to-severe bleeding 36–48 hours after routine gonadectomy.<sup>24</sup> Primary and secondary hemostasis were evaluated preoperatively by measuring platelet count; OSPT, aPTT, and fibrinogen concentration by nephelometry; platelet function with the PFA-100; plasminogen, antiplasmin (AP), and antithrombin (AT) activities; D-dimer concentration; VWF (VWF:Ag) and VWF collagen-binding assay (vWF:CBA) activities; and Factor XIII activity. Hemostasis assays were repeated postoperatively at the time of the detection of the bleeding in those RRGs that had bleeding complications, and in an age- and sex-matched control group of RRGs that had the same surgical procedures at the same time and did not bleed.<sup>24</sup> Of the variables measured, the only differences found were lower AP and AT and higher vWF:CBA activities preoperatively in bleeders compared with non-bleeders. At the time of the bleeding, bleeders had a lower platelet count and HCT, shorter OSPT, and higher fibrinogen concentration. Results suggested that excessive postoperative bleeding in RRGs was not attributable to a defect in primary or secondary hemostasis, but may have been related to enhanced fibrinolysis, as the “bleeders” had lower antiplasmin activities than “non-bleeders” had preoperatively. Furthermore, we proposed that this could be an adaptation to racing or an evolutionary trait intended to prevent clotting of blood with high viscosity during exercise.<sup>24</sup> The pedigrees and racing performances of “bleeders” and “non-bleeders” were not evaluated; thus, it is possible that a genetic alteration leading to defects in clotting or fibrinolysis may play a role in the observed bleeding, as racing Greyhounds are derived from a small genetic pool.<sup>24</sup>

### Clinical chemistry

Clinicians and clinical pathologists should be aware of differences in specific serum biochemical values in Greyhounds<sup>2,6,7,9,11</sup> compared with those in the general canine population to avoid misinterpretations and misdiagnoses. These differences have been confirmed in a recent study of a large number of healthy animals, in which even narrower and more breed-specific reference intervals were established for biochemical analytes in Greyhounds.<sup>34</sup>

#### *Urea nitrogen and creatinine*

Previously published Greyhound-specific reference intervals for serum urea nitrogen and creatinine concentrations were 10–22 mg/dL and 0.8–1.6 mg/dL, respectively.<sup>9</sup> Although mean urea nitrogen concentration was similar to that in other breeds, mean creati-

nine concentrations were significantly higher in Greyhounds (1.6 mg/dL) than in non-Greyhound dogs (1.0 mg/dL).<sup>5</sup> Greyhounds have considerable muscle mass and predictably have higher body stores of phosphocreatine, which may result in higher serum creatinine concentrations. As creatinine is well absorbed from the intestinal tract, diets of animal tissues containing high levels of creatinine fed to many racing Greyhounds may also contribute to the high serum creatinine concentration in the breed. However, high serum creatinine concentrations also occur in retired racers, years after they have left the track.<sup>5</sup> Another possible reason for high serum creatinine would be decreased GFR; however, we found that Greyhounds had significantly a higher GFR than non-Greyhound dogs.<sup>35</sup> Based on these results, the most likely cause of high serum creatinine concentrations in Greyhounds is the large muscle mass, and pursuit of causes of mild increases (1.2–2.1 mg/dL) in serum creatinine concentration in an otherwise healthy Greyhound is not warranted. In the recent report of reference intervals based on a large number of Greyhounds, intervals for urea nitrogen were 11–26 mg/dL and for creatinine 1.1–2.0 mg/dL.<sup>34</sup>

#### *Hepatic enzymes*

Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and of alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are markers of hepatocellular injury and cholestasis, respectively, and reference values for some enzymes have been reported for a small group ( $n = 12$ ) of racing Greyhounds.<sup>36</sup> Mean activities were comparable to those in other breeds, but ranges for ALT and ALP activities were wide in Greyhounds, making their use as indicators of hepatic disease unreliable in Greyhounds.<sup>36</sup> Recently, narrower reference intervals for hepatic enzymes in Greyhounds have been reported with higher ALT activity compared with that in non-breed-specific reference intervals.<sup>34</sup> As muscular dystrophy and subsequent necrosis can increase in increased ALT activity in dogs with no evidence of liver disease,<sup>37</sup> we hypothesize that large muscle mass might be a cause of this high activity in Greyhounds.

