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# Serum protein electrophoresis in retired racing Greyhounds

Manena Fayos, C. Guillermo Couto, María Cristina Iazbik, Maxey L. Wellman

**Background:** Retired racing Greyhounds are becoming common as pets. Because of their unique physiology, results of routine laboratory tests are frequently outside the reference interval for dogs. Compared with other breeds, Greyhounds have low serum protein concentrations, but the concentrations of different serum protein fractions have not been reported. **Objectives:** Our objectives were to evaluate the results of serum protein electrophoresis (SPE) in healthy, retired racing Greyhounds and compare them with a control group of age- and gender-matched non-Greyhound dogs. **Methods:** Agarose gel electrophoresis was done using a standard method; the gels were stained with amido black and scanned with a Cliniscan 2 densitometer (Helena Laboratories, Beaumont, TX, USA). Protein fractions were identified by visual inspection of the electrophoretogram. A Student's *t*-test assuming equal variances was used to compare the concentration of the different fractions between groups. **Results:** The concentrations of total protein, total globulins, and  $\alpha$ -1-,  $\alpha$ -2-,  $\beta$ -1-, and  $\beta$ -2-globulins were significantly lower and the albumin to globulin (A:G) ratio was significantly higher in Greyhounds than in non-Greyhound dogs ( $P < .05$ ). There was no significant difference in albumin or  $\gamma$ -globulin concentrations. **Conclusions:** Low serum protein concentrations in Greyhounds are the result of low concentrations of  $\alpha$ - and  $\beta$ -globulins. These results should be kept in mind when evaluating both healthy and sick Greyhounds. Additional studies are needed to identify the individual proteins associated with low  $\alpha$ - and  $\beta$ -globulin concentrations in Greyhounds. (*Vet Clin Pathol.* 2005;34:397–400)

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**Key Words:** Acute phase protein, alpha-globulin, beta-globulin, Greyhound, hypoproteinemia

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With the increasing popularity of retired rescued Greyhounds, veterinarians are likely to deal with dogs of this breed more frequently in their practices. In the United States, it is estimated that approximately 120,000 Greyhounds live in homes as pets, compared with 55,000 Greyhounds at race-tracks. In the past few years, private Greyhound adoptions have exceeded 18,000 per year (Gary Guccione, National Greyhound Association, personal communication). Therefore, it is important for veterinarians and other animal health care professionals to recognize the physiologic peculiarities of this breed. For instance, mean PCV, hemoglobin concentration, RBC count, whole blood viscosity, serum creatinine concentration, and serum transaminase activities are significantly higher, whereas WBC, neutrophil, and platelet counts are lower in Greyhounds than in other breeds.<sup>1-5</sup>

At The Ohio State University Veterinary Teaching Hospital (OSU-VTH), we evaluate a high number of Greyhounds that are part of the Blood Donor Program or the Greyhound Outreach Service. Based on our experience and compared with the canine reference intervals for our laboratory, it was apparent that plasma and serum protein concentrations were lower in Greyhounds than in non-Greyhound dogs. Porter and Sullivan<sup>1,3</sup> reported a low total protein concentration in this breed; more recently, Steiss<sup>4</sup> found that the calculated globulin concentration was signif-

icantly lower in Greyhounds than in control dogs. To our knowledge, there are no previous studies involving serum protein electrophoresis (SPE) in Greyhounds to help explain the differences in these values. Because hypoproteinemia is associated with a variety of diseases (eg, blood loss, protein-losing enteropathy, protein-losing nephropathy, and chronic liver disease), it is important to recognize breed-specific differences in which the protein concentration may fall below the reference interval for other dog breeds.

SPE is a useful clinical tool for the diagnosis, monitoring, and prognosis of diseases involving changes in the concentrations of albumin,  $\alpha$ -1 globulins,  $\alpha$ -2 globulins,  $\beta$ -1 globulins,  $\beta$ -2 globulins, and  $\gamma$ -globulins.<sup>6-10</sup> In this study we evaluated the SPE patterns in healthy, retired racing Greyhounds and in an age- and gender-matched control group of non-Greyhound dogs.

