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BRIEF COMMUNICATION

Plasma N-terminal pro-B-type natriuretic peptide concentration in healthy retired racing Greyhounds

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Key Words
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Background: N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a cardiac biomarker whose plasma concentration is high in some dogs with cardiopulmonary disease. NT-proBNP is a diagnostic tool that can be used to help determine if a patient has congestive heart failure. Greyhounds have functional heart murmurs, relative cardiomegaly, and high serum cTnI concentration.
Objectives: The purpose of the study was to evaluate the plasma concentration of NT-proBNP in healthy Greyhounds and compare it to non-Greyhound dogs.
Methods: We prospectively evaluated healthy client-owned dogs including retired racing Greyhounds and non-Greyhounds. Plasma was obtained and transferred into tubes containing a protease inhibitor and submitted for a specific NT-proBNP ELISA assay.
Results: The plasma NT-proBNP concentration in Greyhounds was significantly higher than in non-Greyhound control dogs (946 vs 632 pmol/L; $P < .005$); 46% of Greyhounds had NT-proBNP > 1000 pmol/L.
Conclusions: Plasma NT-proBNP concentration in Greyhounds is high and should be interpreted with caution.

Retired racing Greyhounds have become more popular as household rescue pets in the recent years, which has sparked an interest in their differing physiologic and clinicopathologic variables compared to dogs of other breeds. Previous research has shown that the reference intervals (RI) for RBC count, PCV, HGB concentration, serum sodium, chloride, bicarbonate, urea, and creatinine concentrations, ALT and AST activities, and cardiac troponin I (cTnI) concentrations are higher in Greyhounds than non-Greyhound breeds.[1] In contrast, lower RI have been determined for WBC and platelet counts, and serum concentrations of total protein, α- and β-globulin, serum potassium, phosphate, ionized calcium, magnesium, and thyroid hormone in Greyhounds.[1] These findings have led to an interest in defining RI for other variables in Greyhounds and other sighthounds.[2]

B-type natriuretic peptide (BNP) is produced by cardiac muscle tissue and released in response to various stimuli, including volume overload, cardiac myocyte hypertrophy, and hypoxia.[3–6] In people, BNP can serve as a marker for underlying cardiac dysfunction and is used to diagnose congestive heart failure (CHF), to differentiate etiologies in patients with lower respiratory signs, and to provide information regarding morbidity and mortality.[5,6] Recent studies addressed potential applications of N-terminal pro-B-type natriuretic peptide (NT-proBNP) testing in dogs and cats, and gave recommendations for different clinical scenarios.[4,7–9] High concentrations of plasma NT-proBNP have been observed in dogs with CHF as a cause of respiratory signs, and in dogs with occult cardiomyopathy.[4,7,9–12] However, although breed-related cutoff values are currently being investigated[13,14], an NT-proBNP concentration of > 400 pmol/L was proposed for detection of left ventricular systolic dysfunction in Doberman Pinschers[8]; furthermore, NT-proBNP concentration depends on the type of underlying pathology.[7,10,12]

Cardiovascularly, it has been well documented that Greyhounds have a higher heart-to-body weight ratio, vertebral heart scores, serum cTnI concentrations, and greater left ventricular free wall thickness than non-Greyhound dogs.[15,16] Additionally, many Greyhounds have relative aortic stenosis murmurs with no detectable structural or physiologic abnormalities.[17] We also incidentally observed high serum NT-proBNP concentrations in some healthy retired racing Greyhounds evaluated at the Ohio State University Veterinary Medical Center (OSU-VMC). Therefore, we hypothesized that healthy Greyhounds have higher plasma NT-proBNP concentrations than non-Greyhound dogs.

All dogs used in this study were blood donors at the OSU-VMC. An animal use protocol was approved by the Institutional Animal Care and Use Committee. Twelve non-Greyhound dogs were owned by OSU-VMC students or staff who gave signed owner consent for the study. All Greyhounds were retired healthy racers fostered in homes for at least one year. All dogs had normal results of a physical examination, and a CBC and serum chemistry profile within the RI, where Greyhound-specific RIs were used to assess clinicopathologic results in Greyhounds. Most Greyhounds had a 1–2/6 systolic left basilar systolic murmur, as previously reported[2]; however, that information was not recorded in the database. All the dogs in the study had a body condition score of 2.5–3.5/5.

