Background: Retired racing Greyhounds are popular as pets. Greyhounds have several differences in physiological values compared with other breeds, including lower serum α- and β-globulin concentrations. We hypothesized that lower acute phase protein (APP) concentrations could contribute to lower α- and β-globulin concentrations in this breed.

Objectives: The purpose of this study was to compare serum concentrations of several APPs in Greyhounds with those of other dog breeds.

Methods: We measured the serum concentrations of C-reactive protein (CRP), haptoglobin (Hp), acid-soluble glycoprotein (ASG), ceruloplasmin (CP), and serum amyloid A (SAA) in 15 clinically healthy retired racing Greyhounds and 11 age- and gender-matched healthy nonGreyhound controls using previously validated methods. Results were compared by Student’s t-tests.

Results: The concentration of Hp by both colorimetric and immunoturbidimetric methods was significantly lower in Greyhounds than in nonGreyhound dogs (P=.0009 and .019, respectively). The concentration of ASG was also significantly (P=.007) lower in Greyhounds, but CRP and CP concentrations were not significantly different between groups. SAA concentration was below the detection limit of the method in all dogs.

Conclusions: The low serum concentrations of Hp and ASG should be taken into consideration when interpreting APP results in Greyhounds. Because both Hp and some ASG migrate in the α-globulin fraction, these results may explain the low α-globulin concentrations in Greyhounds.

Retired racing Greyhounds have become popular as pets. There are now more than twice as many Greyhounds that live in homes as pets as there are in the racetrack. Greyhounds have several differences in physiological values compared with other breeds, including higher PCV, serum creatinine concentration, and liver transaminase activities; and lower WBC, neutrophil, and platelet counts.1-4 We recently reported that the hypoproteinemia seen in Greyhounds is due to low serum α- and β-globulin concentrations5; albumin and γ-globulin concentrations were not different from the control group. Alpha- and β-globulins include most of the positive acute phase proteins (APPs) in dogs, including C-reactive protein (CRP), haptoglobin (Hp), acid-soluble glycoprotein (ASG), ceruloplasmin (CP), and serum amyloid A (SAA).6 We hypothesized that lower APP concentrations in Greyhounds could contribute to lower α- and β-globulin concentrations in this species. The purpose of this study was to measure the serum concentrations of CRP, Hp, ASG, CP, and SAA in Greyhounds, in comparison with nonGreyhound breeds.

We measured APP concentrations in 15 healthy retired racing Greyhounds that were part of the Blood Donor Program at The Ohio State University Veterinary Teaching Hospital (OSU-VTH), and in 11 age- and gender-matched healthy nonGreyhound controls that were presented to The OSU-VTH for elective

procedures or were owned by students or staff. The blood donor protocol was approved by The OSU Institutional Laboratory Animal Care and Use Committee; nonGreyhound samples were collected with signed owner consent as part of a project to determine reference intervals for our laboratory. All dogs underwent a complete physical examination, and CBC and serum chemistry results were within the reference interval for Greyhounds and nonGreyhound dogs, respectively. All dogs were current on vaccination and heartworm preventative and were routinely dewormed, but had not received medication within 2 weeks of sample collection. All dogs were rested for at least 6 hours before sample collection.

Fasting blood samples were collected by direct jugular venipuncture and placed in a tube without anticoagulant, as previously described.\(^5\) Samples were allowed to clot at room temperature for at least 20 minutes and centrifuged at 1300 \(g\) for 10 minutes. Serum was frozen immediately at \(-30^\circ C\) until assayed within 90 days.