We evaluated 28 healthy, retired racing Greyhounds that were part of the Blood Donor Program at the OSU-VTH and 18 age- and gender-matched healthy non-Greyhound controls that were presented to the OSU-VTH for elective procedures or were owned by students or staff. There were 9 female spayed and 19 male castrated Greyhounds, ranging in age from 2 to 9 years (mean  $\pm$  SD, 5.7  $\pm$  1.7) and 9 female spayed and 9 castrated male mixed breed dogs, ranging in age from 1 to 9 years (3.78  $\pm$  2.2). The Blood Donor Protocol has

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From the College of Veterinary Medicine, Universidad Autónoma de Barcelona, Barcelona, Spain (Fayos); and the Departments of Veterinary Clinical Sciences and Transfusion Medicine Service, Veterinary Teaching Hospital (Couto, Iazbik); the Department of Veterinary Biosciences (Wellman), College of Veterinary Medicine; and the OSU Comprehensive Cancer Center (Couto), The Ohio State University, Columbus, OH. Corresponding author: C Guillermo Couto, DVM, Dipl ACVIM (couto.1@osu.edu). ©2005 American Society for Veterinary Clinical Pathology

## Serum Protein Electrophoresis in Greyhounds

**Table 1.** Mean, median, and 2.5–97.5 percentiles for total protein, albumin, and globulin concentrations (g/dL) and A:G ratios in non-Greyhound control dogs and retired racing Greyhounds.

	Non-Greyhound Dogs (n = 18)				Greyhounds (n = 28)				P Value
	Mean ± SD	Median	2.5 %	97.5%	Mean ± SD	Median	2.5%	97.5%	
Total protein	6.07 ± 0.45	5.90	5.60	7.12	5.56 ± 0.39	5.52	4.90	6.30	.0002
Albumin	3.23 ± 0.33	3.30	2.54	3.66	3.34 ± 0.25	3.30	2.97	3.80	.2538
α-1-globulins	0.46 ± 0.14	0.40	0.30	0.82	0.33 ± 0.05	0.30	0.30	0.40	<.0001
α-2-globulins	0.47 ± 0.14	0.50	0.30	0.70	0.27 ± 0.10	0.30	0.10	0.47	<.0001
β-1-globulins	0.32 ± 0.12	0.30	0.20	0.57	0.20 ± 0.06	0.20	0.10	0.30	<.0001
β-2-globulins	0.34 ± 0.09	0.30	0.20	0.50	0.21 ± 0.07	0.20	0.10	0.30	<.0001
γ-globulins	1.23 ± 0.23	1.10	1.00	1.73	1.23 ± 0.25	1.20	0.87	1.70	.9696
A:G ratio	1.17 ± 0.18	1.17	0.78	1.41	1.51 ± 0.18	1.52	1.25	1.87	<.0001

been approved by The Ohio State University ILACUC. For non-Greyhound dogs, serum samples obtained with signed owners' consent and used to generate reference intervals in our laboratory were used. Both Greyhounds and non-Greyhounds underwent a complete physical examination and had CBC and serum chemistry results within the reference intervals for our laboratory for Greyhounds and for other dog breeds, respectively. All dogs were current on vaccinations and were routinely dewormed and given heartworm preventative.

Fasting blood samples (3 mL) were collected by direct jugular venipuncture and placed in a tube without anticoagulant. The sample was allowed to clot, centrifuged at 1300g for 10 minutes, and the serum removed and frozen immediately at -30°C until assayed. All samples were batched and assayed within 30 days of collection.