All dogs were equally up to date on vaccinations and were receiving heartworm and flea & tick prophylactic treatment. All dogs were serologically negative for *Ehrlichia canis*, *Anaplasma phagocytophylum,* and *Borrelia burgdorferi,* and were negative for *Dirofilaria immitis* (SNAP-4DX; IDEXX Laboratories, Westbrook, ME, USA). Subjects were also tested serologically or molecularly negative for *Babesia canis, Babesia gibsoni, and Bartonella henselae* for allowing entry into the blood donor program at OSU-VMC; they were clinically free of external and intestinal parasites.

During the initial physical examination, blood samples were obtained from the jugular vein using a 21 Ga butterfly catheter and tube containing EDTA (BD Vacutainer, Franklin Lakes, NJ, USA), or 20 Ga needle and 12 mL syringe. All samples were collected between 8 am and 12 pm over a 4-month period of time. All samples were centrifuged within one hour of collection and plasma was aliquoted into 0.5 mL aliquots, placed into blood tubes containing a protease inhibitor provided by IDEXX, inverted gently several times, and then stored frozen at −80°C for 3–4 weeks. Samples were shipped over a 4-month period to IDEXX on dry ice to grant for continuous freezing and sample stability. Analysis of plasma NT-proBNP concentrations in all samples was performed with an ELISA (Cardiopet proBNP testp; IDEXX Laboratories) validated for use in dogs, as previously described.[11]

Normality was evaluated using the D'Agostino method. The ages between groups were compared using a Student's *t*-test. The NT-proBNP concentrations between Greyhound and non-Greyhound dogs were compared using a Mann–Whitney test. Significance was set as $P < .05$. Fisher's exact test was used to compare sex distribution between Greyhounds and non-Greyhound dogs. A chi-square test was used to calculate the proportion of Greyhounds and non-Greyhound dogs with NT-proBNP concentrations > 1000 pmol/L.

In total, 24 retired racing Greyhounds and 29 dogs of other breeds were included in the study. The non-Greyhound group included 3 Boxers, 3 Great Danes, 1 German Shepherd Dog, 1 Doberman, 1 American Staffordshire Terrier, 1 Rottweiler, 2 Labrador Retrievers, 1 Golden Retriever, 2 Siberian Huskies, 1 Standard Poodle, 1 Coonhound, 2 Galgos, and 10 mixed breed dogs.

Sexes were equally distributed ($P = 1$) and there were no age differences between groups ($P = .3$). The mean age in the Greyhounds was 5.1 years (range 3–8 years), and in the non-Greyhounds, it was 4.2 years (range 2–11 years).

The median NT-proBNP concentration in Greyhounds was 945 pmol/L (range 512–2127 pmol/L) and for non-Greyhound dogs, it was 632 pmol/L (range 126–1952 pmol/L). Two of the non-Greyhound dogs were Galgos (Spanish Greyhounds), with NT-proBNP concentrations of 1054 and 1704 pmol/L, respectively; they were excluded from statistical analysis because they are considered sighthounds. The NT-proBNP concentration in Greyhounds ($n = 24$) was significantly higher ($P = .003$) than that in non-Greyhound dogs ($n = 27$) (Figure 1). Eleven of 24 Greyhounds and 5/27 of the non-Greyhound dogs had NT-proBNP concentrations > 1000 pmol/L, the most frequently used cutoff for an abnormal test result ($P = .036$).

The natriuretic peptides, synthesized and secreted by the atrial and ventricular myocardium, are counterregulatory hormones with pleiotropic effects. Specifically, stimulation of a guanylate cyclase coupled to natriuretic peptide receptors results in natriuresis, vasorelaxation, inhibition of renin and aldosterone, inhibition of fibrosis, and increases in lusitropy (myocardial relaxation), among others. Within this group of hormones, BNP has been evaluated as a useful tool in the triage of dyspneic patients and classification of heart failure.[3,9]

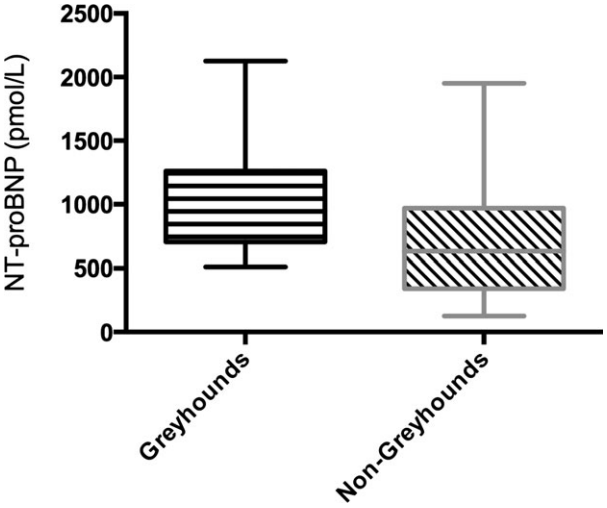

Figure 1. Boxplots showing distribution of N-terminal pro-B-type natriuretic peptide concentrations in Greyhounds and non-Greyhound dogs. The box represents the 25th and 75th percentiles, and the whiskers the 5th and 95th percentiles, respectively. There was a significant difference between Greyhounds and non-Greyhound dogs ($P < .005$).