#### *Serum electrolytes and acid-base balance*

Higher serum concentrations of sodium (Na) and chloride (Cl) in Greyhounds than in non-Greyhound dogs have been reported.<sup>2,6,9,38</sup> Published Greyhound-specific reference intervals for Na and Cl were 149–157 mmol/L and 110–122 mmol/L, respectively.<sup>9</sup> Greyhounds were also reported to have increased serum total CO<sub>2</sub> concentration compared with that of

non-Greyhound dogs. Although increased total CO<sub>2</sub> concentration is typically indicative of metabolic alkalosis, none of the dogs in the study had any findings consistent with alkalosis.<sup>9</sup>

Mean serum total calcium concentrations in racing Greyhounds tended to decrease during the racing season; however, values were within reference interval.<sup>6</sup> In a large population of healthy nonracing Greyhounds, the reference interval for calcium was lower than that of the nonbreed-specific interval.<sup>34</sup> In a study of retired racing Greyhounds using the STP CCX Analyzer (Nova Biomedical, Waltham, MA, USA), a point-of-care analyzer, potassium, ionized calcium, and ionized magnesium concentrations were also lower than in the non-Greyhound group.<sup>39</sup> In contrast, Greyhounds had a higher glucose concentration when using this instrument, but surprisingly lower glucose concentration when using a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN, USA; data not published) than the non-Greyhounds; thus, clinicians and clinical pathologists must be cautious when using reference intervals established on different populations or instruments. As previously reported,<sup>5,9</sup> bicarbonate concentrations obtained using the STP CCX were also higher than in non-Greyhounds.<sup>39</sup>

#### *Serum Proteins*

Recently published Greyhound-specific reference intervals for serum total protein, albumin, and globulin concentrations were 5.2–6.7 g/dL, 2.7–3.7 g/dL, and 2.2–3.3 g/dL, respectively.<sup>34</sup> Others have also reported lower plasma and serum protein concentrations<sup>2,3,40</sup> and lower serum globulin concentration.<sup>9</sup> Hypoglobulinemia in Greyhounds was investigated by analyzing serum proteins using agarose gel protein electrophoresis (Table 4).<sup>40</sup> The concentrations of total protein (mean ± SD, 5.56 ± 0.39 g/dL), total globulins (2.23 ± 0.24 g/dL), and α-1, α-2, β-1, and β-2 globulins (0.33 ± 0.05 g/dL, 0.27 ± 0.10 g/dL, 0.20 ± 0.06 g/dL, and 0.21 ± 0.07 g/dL, respectively) were significantly lower and the albumin-to-globulin (A:G) ratio (1.23 ± 0.25 g/dL) was significantly higher in Greyhounds than in non-Greyhounds, whose respective values for concentrations of total protein, total globulins, and α-1, α-2, β-1, and β-2 globulins and A:G ratios were 6.07 ± 0.45 g/dL, 2.83 ± 0.35 g/dL, 0.46 ± 0.14 g/dL, 0.47 ± 0.14 g/dL, 0.32 ± 0.12 g/dL, 0.34 ± 0.09 g/dL, and 1.17 ± 0.18 g/dL. No significant differences were found in albumin or γ-globulin concentrations. Further studies are necessary to identify the individual proteins associated with low α- and β-globulin concentrations in Greyhounds. Differences in serum concentrations of acute phase proteins may help explain the

low α-globulin concentration (see below), and lower IgA and IgM concentrations in Greyhounds may contribute to the low β-globulin concentrations in Greyhounds.<sup>41</sup> Possible mechanisms of hypoglobulinemia include plasma volume expansion associated with chronic conditioning and training; however, this mechanism does not explain why only some protein fractions are affected or why they persist after Greyhounds retired from racing. As hyperviscosity has been associated with hyperglobulinemia in people and dogs with myeloma<sup>65,66</sup> and in people receiving immunoglobulin therapy,<sup>67</sup> we hypothesize that the opposite, ie, hypoviscosity, might occur with hypoproteinemia and hypoglobulinemia in Greyhounds, being an adaptive mechanism to decrease serum viscosity in this breed with higher PCVs and blood viscosity.<sup>32</sup>