Total serum protein concentration was determined by the biuret method using a Hitachi 911 automated chemistry analyzer (Roche-Boehringer Mannheim, Indianapolis, IN, USA). Albumin and α-, β-, and γ-globulins were measured using agarose gel protein electrophoresis (Helena Laboratories, Beaumont, TX, USA). Although albumin concentration can be measured on the Hitachi using a dye-binding method, SPE is preferred because there is less variation in albumin concentration.<sup>11</sup> Gels were stained with amido black and scanned with a Cliniscan 2 densitometer (Helena). Protein fractions were identified by visual inspection of the electrophoretogram and by relative mobility (Rf) values. Rf values were determined from the stained gels by measuring the distance from the point of application to the midpoint of the area of maximum staining intensity for each fraction and dividing this value by the distance from the point of application to the midpoint of the area of maximum staining intensity for the albumin fraction (100%).<sup>12</sup> The quantity of protein in each fraction was calculated by the densitometer using the area under the curve on the electrophoretogram multiplied by the total serum protein concentration as determined by the biuret method. The globulin concentration was calculated by subtracting the albumin from the total protein concentration.

A Student's *t*-test assuming equal variances (Graph Pad Software, San Diego, CA, USA) was used to compare

the concentration of total protein, albumin, total globulins, and α-1-, α-2-, β-1-, β-2-, and γ-globulin concentrations, as well as the albumin:globulin (A:G) ratio between groups. Values of *P* < .05 were considered significant.

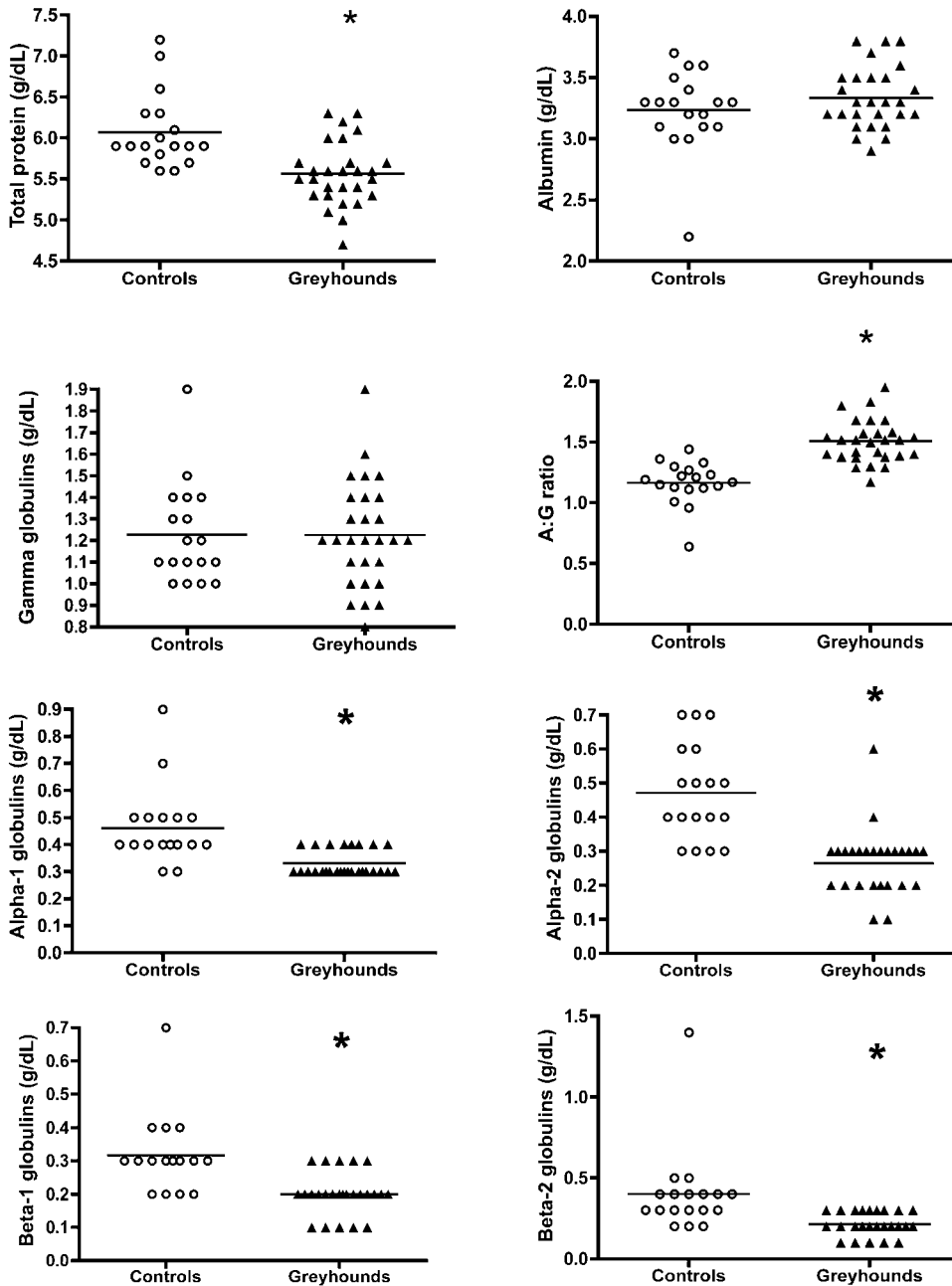
The concentrations of total protein, total globulins, and α-1-, α-2-, β-1-, and β-2-globulins were significantly lower in Greyhound samples than in non-Greyhound samples (Table 1, Figure 1). The A:G ratio was significantly higher in Greyhounds than in non-Greyhounds. There were no significant differences between groups for albumin or γ-globulin concentrations. The differences in globulin fraction concentrations were evident in electrophoretograms from Greyhound and non-Greyhound dogs (Figure 2).

