

BRIEF COMMUNICATION

Serum concentrations of IgG, IgA, and IgM in retired racing Greyhound dogs

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Background: Greyhound dogs have significant physiologic, hematologic, and biochemical differences when compared with other breeds, including significantly lower serum globulin concentration owing to decreases in the α - and β -globulin fractions. The specific proteins that account for differences in globulin concentrations are not known, but IgA and IgM, both β -globulins, are potential candidates.

Objectives: The aims of this study were to measure serum IgG, IgA, and IgM in clinically healthy retired racing Greyhounds and compare the results with those of age- and sex-matched non-Greyhound dogs.

Methods: Study animals included 25 Greyhound and 20 non-Greyhound dogs. Total protein, albumin, and total globulin concentrations were determined. IgG, IgA, and IgM concentrations were measured using a commercially available radial immunodiffusion kit. The Student *t*-test assuming equal variances was used to compare concentrations of immunoglobulins between groups.

Results: Serum concentrations of IgA and IgM in Greyhounds (IgA = 49 ± 20 mg/dL; IgM = 132 ± 47 mg/dL) were significantly lower than concentrations in non-Greyhound dogs (IgA = 70 ± 39 mg/dL; Ig M = 212 ± 78 mg/dL). Concentrations of IgG did not differ between groups.

Conclusions: Mean serum IgA and IgM concentrations in Greyhounds were lower than those in non-Greyhound dogs. This may contribute to low serum concentrations of β -globulins in Greyhounds. Specific reference intervals are recommended for Greyhounds to avoid possible misdiagnosis of IgA or IgM deficiency.

Physiological differences intrinsic to Greyhound dogs affect clinicopathologic measurements.¹ For example, PCV, hemoglobin concentration, RBC concentration, whole blood viscosity, serum creatinine concentration, and hepatic transaminase activities in Greyhounds are higher than in non-Greyhound dogs. Total WBC, neutrophil, and platelet counts and concentrations of thyroxine, free thyroxine, and total protein in Greyhounds are lower than in other breeds.¹⁻⁷ Serum albumin and γ -globulin concentrations determined by electrophoresis are not different, however, and relative hypoproteinemia in Greyhounds results from significantly lower α - and β -globulin serum concentrations.⁷ Determination of serum immunoglobulin (Ig) concentrations is used to assess humoral immune competence in dogs,⁸

and decreased concentrations of either total Ig or specific Ig classes have been associated with secondary infections.⁹ As Greyhounds have significantly lower α - and β -globulin serum concentrations and IgA and IgM migrate in the β region on the electrophoretogram,^{10,11} there may be differences in serum Ig concentrations in Greyhounds compared with those of other breeds. In this study we quantified serum total protein, albumin, globulin, IgG, IgM, and IgA concentrations in healthy retired racing Greyhounds and compared the concentrations with those of non-Greyhound dogs.

Greyhounds used in the study were clinically healthy retired racing dogs enrolled in the Blood Donor Program at The Ohio State University Veterinary Teaching Hospital. The group consisted of 25 Greyhounds, 9

spayed females and 16 neutered males, ranging in ages from 3 to 8 years (mean \pm SD, 5.7 ± 1.4 years). The control group consisted of 20 clinically healthy non-Greyhound dogs that were either enrolled in the Blood Donor Program ($n=18$) or were owned by students or staff ($n=2$). There were 8 spayed females and 12 neutered males, ranging in ages from 1 to 5 years (mean \pm SD, 3.8 ± 1.3 years). Breeds included in this group were Golden Retrievers ($n=5$), Labrador Retriever ($n=4$), German Shepherds ($n=3$), mixed-breed dogs ($n=2$), and 1 each of Coonhound, Poodle, German Shorthair Pointer, Beagle, Siberian Husky, and Rottweiler dogs. This study was approved by The Ohio State University Institutional Animal Care and Use Committee.

Blood samples were collected by direct jugular venipuncture into tubes without anticoagulant (Beckton Dickinson and Company, East Rutherford, NJ, USA). Samples were allowed to clot and were then centrifuged (Centrifuge Model 228, Fisher Scientific, Pittsburgh, PA, USA) at 1380 *g* for 10 minutes. Serum was removed and frozen immediately at -30°C until assayed. All samples were batched and assayed within 90 days of collection.

