Original Study

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Blood gas analysis and cooximetry in retired racing Greyhounds

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Abstract

Objective – The purposes of this study were to evaluate the oxygen affinity of hemoglobin (Hb) in healthy retired racing Greyhounds via cooximetry, and to establish reference intervals for blood gases and cooximetry in this breed.
Design – Prospective clinical study.
Setting – University Teaching Hospital.
Animals – Fifty-seven Greyhounds and 30 non-Greyhound dogs.
Interventions – Venous blood samples were collected from the jugular vein and placed into heparinized tubes. The samples were analyzed within 30 minutes of collection using a blood gas analyzer equipped with a cooximeter.
Measurements and Main Results – Greyhounds had significantly higher pH, PO_2, oxygen saturation, oxyhemoglobin, total Hb, oxygen content, and oxygen capacity and significantly lower deoxyhemoglobin and P_{50} when compared with non-Greyhound dogs.
Conclusion – These findings support the fact that this breed is able to carry a higher concentration of total oxygen in the blood. As reported previously, this breed also has lower P_{50} and, therefore, high oxygen affinity. In light of recent findings suggesting that in certain tissues a high affinity for oxygen is beneficial, this adaptation may be of benefit during strenuous exercise.

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Keywords: cooximeter, dogs, gas exchange, hemoglobin, oxygen affinity

Introduction

Greyhounds have high mean hematocrit (HCT), hemoglobin (Hb) concentration, RBC counts,[1] lower WBC, neutrophil, and platelet counts, and atypical eosinophil morphology, when compared with dogs of other breeds.[1–3] Greyhounds may have the highest resting HCT of any mammalian species, which increases significantly during a race due to splenic contraction, RBC release from the bone marrow,[3–5] or by translocation of water out of the vascular space and subsequent hemoconcentration.[1,3,4,6,7] Some of these mechanisms have been hypothesized to be physiologic adaptations to racing in order to increase oxygen delivery to tissues. However, little is known about Hb function, including oxygen affinity in Greyhounds compared with non-Greyhound breeds. In a previous study, Hb function in Greyhounds was evaluated by determining the PO_2 at which Hb is 50% saturated (P_{50}), using oxyhemoglobin dissociation curves (ODC).[6] The P_{50} values were lower in Greyhounds than in other breeds, representing a left shift in the ODC, and therefore a higher affinity for oxygen.[6] The RBC 2,3-diphosphoglycerate (2,3-DPG) content was not significantly different between the Greyhounds and the non-Greyhounds in that study.[6]

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Based on those results, the authors proposed that decreased oxygen release to the tissues could cause increases in erythropoietin production and increased RBC production. They also proposed this higher oxygen affinity, rather than breeding selection, as the likely cause of the high Hb and HCT in this breed.[6]

Gas exchange and Hb function can be assessed using blood gas analyzers and cooximetry, respectively. Cooximeters are instruments that measure percentages of the 4 Hb moieties spectrophotometrically: oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb), carboxyhemoglobin (COHb), and methemoglobin (MetHb).[8] The objectives of this study were to evaluate the oxygen affinity of Hb in healthy retired racing Greyhounds using a blood gas analyzer with cooximeter, and to establish reference intervals for blood gases and cooximetry in this breed.

Materials and Methods

Animals

Blood samples were obtained from 57 healthy, adult, retired racing Greyhound dogs and 30 healthy, adult dogs of other breeds. Only healthy animals, determined based on lack of abnormal clinical signs or findings on physical examination, were included in the studies. Greyhounds and some of the non-Greyhound dogs were part of the blood donation program at The Ohio State University Animal Blood Bank, with all samples being collected before blood donation. Other non-Greyhound dogs belonged to staff of the Veterinary Medical Center and students in the College of Veterinary Medicine at this University. The Animal Blood Bank has a current animal use protocol approved by the Institutional Animal Care and Use Committee for collection of blood to establish reference intervals. Blood samples in nondonor dogs were collected with signed informed owner consent.

Blood collection procedures

After occlusion for <20 seconds, 3 mL of blood were obtained by venipuncture of the external jugular vein using a 20-G needle and a 3 mL plastic syringe. The sample was then immediately placed into a 3 mL vacuum-sealed glass tube containing lithium heparin,[a,9] and analyzed immediately.