Serum concentrations of acute phase proteins, specifically C-reactive protein (CRP), haptoglobin (Hp), acid-soluble glycoprotein (ASG), ceruloplasmin (CP), and serum amyloid A (SAA), were measured and compared between a group of healthy RRGs and age- and sex-matched healthy non-Greyhound controls.<sup>42</sup> The concentrations of Hp (measured by both colorimetric and immunoturbidimetric methods) and ASG were significantly lower in Greyhounds than in non-Greyhounds; CRP and CP concentrations were not significantly different; SAA concentration was below the detection limit in all dogs. Additional studies on Hp concentration in Greyhounds may provide insight into its low concentration and value as an acute phase marker in Greyhounds. ASG comprises a heterogeneous group of proteins that may vary among animal species, and it remains unknown which of the individual proteins are responsible for low ASG concentrations in Greyhounds. Both Hp and ASG migrate in the α-globulin fraction, and lower concentrations in Greyhounds study may explain their low α-globulin concentrations.<sup>40</sup>

#### *Thyroid hormones*

Greyhounds and other sighthounds have basal serum total T<sub>4</sub> (tT<sub>4</sub>) concentrations below non-breed-specific reference intervals.<sup>43–51</sup> Free T<sub>4</sub> (fT<sub>4</sub>) concentrations may also be low, although not to the same extent as tT<sub>4</sub>, with reported mean values ranging from 6.0 to 11.6 pmol/L.<sup>45,47</sup> Trained and racing Greyhounds had lower tT<sub>4</sub> concentrations than retired racers had, and tT<sub>4</sub> concentrations were higher 5 minutes after racing.<sup>47</sup> Highly variable total T<sub>3</sub> concentrations have been reported<sup>52</sup>; fT<sub>3</sub> concentrations in Greyhounds are usually below nonbreed-specific reference intervals.<sup>47,51,53</sup> Young pretraining Greyhounds (5–13 months) were found to have lower tT<sub>4</sub> and fT<sub>4</sub>

concentrations and a tendency toward higher total T<sub>3</sub> concentrations when compared with non-breed-specific reference intervals.<sup>52</sup> Concentrations of TSH in the young Greyhounds were within the non-breed-specific reference intervals, consistent with previous studies investigating TSH in adult Greyhounds<sup>45,47</sup>; however, tT<sub>4</sub> concentration did not increase after administration of exogenous TSH in adults.<sup>45,51</sup> In studies of scintigraphy of the thyroid gland used to assess thyroid function in Greyhounds suspected of having primary hypothyroidism based on standard thyroid hormone testing, thyroidal (99m) TcO<sub>4</sub>-uptake values (mean ± SD, 0.76 ± 0.26%) were within the reference limits published for euthyroid dogs (0.39–1.86%), making hypothyroidism highly unlikely in the Greyhounds evaluated in this study.<sup>54</sup>

In a retrospective study of serum thyroid hormone concentrations in 398 sighthounds, including Greyhounds (*n* = 347), Borzois (*n* = 22), Salukis (*n* = 11), Irish Wolfhounds (*n* = 14), and Scottish Deerhounds (*n* = 4), hypothyroidism had been diagnosed in 286 of the 398 (71.9%) sighthounds based on low serum concentrations of tT<sub>4</sub> or tT<sub>3</sub> alone; 17 (4.3%) dogs also had low fT<sub>4</sub> or fT<sub>3</sub> concentrations, and 30 (7.5%) had been diagnosed with hypothyroidism despite having thyroid hormone concentrations within reference intervals.<sup>55</sup> Only 65 (16.3%) sighthounds had additional abnormalities, such as high serum TSH concentration or positive thyroglobulin autoantibody, suggestive of hypothyroidism. A cross-sectional study was also performed to assess serum thyroid hormone concentrations in healthy Salukis. When compared with non-breed-specific reference intervals, 154/282 (54.6%) Salukis had tT<sub>4</sub> values and 67/216 (31%) Salukis had fT<sub>4</sub> values below reference limits.<sup>55</sup> Thus, sighthound breeds other than Greyhounds also have low serum tT<sub>4</sub> concentrations.

