BLOOD GASES AND COOXIMETRY IN RETIRED RACING GREYHOUNDS:
UNIQUE HEMOGLOBIN PHYSIOLOGY AND OXYGEN CARRYING
PROPERTIES

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

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ABSTRACT

Greyhounds have differences in many hematological parameters compared to other breeds [i.e. higher PCV and red blood cell counts], attributed to selective breeding, and to a compensatory mechanism for their high oxygen affinity hemoglobin (Hb). The purpose of this study was to evaluate this oxygen affinity of Hb in retired racing Greyhounds (RRGs) using a blood gas analyzer with cooximeter (Nova CCX), and to establish reference intervals in this breed. Venous blood samples from 57 RRGs (G) and 30 non-Greyhounds (NG) were analyzed, and groups were compared using T-test. The G group had significantly higher pH, partial pressure of oxygen (PO₂), oxygen saturation (SO₂), oxyhemoglobin (O₂Hb), total Hb (tHb), oxygen content (O₂Ct), and oxygen capacity (O₂Cap) and significantly lower deoxyhemoglobin (HHb) and P₅₀ when compared to NG, supporting the higher oxygen-carrying capacity in this breed. Consistent with previous reports, P₅₀ was lower (high oxygen affinity). Current studies on Hb-based oxygen carriers have revealed that in tissues which need more oxygen, a high-affinity oxygen carrier is beneficial (i.e. strenuous exercise), potentially explaining the benefits of having a high-affinity Hb.

Given the narrow range found in the P₅₀ value, and Greyhounds’ high mean Hct, we postulated that the high viscosity had affected the results by impairing a constant blood flow through the analyzer. Venous samples from 13 RRGs were obtained, and divided in two heparinized tubes, diluting one to 20% with PBS. Both undiluted and diluted samples
were analyzed simultaneously, and $P_{50}$ was also calculated manually (formula in the user’s manual). No significant differences were found among undiluted and diluted samples, but when calculated manually, mean $P_{50}$ was significantly lower than the one generated by the instrument in both undiluted and diluted samples. In conclusion, the high viscosity did not affect the results obtained in the previous study.

In order to evaluate the $P_{50}$ variation during storage, venous blood from 19 RRGs was analyzed and then stored at 4ºC for 3 weeks. At this time, the analysis was repeated and the two time points were compared. The mean $P_{50}$ value decreased, but unexpectedly, almost half of the values remained the same after 3 weeks.

The Hemox-Analyzer is an instrument that accurately records and plots the oxyhemoglobin dissociation curve (ODC) during deoxygenation, providing information about the delivery of oxygen to the tissues. We evaluated venous samples from two adult dogs (one RRG and one mixed breed); both dogs had blood gas and cooximetry analysis performed too. Although both dogs had the same $P_{50}$ using the Nova CCX, the Greyhound had lower $P_{50}$ than the mixed breed dog using the Hemox-Analyzer.

Overall, we conclude that Greyhounds have high affinity Hb, as reflected by a low $P_{50}$, compared to NG. However, this is not accurately assessed by the Nova CCX analyzer, probably because it is not designed for animals (uses human ODC algorithms). Based on limitations imposed by the machine’s calculation of $P_{50}$, higher $SO_2$ in Greyhounds (>80%) could also make the analyzer assign a default value, explaining such a narrow range in $P_{50}$ values.
DEDICATION

To my parents, Ana and Santiago, and my brother Pablo; for their unconditional love and support. To Jose, for giving me strength, confidence and love.
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PREVALENCE OF DOG ERYTHROCYTE ANTIGENS IN RETIRED RACING GREYHOUNDS. Veterinary Clinical Pathology, in press.

COOXIMETRY IN RETIRED RACING GREYHOUNDS. Journal of Veterinary Emergency and Critical Care (JVECC), accepted for publication.

FIELDS OF STUDY

Major Field: Veterinary Clinical Science
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CHAPTER 1: UNIQUE HEMATOLOGIC PARAMETERS IN GREYHOUNDS

Retired racing Greyhounds (RRGs) are becoming increasingly popular in the United States; currently, over 120,000 live in homes as pets. As the popularity of adopting RRGs expands, the responsibility of small animal veterinarians to this breed also grows. There are several physiological aspects of Greyhounds that are distinct from other canine breeds.\textsuperscript{1-8} For example, Greyhounds have higher troponin I levels,\textsuperscript{9} a physiologic left basilar systolic murmur (usually grade 1-2/6) with no apparent clinical relevance,\textsuperscript{10} and high arterial blood pressures than other breeds of dogs.\textsuperscript{11} In addition, some biochemical and hematological values are significantly different in Greyhounds when compared to non-Greyhound breeds, so they are frequently misinterpreted as abnormal, unless Greyhound specific reference intervals are used.