### Discussion

The results of this study confirmed that Greyhounds have a lower total serum protein concentration than non-Greyhound dogs and were consistent with those of previous studies.<sup>1-4,13</sup> This difference was due to the low concentrations of α- and β-globulins in Greyhounds, whereas albumin and γ-globulin concentrations were similar to those in the control dogs and within the reference interval for other dog breeds. Consequently, the A:G ratio was higher in Greyhounds than in non-Greyhound dogs.

Although it is widely accepted that Greyhounds have acquired a unique evolutionary physiology as racing hounds, the underlying mechanism for the difference in serum protein concentration has not been determined. Ilkiw<sup>2</sup> suggested that the low protein concentration in Greyhounds could be the result of chronic, plasma volume expansion associated with chronic conditioning and training; however, this postulated mechanism does not explain why only some protein fractions are affected, whereas others are normal. Moreover, some of the physiologic traits in Greyhounds, such as relative cardiomegaly, do not appear to be related to conditioning because they are present in both racing and non-racing Greyhounds.<sup>14</sup>

Greyhounds have significantly higher PCVs and blood viscosity than other dog breeds<sup>15</sup>; it is possible that hypoproteinemia is an adaptive mechanism in an attempt to



**Figure 1.** Protein values for non-Greyhound control dogs (n=18) and Greyhounds (n=28). Asterisks denote statistically significant differences between groups.