Greyhounds exhibit many adaptational physiologic traits that are different from other breeds, presumably caused by selection for a high racing performance. For instance, they have a unique musculoskeletal conformation, larger myocardial muscle mass, and a higher concentration of RBC to increase their exercise efficiency.[1] These adaptations include differences in their hematologic and biochemical variables, some of which are only now beginning to be documented.[1]

The main stimulus for BNP release from myocytes is myocardial stretching; therefore, an increase in volume- or pressure-mediated wall stress could explain the higher BNP concentrations in Greyhounds, although other possible causes should be explored.[3,9] In Greyhounds, the higher blood viscosity due to the higher HCT[1], in addition to functional relative aortic stenosis[17], and the role of diastolic function under these conditions could be the main promoting factors for increasing cardiac stretch. However, in people, BNP and cardiac filling pressures are not well correlated.

The role of the breed in canine NT-proBNP plasma concentrations has been described previously[8,13,14], so it is possible that the higher NT-proBNP range in Greyhounds represents just another breed-related finding in healthy dogs, similar to Labradors and Newfoundlands.[13] Therefore, given the high prevalence of physiologic murmurs, radiographic evidence of "cardiomegaly", and "stiff" ventricles on echocardiography, NT-proBNP values in Greyhounds should not be overinterpreted by the clinician.

Other, extracardiac influences on BNP concentration have been described in both people and dogs, including age, sex, renal function, and angiotensin II and endothelin 1 concentrations.[3,4,13,14,18] For instance, NT-proBNP concentrations are higher in azotemic dogs without cardiac disease.[19] While Greyhounds have higher serum creatinine concentrations than non-Greyhound dogs[1], their glomerular filtration rate (GFR) is also higher[20]; therefore, the higher NT-proBNP concentrations are unlikely to be due to reduced renal clearance, although we did not measure GFR in the study dogs.

Obese human patients have lower NT-proBNP concentrations, and NT-proBNP concentrations are inversely related to body mass index (BMI).[18] The proposed mechanism is an alteration of peripheral degradation of BNP due to abundance of adipose tissue. In addition, NT-proBNP concentrations increase after bariatric surgery in obese human patients, suggesting that NT-proBNP production is suppressed in obese individuals.[21] Therefore, it is possible that a contributing factor to the higher NT-proBNP concentration in Greyhounds is their lean body mass. As reported, 2 of the highest NT-proBNP concentrations in non-Greyhounds were determined in Galgos, another sighthound breed. This breed has lean body mass and cardiovascular features similar to those in Greyhounds, and additional studies on their cardiovascular function and cardiac biomarkers are currently in progress (PGO and CGC, data not shown).

Greyhounds have the highest heart-to-body weight ratio of all dog breeds evaluated in the literature, a comparatively larger heart radiographically, and mean values for left ventricular size and thickness are above the RI for non-Greyhound dogs.[15,17] Because the ventricular muscle is the main source for BNP, the relatively large heart size in Greyhounds could explain the high circulating values of NT-proBNP.[3,9]

Interestingly, people with an athlete's heart have features of left ventricular hypertrophy that resembles that seen in Greyhounds.[22] However, NT-proBNP concentrations in clinically healthy endurance athletes with athlete's hearts are not significantly different from healthy untrained controls.[22] Therefore, the high NT-proBNP concentrations in Greyhounds cannot be solely explained on the basis of an athlete's heart.

A preanalytic increase in NT-proBNP concentration due to handling or sampling in our study is

unlikely, as all samples from both groups of dogs were processed using the same protocol. Samples were all collected in the morning hours, and thus it is unlikely that the significant difference between study groups was due to time of day variance.

Prospective cardiac studies correlating NT-proBNP concentration with echocardiographic findings should be performed to ascertain the role of cardiac stretching in Greyhounds without cardiac disease and their potential role in their high NT-proBNP concentration. A separate cutoff value may need to be used for Greyhounds, so that unnecessary testing is not pursued when a high plasma NT-proBNP concentration is found in a healthy dog presenting with a murmur or other signs of potential heart disease.

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