In the hematologic analysis or complete blood cell count (CBC), previous reports indicate that Greyhounds have a higher packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV) and red blood cell count (RBC), as well as lower white blood cell (WBC) and platelet (PLT) counts, when compared to other breeds.\textsuperscript{7,12} RBC in Greyhounds have a shorter circulating lifespan than those in other breeds,\textsuperscript{13} and eosinophils often have a different morphology.\textsuperscript{14} Greyhounds have a tendency to bleed profusely after surgical procedures. Although in affected individuals results of conventional coagulation tests (prothrombin time, activated partial
thromboplastin time) that are within normal limits.\textsuperscript{15} viscoelastic analysis of blood coagulation with thromboelastography (TEG) revealed that Greyhounds have slower clotting kinetics and weaker clot strength compared to other breeds.\textsuperscript{16}

There are several studies reporting the hematologic effects of maximum exercise in racing Greyhounds.\textsuperscript{17-22} During a race, they experience extreme changes, such as markedly higher packed cell volume (PCV), lactate concentration and body temperature, and extremely low pH (when compared to humans and horses performing sprint exercise).\textsuperscript{20} Immediately after racing, the PCV remains slightly elevated, with Hb content, RBC counts, WBC and total proteins (TP) higher than prior to the race, but return to normal a few hours after racing.\textsuperscript{17-18,21-22} This breed has an extremely high resting PCV (possibly the highest of any mammalian species), which increases even further during racing. This could be attributed to splenic release of RBCs,\textsuperscript{22} and/or a decrease in plasma volume (higher plasma TP)\textsuperscript{22-23} or the increases in stroke volume and cardiac output.\textsuperscript{24}

Hemoglobin is the iron-containing oxygen-transport metalloprotein in RBCs of vertebrates. In mammals, this protein comprises about 97\% of the dry matter of RBCs, and about 35\% of the total content that includes water. Hemoglobin transports oxygen from the lungs to other tissues, such as the muscles, where it releases the oxygen at the capillary level for use by various tissues. The affinity of Hb for oxygen is measured using the $P_{50}$ value, defined as the amount of oxygen needed to saturate 50\% of Hb. The relationship between oxygen and Hb is described by the oxygen dissociation curve of Hb (ODC) [Figure 1], which plots the oxygen saturation of Hb (SO$_2$\%) on the Y axis and the
partial pressure of oxygen (PO₂) on the X axis. The sigmoidal shape of this curve is a function of the interaction between the Hb subunits. When a Hb subunit releases a molecule of oxygen, the other subunits undergo a conformational change, which facilitates release of the remaining oxygen molecules. Conversely, binding of oxygen to one subunit causes a conformational change which increases affinity and promotes binding to the other subunits. Therefore, this relationship and the shape of the ODC promotes rapid uptake of oxygen in the lungs and facilitates efficient delivery of oxygen to the tissues. Hemoglobin affinity for oxygen is affected by pH, partial pressure of carbon dioxide (PCO₂), concentration of 2,3-diphosphoglycerate (2,3-DPG), and temperature. Increases in PCO₂, 2,3-DPG, and temperature, and a decrease in pH, shifts the curve to the right, which causes a decrease in oxygen affinity of Hb (and therefore an easier release of oxygen). These conditions occur in tissues, being especially advantageous in those that are metabolically active (i.e. exercising muscle), which are accumulating lactate and CO₂ due to a transient hypoxia. Conversely, decreases in CO₂, 2,3-DPG, and temperature and increases in pH cause a shift to the left and increase affinity, as occurs in the lungs. In standard conditions (37ºC, pH 7.4, PCO₂ 40 mmHg), species variations in ODC are determined mainly by the primary structure of the Hb and the chemical composition of RBCs. Differences in P₅₀ between breeds and species are due to genetic changes in Hb structure or 2,3-DPG levels, which are higher in dogs compared with cattle and humans. The effect of temperature on the ODC in dogs, cattle and horses is less than in humans. In contrast, the effect of pH on the ODC in dogs, horses and humans is more pronounced than in cattle.
In human athletes, the Hb affinity is typically low; thus the oxygen off-loading and exchange is more efficient at the tissue level. Adequate oxygen exchange is necessary to maintain cellular aerobic metabolism in adapted racing individuals, as has been demonstrated in previous studies related to exercise physiology in humans, horses, and dogs. Our preliminary observations indicate that Greyhounds have high oxygen affinity, contradicting this theory, since oxygen would be more likely to remain bound to Hb and therefore unavailable for tissues. Hb function in Greyhounds will be discussed in detail in Chapter 2.