Blood analyses

Blood samples were analyzed using a blood gas analyzer[b] with a cooximeter. All samples were analyzed following the manufacturer's instructions.[c] The analyzer directly measures pH, PCO_2, PO_2, oxygen saturation (SO_2%), HCT, and Hb. The methods used to measure these parameters were specific electrode (pH), Severinghaus method (PCO_2), amperometric (PO_2), optical reflectance (SO_2%), conductivity/Na correction (HCT), and multiple wavelength/conductivity correction (Hb). The cooximeter directly measures by multiple wavelength spectophotometry the percentages of O_2Hb%, HHb%, COHb%, and MetHb%. The instrument's software automatically calculates other parameters such as P_{50}, oxygen content (O_2Ct), and oxygen capacity (O_2Cap).

Statistical analysis

The dogs were divided into 2 groups, Greyhounds and non-Greyhounds, and the data were analyzed using commercial statistical software.[d] Variables were analyzed using descriptive statistics and evaluated for normality using the D'Agostino and Pearson omnibus normality test. Unpaired 2-tailed Student's *T*-tests were used to compare values between both groups when data were normally distributed, and a Mann-Whitney test was used when data did not have normal distribution. Statistical significance was set at $P<0.05$. Reference intervals for Greyhounds and non-Greyhounds were established using the central 95% of values (mean $\pm$ 2 SD) when data were normally distributed. For variables that did not follow Gaussian distribution, observed ranges are listed (method used based on the small number of data points, and nonnormal distribution).[10]

Results

The Greyhound group comprised 30 males (53%) and 27 females (47%), with a mean age of 5.7 years (SD 1.65 years), and a mean weight of 32 kg (SD 4.49 kg). The non-Greyhound group comprised 20 males (67%) and 10 females (33%), with a mean age of 4.7 years (SD 2.51 years), and a mean weight of 31.72 kg (SD 12.30 kg). All the dogs included in the study were neutered. The other breeds included a wide range of weights and muscle masses. All the dogs included in this study were pets with a similar range of activity. The non-Greyhound group included a variety of large and small breeds. All the data were normally distributed with the exception of MetHb in both groups, and pH and P_{50} in the Greyhounds group. As has been reported previously,[1,11] we found a significantly higher HCT in Greyhounds ($P<0.0001$) compared with non-Greyhounds. Values from the blood gas analyzer and cooximeter are shown in Table 1. Greyhounds had significantly higher pH, PO_2, SO_2%, O_2Hb%, total Hb (tHb), O_2Ct, O_2Cap, and lower HHb% and P_{50} compared with non-Greyhounds. The remaining parameters (PCO_2, COHb%, MetHb%) were not statistically different between Greyhounds and non-Greyhounds. As shown in Figure 1, the distribution of the P_{50} values was much narrower in

Table 1: Venous cooximetry and blood gas mean values ± SD (unless otherwise indicated) of 57 Greyhounds and 30 non-Greyhounds exhibiting significant difference ($P < 0.05$)

Value	Greyhounds	Non-Greyhounds	*P*-value
pH	7.41 ± 0.03	7.40 ± 0.03	= 0.034
HCT (%)	51.7 ± 3.9	46.1 ± 2.8	< 0.0001
PO_2 (mm Hg)	60.3 ± 12.0	52.1 ± 8.7	= 0.0014
Oxygen saturation (SO_2, %)	89.2 ± 5.3	77.0 ± 11.3	< 0.0001
Oxyhemoglobin (O_2Hb, %)	86.5 ± 5.5	75.4 ± 10.3	< 0.0001
Deoxyhemoglobin (HHb, %)	10.8 ± 5.2	21.4 ± 9.3	< 0.0001
Total hemoglobin (tHb, g/dL)	21.5 ± 1.7	18.2 ± 1.6	< 0.0001
P_{50} (mm Hg)	26.5 ± 0.3*	29.9 ± 4.3	< 0.0001
Oxygen content (O_2Ct, mL/dL)	25.9 ± 3.1	19.0 ± 2.8	< 0.0001
Oxygen capacity (O_2Cap, mL/dL)	29.0 ± 2.6	24.3 ± 2.1	< 0.0001

*Data nonnormally distributed, presented as median and interquartile range.

Greyhounds (range 26.00–28.40 mm Hg; SD = 0.40) than in the non-Greyhounds (range 25.90–38.50 mm Hg; SD = 4.28).