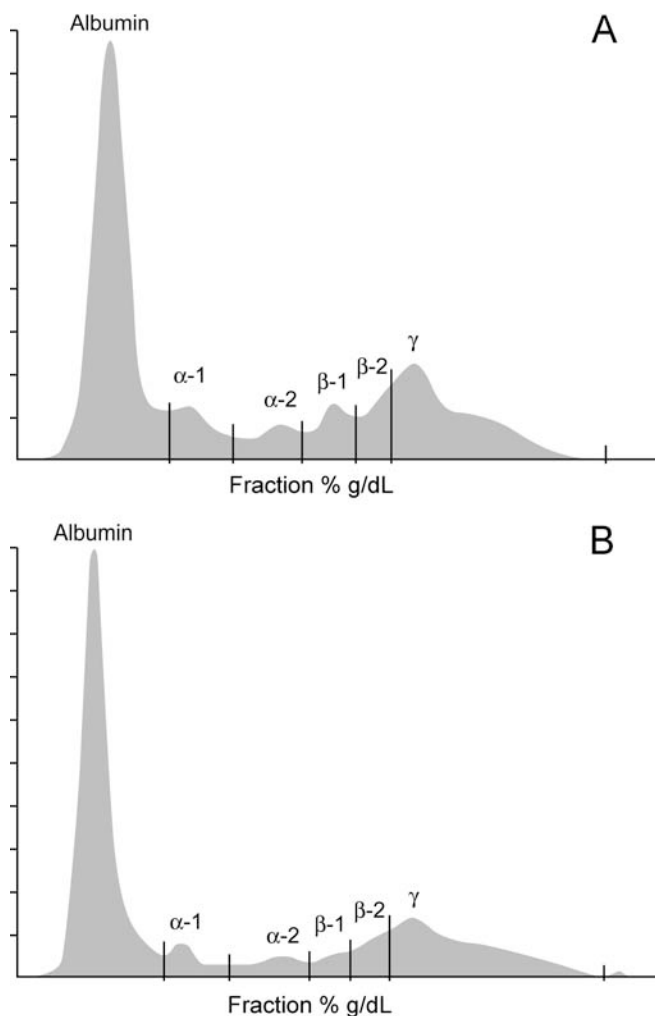
decrease serum viscosity. It is likely that in Greyhounds some proteins exert more of an effect on viscosity than others.

Climate and husbandry (eg, exercise, stress, nutrition) also have been proposed as factors that influence serum protein concentration.<sup>6,16</sup> However, the protein concentrations in dogs were similar in several studies conducted in different environments. Pregnancy, lactation, advanced age, and hormonal factors<sup>6-8</sup> also can influence serum protein concentrations; however, the Greyhounds and non-Greyhounds evaluated in this study were clinically and physiologically homogeneous.

The globulin fractions on electrophoretograms include a number of individual proteins. Alpha-globulins include thyroid-binding globulin,  $\alpha$ -fetoprotein,  $\alpha$ -1-antitrypsin, or-

somucoid, some lipoproteins,  $\alpha$ -2-macroglobulin, prealbumin, ceruloplasmin, haptoglobin, antithrombin, and erythropoietin.  $\beta$ -Globulins include transferrin, hemopexin, complement, and plasminogen. A small proportion of immunoglobulins, such as IgA and IgM, can also be found in the  $\beta$ -globulin fractions.<sup>6</sup> Preliminary data in our laboratory suggest that Greyhounds may have lower plasma plasminogen and antithrombin concentrations (Couto, Iazbik, Lara, Brooks; unpublished observations), which could contribute to the lower  $\alpha$ - and  $\beta$ -globulin concentrations seen in this study.

Additional studies are needed to identify the individual proteins associated with the low  $\alpha$ - and  $\beta$ -globulin concentrations in Greyhounds. High-resolution electrophoresis



**Figure 2.** Serum protein electrophoretograms from (A) a non-Greyhound control dog and (B) a Greyhound. Note the differences in height in the globulin regions between Greyhounds and non-Greyhounds.

(HRE), recently validated in dogs,<sup>13</sup> allows researchers to separate “bands” of single proteins, mainly by immune fixation and other methods. This technique, as well as quantification of acute phase proteins, should be helpful in determining the main proteins responsible for the lower  $\alpha$ - and  $\beta$ -globulin concentrations in Greyhounds.

We frequently receive requests for consults or referrals from general practitioners or Greyhound owners about “hypoproteinemia” in otherwise healthy dogs. Some of these dogs have been extensively evaluated for liver, renal, or intestinal disease. The results of this study suggest that breed-specific reference intervals should be determined for most

protein values in Greyhounds to avoid false positive diagnoses of hypoproteinemia. In particular, because serum creatinine concentrations are higher in Greyhounds,<sup>5</sup> hypoproteinemia in a seemingly azotemic dog may result in an unnecessary work-up for protein-losing nephropathy.

**Acknowledgements**

Supported in part by grant P30 CA16058, National Cancer Institute, Bethesda, MD and by the Barry French Poodle and Savannah Memorial Fund.

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