Figure 1: Diagram of the oxyhemoglobin dissociation curve (ODC).
CHAPTER 2: BLOOD GAS ANALYSIS AND COOXIMETRY

As mentioned in chapter 1, Greyhounds have many hematologic differences from other breeds. Some of these features have been hypothesized to be physiological adaptations to racing in order to increase oxygen delivery to tissues. However, little is known about Hb function related to oxygen transport in RRGs compared to non-Greyhound breeds. In a previous study, Hb function in Greyhounds was evaluated by determining $P_{50}$ values using ODCs, 2,3-DPG determinations, and Hill coefficients which express the degree to which cooperative binding of oxygen occurs on the Hb molecule. The values for $P_{50}$ were found to be lower in Greyhounds than in other breeds, indicating a left shift of the ODC and therefore a higher affinity for oxygen. The RBC 2,3-DPG content was not different between Greyhounds and non-Greyhounds, but the Hill’s coefficient was lower in Greyhounds than in the non-Greyhound group. Based on those results, the authors proposed that decreased oxygen release to the tissues could cause increases in erythropoietin production and increased RBC production, leading to secondary erythrocytosis. They also proposed this higher oxygen affinity, rather than breeding selection, as the likely cause of the high Hb and Hct in Greyhounds.

Gas exchange and Hb function can be assessed using blood gas analyzers and cooximetry, respectively. Cooximeters are instruments that measure percentages of the four Hb moieties spectrophotometrically: oxyhemoglobin ($O_2$Hb), deoxyhemoglobin
(HHb), carboxyhemoglobin (COHb), and methemoglobin (MetHb). The purpose of this study was to further characterize the Hb molecule in healthy RRGs, using a blood gas analyzer equipped with cooximeter, and compare Hb function in Greyhounds to non-Greyhound dogs.

2.1. Materials and Methods

Blood samples were obtained from 57 healthy, adult, RRGs and 30 healthy, adult dogs of non-Greyhound breeds. RRGs and some of the non-Greyhound dogs were part of the blood donation program at The Ohio State University Animal Blood Bank (OSUABB), and samples were collected prior to blood donation. Other non-Greyhound dogs belonged to staff or students in the College of Veterinary Medicine at OSU. The OSUABB has a current animal use protocol approved by the Institutional Animal Care and Use Committee at OSU for collection of blood to establish reference intervals. Blood samples in non-donor dogs were collected after signed owner’s consent.

Three milliliters (ml) of blood were obtained by venipuncture of the external jugular vein using a 20-gauge needle and a 3 ml plastic syringe. Blood was immediately placed into a 3 ml glass tube containing lithium heparin (Monoject™ Green Stopper, Tyco Healthcare, Mansfield, MA) and analyzed within 30 minutes of collection. Blood samples were analyzed using a Stat Profile® Critical Care Xpress Analyzer (Nova CCX, Nova Biomedical, Waltham, MA) with a cooximeter following the manufacturer’s instructions. The analyzer directly measures pH, partial pressure of carbon dioxide (PCO\(_2\)), partial pressure of oxygen (PO\(_2\)), oxygen saturation (SO\(_2\)%), hematocrit (Hct) and
hemoglobin (Hb). The methods used to measure these parameters were specific electrode (pH), Severinghaus method (P\textsubscript{CO\textsubscript{2}}), amperometric (P\textsubscript{O\textsubscript{2}}), optical reflectance (SO\textsubscript{2}%), conductivity/Na correction (Hct), and multiple wavelength/conductivity correction (Hb). The cooximeter directly measures by multiple wavelength spectrophotometry the percentages of oxyhemoglobin (O\textsubscript{2}Hb%), deoxyhemoglobin (HHb%), carboxyhemoglobin (COHb%), and methemoglobin (MetHb%). The instrument’s software automatically calculates other parameters such as P\textsubscript{50}, oxygen content (O\textsubscript{2}Ct) and oxygen capacity (O\textsubscript{2}Cap). The O\textsubscript{2}Ct is the total amount of oxygen in the blood (dissolved oxygen and oxygen bound to Hb) and is calculated by the analyzer using the equation 1.39 (Hb x SO\textsubscript{2}% + 0.003 x P\textsubscript{O\textsubscript{2}}. O\textsubscript{2}Cap is the total amount of oxygen that Hb can carry and is calculated by the analyzer using the equation 1.39 x (O\textsubscript{2}Hb%+HHb%)/100 x [tHb].