#### *Cardiac troponin*

Healthy Greyhounds have a higher heart weight-to-body weight ratio, higher left ventricular free wall thickness, functional murmurs with no detectable structural or physiologic abnormalities, and a higher vertebral heart scores (VHS) than non-Greyhounds have.<sup>25–27,56–60</sup> Cardiac troponin I (cTnI) is a polypeptide found specifically in cardiac muscle, and serum concentrations of cTnI have been used as a diagnostic and prognostic indicator of heart disease, including cardiac infarction in people and cardiomyopathy in dogs. Serum cTnI concentrations have been compared between RRGs, Boxers with and without arrhythmogenic right ventricular cardiomyopathy (ARVC), and non-Boxer control dogs.<sup>61</sup> Greyhounds had signifi-

cantly higher serum cTnI concentrations compared with non-Greyhound dogs, but significant differences in serum cTnI between Greyhounds and Boxers with and without ARVC were not found; interestingly, several Greyhounds had cTnI concentrations within or above the range of concentrations in the Boxers with ARVC. Undetected underlying myocardial disease in Greyhounds in the study was also a possible explanation, but was considered less likely as the Greyhounds were healthy, had no arrhythmias on auscultation, and remained asymptomatic for heart disease months after completing the study. High cTnI concentrations in Greyhounds may be, in part, the result of a higher heart weight-to-body weight ratio.<sup>57</sup> Greyhounds with a heart murmur, high VHS, and high serum cTnI concentrations could be incorrectly diagnosed with myocardial disease; thus, until a Greyhound-specific reference interval is established, caution should be used when interpreting serum cTnI concentrations in Greyhounds with suspected cardiac disease.<sup>61</sup>

#### **Urinary Fractional Excretion of Electrolytes**

The fractional excretion (FE) of an electrolyte is an expression of the proportion of the electrolyte excreted in the urine compared with the proportion filtered by the glomerulus. As serum sodium and chloride concentrations are higher in Greyhounds than in non-Greyhounds,<sup>2,6,9</sup> the use of Greyhound-specific reference intervals for urinary FE of electrolytes is recommended when investigating renal tubular disease in the breed. Reference intervals for urinary FE of electrolytes from 48 Greyhounds were established using a bootstrap estimate; non-Greyhound control dogs were not used for comparison.<sup>62</sup> Although the fractional excretions of potassium, chloride, calcium, and phosphate were significantly lower in Greyhounds compared with published nonbreed-specific intervals, they may not have been clinically relevant. Veterinary practitioners may use these reference intervals when investigating renal tubular disease in Greyhound dogs.<sup>62</sup>

#### **Preliminary Reference Intervals for Greyhounds at The Ohio State University**

Establishment of Greyhound-specific reference intervals has long been a priority at The Ohio State University Veterinary Medical Center. Studies of different aspects of the physiology (including hematology, coagulation, clinical chemistry, and blood gases) have been conducted to characterize differences and propose reference intervals that are valid and specific for the

breed. Although the sample sizes in these studies did not meet the recommended numbers of reference individuals for establishing reference intervals, these preliminary intervals should benefit clinicians by providing a better understanding of the hematologic and biochemical differences between Greyhounds and non-Greyhound dogs, and by helping to prevent misdiagnoses based on use of reference intervals that are inappropriate for sighthound breeds.