Total serum protein concentration was determined by the biuret method using a Hitachi 911 automated chemistry analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA). Albumin concentration was measured on the same analyzer using the bromocresol green dye-binding method. Globulin concentration was calculated by subtracting albumin concentration from total protein concentration. Serum concentrations of IgG, IgA, and IgM were measured using radial immunodiffusion (Bethyl VET-RID Kit, Bethyl Laboratories Inc, Montgomery, TX, USA). Igs were assayed in triplicate for each dog, and mean values were used for comparisons. The VET-RID assay is based on an antigen-antibody reaction occurring in an agarose gel support medium that is visible as an opaque precipitating ring. The reference standards and the unknown concentration samples provided with the kit were used

with each plate. After 24 hours of incubation at room temperature (20°C), 2 observers measured the diameter in millimeter of the precipitating ring for the reference standards and the unknown samples by visual inspection using a magnifying eyepiece. A reference curve was constructed for each standard. Ig concentrations (logarithmic scale) were plotted against the diameters of precipitating rings (linear scale), generating a reference curve. Protein concentrations of unknown samples were determined by locating the precipitating ring diameters of each sample on the reference curve and reading the concentration on the logarithmic scale.

Graph Pad Prism, version 40 b (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. To determine if Greyhounds and non-Greyhound dogs were comparable and had equal variances, population attributes of both groups were compared using a χ^2 -test for age and the Levene test for sex. The Student *t*-test was used to compare concentrations of total protein, albumin, total globulin, IgG, IgA, and IgM and albumin:globulin (A:G) ratios between Greyhounds and non-Greyhound dogs and between neutered males and spayed females. A Spearman test was performed to determine the correlation between age and serum concentrations of IgA, IgM, and IgG in Greyhounds and non-Greyhound dogs. Differences and correlations were considered statistically significant at $P < .05$.

Aside from breed, the 2 groups of dogs were determined to be matched with no significant differences found in age or sex between groups. Concentrations of IgA, IgM, and IgG were consistently within detection limits of the RID kit used. Mean concentrations of total protein, total globulin, IgA, and IgM in Greyhounds were significantly lower than in non-Greyhound dogs (Table 1, Figure 1). The mean A:G ratio in Greyhounds was significantly higher than in non-Greyhound dogs. There were no significant differences between groups for albumin or IgG concentrations. IgA concentrations

Table 1. Concentrations of total protein, albumin, globulin, IgG, IgA, and IgM and albumin:globulin ratios in retired racing Greyhound and non-Greyhound dogs.

	Greyhound Dogs ($n=25$)				Non-Greyhound Dogs ($n=20$)				<i>P</i> Value
	Mean \pm SD	Median	25th Percentile	75th Percentile	Mean \pm SD	Median	25th Percentile	75th Percentile	
Total protein (g/dL)	5.4 ± 0.8	5.6	5.0	6.0	6.3 ± 1.0	6.5	5.8	7.0	< .01
Albumin (g/dL)	3.7 ± 0.3	3.7	3.6	4.0	3.6 ± 0.4	3.7	3.5	3.8	.19
Globulin (g/dL)	2.2 ± 0.3	2.1	1.9	2.4	2.5 ± 0.4	2.5	2.3	2.8	< .01
IgG (mg/dL)	1789 ± 886	1900	1150	1900	2030 ± 989	1900	1150	2175	.39
IgA (mg/dL)	49 ± 20	45	33	62	70 ± 39	62	39	86	< .03
IgM (mg/dL)	132 ± 47	130	130	130	212 ± 78	200	130	300	< .01
A:G ratio	1.8 ± 0.27	1.7	1.5	2.0	1.5 ± 0.2	1.5	1.4	1.6	< .01