Hp was determined using 2 biochemical methods: a hemoglobin-binding method (Tridelta Phase, Tridelta Development Ltd., Bray, Ireland) and an immunoturbidimetric method using polyclonal goat antihuman antisera (ITC Diagnostics, Barcelona, Spain). Cross-reactivity between the polyclonal goat antihuman Hp antisera and canine Hp was previously demonstrated by radial immunodiffusion and ELISA tests.\(^7\) Both methods were performed using a biochemistry autoanalyzer (Cobas Mira Plus, ABX Diagnostics, Montpellier, France). Results were reported in gram per liter. Hp was also evaluated by electrophoresis of pooled serum samples from Greyhounds and nonGreyhound dogs. A portion of serum was fractionated with 50–70% saturated ammonium sulfate before electrophoresis.\(^8\) All sera were assessed by 1-dimensional sodium-dodecylsulfate polyacrylamide gel electrophoresis (Mini-PROTEAN 3, Bio-Rad Laboratories, Hercules, CA, USA) with 12% polyacrylamide under reducing conditions (100°C for 5 minutes in the presence of \(\beta\)-mercaptoethanol) with a Benchmark Protein Ladder (Invitrogen, Carlsbad, CA, USA) for calibration of molecular mass. The gel was stained with Coomassie Brilliant Blue R-250 (AnaSpec, San Jose, CA, USA). The concentration of total protein was estimated by a biuret assay (Bio-Rad Laboratories), using bovine serum albumin as a standard. The same concentration of protein per sample was loaded into each well.

CRP was measured using a time-resolved immunofluorometric assay previously validated for canine samples using goat anticanine polyclonal antibodies.\(^9\) The fluorescence, proportional to the concentration of CRP in the sample, was measured in a VICTOR 1420 multilabel counter (Perkin-Elmer Lifesciences, Wallac Oy; Turku, Finland). This method has a lower limit of detection and lower between-run imprecision compared with other commercial kits for CRP analysis. Results were reported in \(\mu g/mL\).

ASG was determined using the method described by Nahagata et al\(^{10}\) and modified by Eckersall et al.\(^{11}\) Serum was precipitated with 0.6 M perchloric acid. Samples were then centrifuged at 1750 \(g\) for 10 minutes. The protein content in the supernatant was determined in a Cobas Mira Plus analyzer (ABX Diagnostics) after mixing 20 \(\mu L\) of supernatant with 196 \(\mu L\) of bicinchoninic acid (Sigma Chemical Company, St. Louis, MO, USA) at 37°C, incubating for 25 seconds, and adding 4 mL of copper sulfate (Sigma) to initiate the reaction. Absorbance was recorded at a wavelength of 550 nm after 325 seconds. Results were reported in gram per liter. CP concentration was measured using a spectrophotometric method based on the in vitro oxidase activity of CP with \(p\)-phenylenediamine on a Cobas Mira Plus (ABX Diagnostics).\(^{12}\) Results were reported as the change in absorbance per minute at 550 nm. SAA concentration was measured using the Tridelta Phase Range assay. Final absorbance of the samples was measured by use of a microtiter plate reader (Powerwave XS, Biotek Instruments, Carson City, NV, USA) at 450 nm using 630 nm as the reference. Because this assay has high between-run imprecision (16%),\(^{13}\) all samples were assayed for SAA on the same day.

All methods were previously validated in the laboratory of one of the authors (J.J.C.) for canine serum.\(^{7,9,12,13}\) Student’s \(t\)-tests assuming equal variances (GraphPad Software, San Diego, CA, USA) were used to compare the concentration or activity of the APPs between Greyhound and nonGreyhound dogs. Significance was set at \(P < .05\).

Twelve male castrated and 3 female spayed Greyhounds ranging in age from 2 to 11 years (mean 7.2 years) were included in the study. Control dogs included 4 castrated males and 7 female spayed dogs, ranging in age from 2 to 7 years (mean 4.8 years). The breed distribution was as follows: mixed breed (\(n = 3\)), Golden Retriever (2), Labrador Retriever (1), Pug (1), Wirehaired Foxterrier (1), Boxer (1), Rottweiler (1), and Great Pyrenees (1).

The Hp concentration obtained by both the colorimetric and immunoturbidimetric methods was significantly lower in Greyhounds than in nonGreyhound dogs (Figure 1). Bands corresponding to \(\alpha\) and \(\beta\) chains...
of Hp (16 and 37 Kd, respectively) were observed in pooled serum from nonGreyhound dogs, whereas these bands were not seen in pooled serum from Greyhounds (Figure 2). The concentration of ASG was also significantly lower in Greyhounds than in nonGreyhounds (Figure 3). The concentrations of CRP and CP were not significantly different between groups (Figures 4 and 5). Only 2 samples contained detectable concentrations of SAA.