Dogs used for generation of reference intervals were from 2 different populations. The first group was from the Greyhound Spay/Neuter/Dental Clinic conducted for third or fourth year veterinary medical students at The Ohio State University. Greyhounds from an adoption organization (Greyhound Adoption of Ohio, Chagrin Falls, OH, USA; www.greyhoundadoptionofoh.org) are spayed/neutered before being placed in adoptive homes, and samples for reference interval studies were collected over 5 years. The second group was the Greyhound blood donor population, which is a homogeneous population of healthy RRGs. Animals from both groups were considered healthy based on a normal physical examination, negative serology for *Ehrlichia canis*, *Anaplasma phagocytophylum*, *Borrelia burgdorferi*, and *Dirofilaria immitis*

(Canine SNAP 4Dx Test, IDEXX Laboratories). Studies using both populations of Greyhounds were conducted in compliance with protocols for the ethical care and use of animals (IACUC protocol). Descriptive statistics and D'Agostino and Pearson normality test of the data were obtained using GRAPHPAD PRISM statistical software (GraphPad Software, San Diego, CA, USA). All reference intervals were established by using the 5th and 95th percentiles, although the sample size was not optimal in some cases, and not all data were normally distributed.

Jugular or cephalic venous samples were collected in tubes with sodium EDTA for CBCs, tubes with sodium citrate for hemostasis assays, and tubes without anticoagulant for biochemical profiles (Monocject, Sherwood, St. Louis, MO, USA); samples were processed and analyzed within 4 hours of collection. CBCs were performed using a LaserCyte ( $n = 151$ ) or a ProCyte Dx ( $n = 48$ ) analyzer (IDEXX Laboratories) with the appropriate software settings (Table 2). If flags were obtained, the sample was excluded and the dog was not evaluated owing to lack of additional blood samples. In a subset of dogs ( $n = 28$ ), CBCs were evaluated using a Cell-Dyn 3500 hematology analyzer. Differential WBC counts were performed manually by the

**Table 2.** Greyhound-specific and non-breed-specific hematology reference intervals at The Ohio State University and published reference intervals for Greyhounds.

Analyte	OSU Reference Intervals*			Published Reference Intervals	
	Greyhounds LaserCyte† ( $n = 151$ )	Greyhounds ProCyte† ( $n = 48$ )	Greyhounds Cell-Dyn 3500† ( $n = 28$ )	Greyhounds <sup>§</sup> ADVIA 120/ADVIA 2120† ( $n = 304$ )	Non-Breed-Specific <sup>¶</sup> ADVIA 120† ( $n = 46$ )
Total WBC ( $\times 10^9/L$ )	4.4–10.8 (5.5–16.9)	3.6–6.9 (5.1–16.7)	3.3–7.5 (4.1–15.2)	3.4–8.5	5.8–20.3
Lymphocytes ( $\times 10^9/L$ )	0.2–2.5 (0.5–4.9)	0.8–2.2 (1.1–5.1)	0.4–2.2 (1–4.6)	0.6–2.5	2.0–4.7
Neutrophils ( $\times 10^9/L$ )	2.6–7.4 (2.0–12.0)	2.1–5.2 (2.9–11.6)	2.1–6.1 (4.1–15.2)	2.2–6.5	4.3–9.1
Monocytes ( $\times 10^9/L$ )	0.3–1.1 (0.3–2.0)	0.1–0.3 (0.2–1.1)	0.0–0.5 (0–1.2)	0–0.8	0.2–2.0
Eosinophils ( $\times 10^9/L$ )	0–1.1 (0.1–1.5)	0–1.0 (0.2–1.2)	0.0–0.6 (0–1.3)	0–0.3	0.1–1.2
Basophils ( $\times 10^9/L$ )	(0.00–0.01)	0.0–0.1 (0.0–0.1)	0.0–0.0 (NA)	ND	ND
HCT (%)	42.7–61.5 (37.0–55.0)	51.5–71.0 (37.3–61.7)	46.9–62.5 (36–54)	50.0–68.0	42.0–62.0
HGB (g/dL)	15.1–20.4 (12.0–18.0)	17.4–24.1 (13.1–20.5)	16.3–22.0 (11.9–18.4)	16.9–22.8	13.7–20.3
RBC ( $\times 10^{12}/L$ )	6.0–9.4 (5.5–8.0)	7.4–10.2 (5.6–8.8)	6.7–9.3 (4.9–8.2)	6.7–9.2	5.7–9.1
Reticulocytes ( $\times 10^9/L$ )	17.2–45.7 (14.7–17.9)	10.0–97.7 (6.6–100.7)	ND	ND	ND
MCV (fL)	66–79 (60–77)	63–76 (62–74)	66–72 (64–75)	70–80	63–75
MCHC (g/dL)	29.4–38.2 (30.0–37.5)	33.1–35.1 (32.0–37.9)	34.1–36.0 (32.9–35.2)	ND	ND
MCH (pg)	20.9–28.6 (18.5–30.0)	21.5–26.2 (21.2–25.9)	ND	ND	ND
RDW (%)	14.7–15.9 (14.7–17.9)	16.0–22.2 (13.6–21.7)	14.2–17.2 (13.4–17.0)	ND	ND
Platelet count ( $\times 10^9/L$ )	117–295 (175–500)	112–205 (148–484)	135–235 (106–424)	145–309	173–497
MPV (fL)	6.9–11.8 (NA)	8.6–11.9 (8.7–13.2)	ND	ND	ND