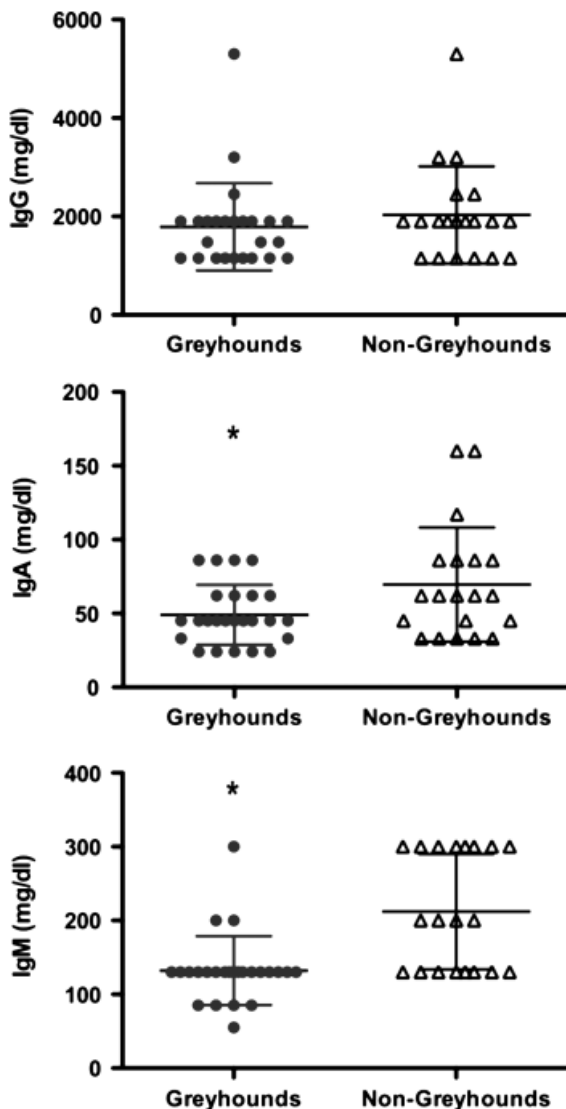


Figure 1. IgG, IgA, and IgM concentrations in Greyhounds and non-Greyhound dogs. Horizontal lines and vertical bar indicate mean and SD, respectively. Asterisks denote significant differences ($P < .05$) between groups.

in 5 (20%) Greyhounds were below the lower limit of a published reference interval of 20–150 mg/dL (method not specified)¹¹; IgA concentrations in the remaining Greyhounds and in all non-Greyhound dogs were > 20 mg/dL. IgM concentration in 1 Greyhound was below the lower limit of a published reference interval of 70–270 mg/dL (method not specified)¹¹; IgM concentrations in the remaining Greyhounds and in all non-Greyhound dogs were within the reference interval.¹¹ Significant correlations between age and serum levels of IgA ($P = .14$ for Greyhounds and $.62$ for non-Greyhounds), IgM ($P = .92$ for Greyhounds and $.60$ for non-Greyhounds), and IgG ($P = .57$ for Greyhounds and $.72$ for non-Greyhounds) were not found.

Discussion

The synthesis of serum proteins is under genetic control; therefore, it is to be expected that variations in their concentrations will occur among individuals, breeds, and species.¹⁰ Greyhound dogs have distinctive physiological features, including hematologic and biochemical characteristics, that can largely be attributed to adaptations related to their function as sight hounds and racing dogs.

Several studies have reported that Greyhounds have total serum protein concentrations that are lower than those of mixed-breed dogs.^{1–3,7} This difference has been determined to result from low concentrations of α - and β -globulins.⁷ Five major classes of immunoglobulins, IgG, IgA, IgM, IgE, and IgD, have been identified and characterized in people and various animal species, including the dog.¹¹ When serum protein electrophoresis is performed, IgA and IgM migrate in the β region.^{10,11} In this study, we determined that IgA and IgM concentrations were significantly lower in Greyhounds. In a previous study, we reported a difference of approximately 250 mg/dL in serum β -globulin concentrations between Greyhounds (mean, 410 mg/dL) and non-Greyhound dogs (mean, 660 mg/dL).⁷ In the current study, the sum of the IgA and IgM concentrations was 100 mg/dL less in Greyhounds compared with non-Greyhounds, which partially explains the lower concentration of β -globulins in Greyhounds. This study did not address the lower serum α -globulin concentrations found in Greyhounds and whether lower acute phase protein or hemostatic protein concentrations may contribute to this difference.

Physiological factors, such as hormonal and nutritional influences and young or advanced age, can be associated with increases or decreases in serum Ig concentrations.¹⁰ Typically, total serum protein and globulin concentrations increase and albumin concentration decreases with advancing age.¹⁰ However, the two groups in our study were age- and sex-matched. In addition, significant correlations between age and concentrations of IgA and IgM were not found in either group. Therefore, age is unlikely to explain the significant differences between groups.

Measurement of serum Ig concentrations is frequently used to assess immune competence in dogs.⁸ Deficiency of either total Igs or specific classes, especially IgA, may be associated with secondary infections.⁹ Consequently, it is important to know that the concentrations of IgA and IgM for clinically healthy Greyhounds are potentially below the lower limits of published canine reference intervals for these proteins, and separate intervals for Greyhounds should

be established to avoid erroneous conclusions when interpreting serum Ig panels.

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