Both SO_2% and O_2Hb% were significantly higher ($P < 0.0001$) in Greyhounds (SO_2 mean, 89.18%; O_2Hb mean, 86.51%) than in non-Greyhounds (SO_2 mean, 77.05%; O_2Hb mean, 75.41%), and HHb% was lower in Greyhounds compared with the non-Greyhound group (10.77% versus 21.36%; $P < 0.0001$). Greyhounds also had higher tHb (mean, 21.53 g/dL; $P < 0.0001$) than non-Greyhounds (mean, 18.16 g/dL). Greyhound-specific reference intervals for parameters measured in this study are shown in Table 2, compared with reference intervals for non-Greyhounds.

Discussion

Greyhounds have higher PO_2, SO_2, O_2Hb, tHb, O_2Ct, and O_2Cap than non-Greyhounds. These parameters are used to assess the oxygenation and function of the Hb molecule, and higher values indicate that Greyhounds are able to carry a higher concentration of total oxygen in the blood. However, Greyhounds also have lower P_{50} than non-Greyhounds, which could be due to higher oxygen affinity. Previous studies have proposed that a decrease in oxygen affinity (higher P_{50}) is beneficial for athletic performance,[12–18] because the oxygen is more easily released from the Hb to tissues. In Greyhounds, the apparent high oxygen affinity Hb does not support this theory.

In people, hemoglobinopathies are the most frequently encountered monogenic disorders worldwide.[19] Over 900 structural Hb variations have been described, including single mutations, deletions, or insertions in the genes that encode either the α- or β-globin chain. In over 95% of these structural variations, there is a single amino acid mutation that leads to changes in stability, solubility, and function. In the 89 reported hemoglobinopathies associated with high oxygen affinity, the decreased release of oxygen to tissues results in tissue hypoxia.[19] This hypoxia triggers

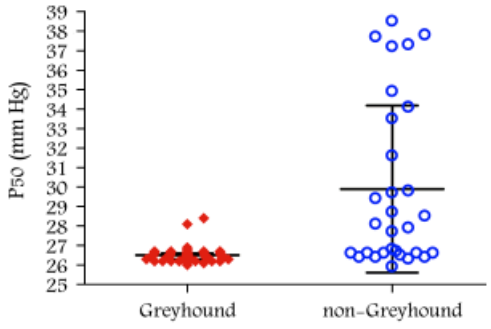

Figure 1: Hemoglobin P_{50} values (PO_2 at which hemoglobin is 50% oxygenated) of healthy Greyhounds and non-Greyhounds. Greyhounds group did not pass the normality test.

Table 2: Venous cooximetry and blood gas reference intervals for Greyhounds and non-Greyhounds

Parameter	Greyhound	Non-Greyhounds
PO_2 (mm Hg)*	36.3–84.3	34.6–69.6
PCO_2 (mm Hg)	25.6–39.9	24.7–44.4
SO_2 (%)*	78.6–99.8	54.4–99.8
tHb (g/dL)*	18.1–25.0	15.0–21.3
O_2Hb (%)*	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_{50} (mm Hg)*	26.0–28.4†	21.4–38.4
O_2Ct (mL/dL)*	19.7–32.0	13.3–24.6
O_2Cap (mL/dL)*	23.8–34.1	20.2–28.5

*Parameters showing significant differences between the two groups (Greyhound and non-Greyhound dogs).
†Data nonnormally distributed. Reference intervals expressed as observed ranges.
SO_2, oxygen saturation; tHb, total hemoglobin; O_2Hb, oxyhemoglobin; COHb, carboxyhemoglobin; MetHb, methemoglobin; HHb, deoxyhemoglobin; O_2Ct, oxygen content; O_2Cap, oxygen capacity.

production of erythropoietin by hypoxia-inducible factors, leading to secondary erythocytosis.[20] Patients with high-affinity Hb have low P_{50}, as is the case in Greyhounds.

The ODC is a graphical representation of the uptake of oxygen in the lungs and the delivery to the tissues.[21] The position of this curve is influenced by pH, temperature, CO_2, and 2,3-DPG concentration. As discussed above, Greyhounds had a higher pH and lower CO_2 than non-Greyhounds, causing a left shift of the ODC, and consequently a lower P_{50}. Under standard conditions (37°C, pH 7.4, PCO_2 40 mm Hg), interspecies variations in the ODC are mainly determined by the primary structure of the Hb molecule and the chemical composition and structure of erythrocytes. A previous report in dogs showed that the ODC is strongly influenced by breed, because the P_{50} value was widely dispersed among breeds, ranging from 25.8 mm Hg in spaniels to 35.8 mm Hg in hounds.[21] In the present study, the unusual minimal dispersion as well as the low P_{50} in Greyhounds suggests that unknown factors have selected for a very specific Hb oxygen affinity in this breed. As previous reports show that 2,3-DPG concentrations in Greyhounds are not different than in other breeds,[6] we suggest that Greyhounds may be more sensitive to pH changes,[22] causing the ODC left shift.