\*Intervals in parentheses are reference intervals for dogs (all breeds) provided by the instrument manufacturer.

†LaserCyte and Procyte, IDEXX Laboratories, Westbrook, ME, USA; Cell-Dyn 3500, Abbott Diagnostics, Abbott Park, IL, USA; Advia 120 and 2120, Siemens Healthcare Diagnostics, Deerfield, IL, USA.

ND, not done; NA, not available.

**Table 3.** Greyhound-specific and non-breed-specific reference intervals for hemostasis profiles at The Ohio State University.

Assay	OSU Reference Intervals		
	Greyhounds ACL 200* (n = 88)	Greyhounds Stago Compact† (n = 62)	Non-Breed- Specific ACL 200*
OSPT (seconds)	6.2–7.6	6.9–8.3	6–7.5
aPTT (seconds)	11.2–18.1	9.7–12.1	9–21
Fibrinogen (mg/dL)	83–190	89–180	100–384

\*Samples collected in 3.8% sodium citrate; ACL 200 (Instrumentation Laboratory, Lexington, MA, USA).

†Samples collected in 3.2% sodium citrate; STA Compact CT (Diagnostica Stago, Parsippany, NJ, USA).

OSPT, one-stage prothrombin time; aPTT, activated partial thromboplastin time.

**Table 4.** Greyhound-specific and non-breed-specific reference intervals for serum biochemical profiles and protein fractions at The Ohio State University.

Serum Biochemistry*	OSU Reference Intervals	
	Greyhounds (n = 100)	Non-Breed- Specific
Urea nitrogen (mg/dL)	11–21	5–20
Creatinine (mg/dL)	1.0–1.7	0.6–1.6
Phosphorus (mg/dL)	2.3–5.3	3.2–8.1
Calcium, total (mg/dL)	9.4–11.4	9.3–11.6
Sodium (mmol/L)	144–156	143–153
Potassium (mmol/L)	3.5–4.4	4.2–5.4
Chloride (mmol/L)	108–119	109–120
Anion gap	9.0–19.9	15–25
Osmolality, calculated (mmol/L)†	285–310	285–304
Bicarbonate (mmol/L)	20–31	16–25
Alanine aminotransferase (U/L)	28–82	10–55
Aspartate aminotransferase (U/L)	24–57	12–40
Alkaline phosphatase (U/L)	19–90	15–120
C-ALP (U/L)‡	0–31	0–6
Creatine kinase (U/L)	76–254	50–400
Cholesterol (mg/dL)	91–210	80–315
Bilirubin (mg/dL)	0.1–0.3	0.1–0.4
Glucose (mg/dL)	77–121	77–126
Total protein (g/dL)	4.8–6.3	5.1–7.1
Albumin (g/dL)	2.9–3.9	2.9–4.2
Globulin (g/dL)	1.7–3.0	2.2–2.9
Albumin:globulin ratio	1.0–2.2	0.8–2.2
Agarose gel electrophoresis		
α-1 globulin (g/dL)	0.3–0.4	0.3–0.9
α-2 globulin (g/dL)	0.1–0.5	0.3–0.7
β-1 globulin (g/dL)	0.1–0.3	0.2–0.7
β-2 globulin (g/dL)	0.1–0.3	0.2–1.4
γ-globulin (g/dL)	0.8–1.7	1.0–1.9

\*All analytes were measured using the COBAS c501 chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA).