APPs are considered to be sensitive markers of inflammation in dogs; Hp and α-1-acid glycoprotein (AGP; a major component of ASG) are moderate APPs whereas CRP and SAA are major APPs, based on the magnitude of increase to an inflammatory stimulus. We are aware of only 1 study that has addressed possible breed effects on the serum concentrations of APPs. Thougaard et al. found significantly lower concentrations of AGP in healthy Yorkshire Terriers and Dachshunds when compared with Poodles, Cocker Spaniels, and German Shepherds. In our study, Greyhounds had lower serum concentrations of Hp and ASG compared with dogs of other breeds. Because both Hp and some ASG migrate in the α-globulin fraction in serum electrophoretograms, our results likely explain the low α-globulin concentrations previously found in Greyhounds.

The most significant difference between Greyhounds and nonGreyhounds was in Hp concentration, which was negligible in all Greyhounds. This finding was based initially on the biochemical results using 2 different methods and was confirmed by protein electrophoresis, in which negligible bands were found in the Hp area. Human patients with Hp deficiency (anhaptoglobinemia) have been described in association with various diseases, including inflammatory bowel disease and sickle cell anemia. In dogs, Hp deficiency may be associated with certain breeds, such as Greyhounds, and may contribute to the development of certain diseases. Therefore, a better understanding of the role of Hp in inflammatory processes in dogs is crucial for the interpretation of blood test results.

Figure 1. Serum haptoglobin concentrations as measured by (A) colorimetric and (B) immunoturbidimetric methods in Greyhounds (n = 15) and nonGreyhound dogs (n = 11). Horizontal lines and vertical bars indicate mean ± SD. A significant difference was found between groups for colorimetric (P = .0009) and immunoturbidimetric (P = .019) methods.

Figure 2. Representative sodium-dodecylsulfate polyacrylamide gel electrophoresis of pooled serum from Greyhounds and nonGreyhound dogs. Lane 1: molecular weight standards. Lanes 2 and 4: Greyhound serum. Lanes 3 and 5: nonGreyhound serum. Serum samples in lanes 2 and 3 were fractionated with 50–70% saturated ammonium sulfate before electrophoresis. Arrows mark the 2 haptoglobin bands corresponding to α and β chains of haptoglobin (Hp). Serum from Greyhounds lacks visible Hp bands whereas nonGreyhounds have clearly visible bands in the Hp region.

Figure 3. Serum acid-soluble glycoprotein concentrations in Greyhounds and nonGreyhound dogs. Horizontal lines and vertical bars indicate mean ± SD. A significant difference was found between groups (P = .007).
with a “silent allele” (Hp0-0) with no gene product.\(^\text{15}\) In addition, low Hp concentrations have been reported in people with hemolytic anemias, where hemoglobin released from erythrocytes binds to and saturates Hp and is removed from the circulation.\(^\text{16}\) Greyhounds have shorter RBC lifespans and macrocytic RBCs compared with other breeds of dogs\(^\text{3,17}\); however, reticulocyte counts, RBC morphology, and bone marrow show no obvious abnormalities suggestive of hemolysis. Further studies to investigate the possible causes for low Hp concentration in Greyhounds are warranted. The findings may have important practical implications in terms of the value of Hp as an acute phase marker in Greyhounds.

ASG constitutes a heterogeneous group of proteins soluble in acid conditions.\(^\text{18}\) In humans, ASG is composed mainly (90%) of AGP, with the remainder consisting of diverse proteins such as \(\alpha\)-1-antitrypsin, \(\beta\)-1-chymotrypsin, \(\beta\)-2-glycoprotein, hemopexin, albumin, and prealbumin. However, a different protein distribution for ASG has been postulated in some animal species,\(^\text{7,11}\) and it is unknown which of these individual proteins may be responsible for the low ASG values in Greyhounds.

Acknowledgment

This study was supported in part by the Savannah and Barry French Poodle Memorial Fund.

References