Although arterial samples are more traditionally used for the assessment of oxygenation, venous samples were used in this study based on the guidelines for routine measurement of blood Hb oxygen affinity,[9] because P_{50} should not vary among them, and venous samples are more commonly obtained and practical to run in the clinical setting.

The O_2Ct is the total amount of oxygen in the blood (dissolved oxygen and oxygen bound to Hb) and is calculated by the cooximeter using the equation $1.39Hb \times SO_2\% + 0.003PO_2$. O_2Cap is the total amount of oxygen that Hb can carry and is calculated by the cooximeter using the equation $1.39(O_2Hb\% + HHb\%)/100[tHb]$. In this study, both O_2Ct and O_2Cap were significantly higher in Greyhounds compared with non-Greyhounds, suggesting that these increases could be a consequence of the high-affinity Hb and stronger binding between Hb and O_2.

It is unclear how an athletic breed such as Greyhounds benefits from a low P_{50}. Recent studies on Hb-based oxygen carriers have revealed that in certain tissues, a high-affinity oxygen carrier is beneficial, suppressing vasoconstriction elicited by early off-loading and over-oxygenating tissues at the level of the precapillary sphincter.[23] In normal individuals, oxygen has a tendency to be released at the arteriolar level before it reaches the capillaries. With higher affinity Hb, the oxygen remains bound longer (ie, through arteriolar circulation), and should therefore be released at a deeper tissue level (ie, capillaries), where oxygen tension is lower.[24] This may allow delivery of oxygen to the tissues which need it most (ie, muscles), which would be of benefit during strenuous exercise. Although counterintuitive to traditional wisdom, these mechanisms could explain the benefits of having a high-affinity Hb in an athletic breed such as Greyhounds.

This study has some potential limitations. First of all, sample handling could have influenced venous $SO_2\%$, PCO_2, and PO_2.[25] However, all the samples were handled by the same operator (S.Z.L.), and $SO_2\%$ and PO_2 in Greyhounds are still significantly higher than in non-Greyhounds. A potential reason for the high $SO_2\%$ observed may be that the high oxygen affinity Hb (low P_{50}) found in the Greyhound makes Greyhound Hb more likely to remain bound to oxygen, therefore leading to the higher than expected $SO_2\%$. It is also important to note that, although this instrument[b] is widely used in hospitals and emergency practices,[26–28] no validation for its use in dogs has been reported. The only parameter that has been validated in vitro is $SO_2\%$.[29]

The cooximetry results in Greyhounds, in combination with the previous findings of decreased cooperative binding of Hb,[6] indicate that Greyhounds may have a unique structural variation in the Hb molecule. Alternatively, given the high HCT (mean, $51.70 \pm 3.92\%$), the high viscosity in Greyhounds could have impeded a constant flow rate through the channel in the instrument, thus altering the cooximeter values. Preliminary data from electrophoresis of Hb from retired racing Greyhounds (data not shown) did not reveal any different mobility patterns compared with non-Greyhounds. However, this technique may not be useful in the evaluation of high-affinity Hb because many of the mutations are electrophoretically silent.

Additional studies are currently being performed in order to evaluate whether viscosity is a factor in cooximetry evaluation of Greyhound blood. Crystallography may be helpful in characterizing this high-affinity Hb structure,[30,31] these studies being currently under way. Arterial blood samples from Greyhounds should also be analyzed with cooximetry, and comparison with venous samples would allow determination of oxygen extraction ratios, which may lend further support to evidence for increased Hb affinity for oxygen in Greyhounds compared with other breeds of dogs. In conclusion, further investigation into the unique Hb oxygen affinity of Greyhounds through the use of techniques such as electrophoresis, high-performance liquid chromatography, or molecular methods is warranted.

Footnotes

[a] Monoject™ Green Stopper, Tyco Healthcare, Mansfield, MA.
[b] STP CCX Analyzer, Nova Biomedical, Waltham, MA.
[c] Nova Biomedical STP CCX Analyzer User Manual, 2003.
[d] GraphPad Software Inc, San Diego, CA.

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