†Osmolality was calculated using the following formula:  $1.86 \times Na + (Glucose/18) + (UN/2.8) + 9$ .

‡Corticosteroid-induced alkaline phosphatase.

staff in the Clinical Pathology Laboratory at OSU on blood smears stained with Wright-Giemsa by counting 100 cells/smear.

For conventional hemostasis assays, OSPT, aPTT, and fibrinogen concentration, 2 analyzers were used: the ACL 200 coagulation analyzer (Instrumentation Laboratory, Lexington, MA, USA) and the Stago Compact analyzer (Diagnostica Stago, Parsippany, NJ, USA) (Table 3). Serum biochemistry profiles (n = 100) were performed on a COBAS c501 chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA) (Table 4).

Thrombelastography was performed as previously described.<sup>31</sup> Initially, 20 µL CaCl<sub>2</sub> were placed in the prewarmed cup of the TEG-5000 (Thrombelastograph, TEG Haemoscope, Niles, IL, USA); 340 µL citrated blood were then added for a total volume of 360 µL. No activator was used. Tracings were obtained after

**Table 5.** Reference intervals for thromboelastographic (TEG) measurements in Greyhounds using the native calcium chloride method on the TEG-5000 (Thrombelastograph, TEG Haemoscope, Niles, IL, USA).

Measurement	Reference Interval Greyhounds (n = 129)	Reference Interval Non-Breed-Specific <sup>31</sup> (n = 15)
R-time (minutes)	2.5–8.8	2.0–8.2
K-time (minutes)	1.9–6.4	0.8–3.4
Angle (degrees)	34.5–62.9	49.4–80.5
MA (mm)	38.6–61.2	43.8–74.2
G (dyn/cm <sup>2</sup> )	3148–7900	3843–7810
LY60 (%)	0–8.9	0–8.8

**Table 6.** Greyhound-specific and non-breed-specific reference intervals for venous cooximetry and blood gas analysis at The Ohio State University using the STP CCX Analyzer (Nova Biomedical, Waltham, MA).<sup>39</sup>

Analyte	Reference Interval Greyhounds (n = 57)	Reference Interval Non-Breed-Specific (n = 30)
pO <sub>2</sub> (mmHg)	36.3–84.3	34.6–69.6
pCO <sub>2</sub> (mmHg)	25.6–39.9	24.7–44.4
SO <sub>2</sub> (%)	78.6–99.8	54.4–99.8
tHb (g/dL)	18.1–25.0	15.0–21.3
O <sub>2</sub> Hb (%)	75.6–97.4	54.7–96.1
COHb (%)	0.9–3.9	0.4–4.5
MetHb (%)	0.0–2.2	0.1–2.8
HHb (%)	0.4–21.2	2.7–40.0
P <sub>50</sub> (mmHg)	26.0–28.4	21.4–38.4
O <sub>2</sub> Ct (mL/dL)	19.7–32.0	13.3–24.6
O <sub>2</sub> Cap (mL/dL)	23.8–34.1	20.2–28.5

pO<sub>2</sub>, partial pressure of O<sub>2</sub>; pCO<sub>2</sub>, partial pressure of O<sub>2</sub>; SO<sub>2</sub>, oxygen saturation; tHb, total hemoglobin; O<sub>2</sub>Hb, oxyhemoglobin; COHb, carboxyhemoglobin; MetHb, methemoglobin; HHb, deoxyhemoglobin; O<sub>2</sub>Ct, oxygen content; O<sub>2</sub>Cap, oxygen capacity.

120–180 minutes of running time at 37°C (Table 5). Reference intervals for blood gases are reported from our previous study (Table 6).<sup>10</sup>

## Conclusion

Greyhounds have hematologic and serum biochemical values that frequently differ from those of non-Greyhound dogs, suggesting that they have differences in many aspects of their physiology. Establishment of reference intervals specific to the breed or group, ie, sighthounds, is essential for correct diagnosis and management of medical conditions that are monitored by laboratory testing. As RRG adoptions increase in the US, practicing veterinarians and clinical pathologists will be faced with the challenge of interpreting laboratory values in light of characteristics that typify the breed.

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