The dogs were divided into two groups, Greyhounds (G) and non-Greyhounds (NG), and the data were analyzed using GraphPad Prism® statistical software. Variables were analyzed using descriptive statistics and evaluated for normality using the D’Agostino & Pearson omnibus normality test. Unpaired two-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 Gaussian distribution. Statistical significance was set at P<0.05. Reference intervals for G and NG were established using the central 95% of values (mean ±2SD) 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 non-normal distribution\textsuperscript{30}).
2.2. Results.

The Greyhound group had 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 had 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 spayed/neutered. The other breeds included a wide range of weights and muscle masses. All the dogs included in this study were pets with similar lifestyles. The non-Greyhound group included a variety of large and small breeds. All data were normally distributed with the exception of MetHb in both groups, and pH and \( P_{50} \) in the G group. A significantly higher Hct was found in Greyhounds (P<0.0001) compared to non-Greyhounds. Values from the blood gas analyzer and cooximeter are shown in Table 1. Greyhounds had significantly higher pH, \( P_{O_2} \), \( SO_2 \% \), \( O_2 Hb\% \), \( tHb \), \( O_2 Ct \), \( O_2 Cap \), and lower HHb% and \( P_{50} \) compared to non-Greyhounds. The remaining parameters (\( P_{CO_2} \), COHb%, MetHb%) were not statistically different between Greyhounds and non-Greyhounds. As shown in Figure 2, the distribution of the \( P_{50} \) values was much narrower in Greyhounds (range 26.00-28.40 mm Hg; SD=0.40) than in the non-Greyhounds (range 25.90-38.50; SD=4.28).

Both \( SO_2 \% \) and \( O_2 Hb\% \) were significantly higher (p<0.0001) in Greyhounds (\( SO_2 \) mean, 89.18%; \( O_2 Hb \) mean, 86.51%) than in non-Greyhounds (\( SO_2 \) mean, 77.05%; \( O_2 Hb \) mean, 75.41%). This is further reflected by the lower percentage of HHb% in Greyhounds compared to the non-Greyhound group (10.77% and 21.36%, respectively;
Greyhounds also had higher total 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 to reference intervals for non-Greyhounds.

2.3. Discussion

Greyhounds have higher O₂Hb, tHb, O₂Ct, and O₂Cap than non-Greyhounds. These parameters assess the oxygenation and function of the Hb molecule, and higher values support the fact that Greyhounds are able to carry a higher concentration of total oxygen in the blood. Greyhounds also have higher PΟ₂, SO₂, and lower P50 than non-Greyhounds, which is likely due to higher oxygen affinity, as they represent decreased uptake by the tissues, while the blood passes through the microcirculation. Previous studies have proposed that a decrease in oxygen affinity (higher P50) is beneficial for athletic performance, since the oxygen is more easily released from the Hb to the tissues. In Greyhounds, the apparent high-oxygen affinity Hb seems to contradict this theory.

In people, hemoglobinopathies are the most frequently found monogenic disorders worldwide. Over 900 Hb structural 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. This hypoxia triggers production of erythropoietin by hypoxia-inducible
factors (HIF), leading to secondary erythocytosis. Sullivan et al suggested that the high Hb concentration and PCV in Greyhounds could be a compensation for the low P50 (and therefore higher oxygen affinity) found in this breed.

As mentioned in Chapter 1, the ODC is influenced by pH, temperature, CO₂, 2,3-DPG concentration, and breed. In the present study, the unusual minimal dispersion as well as the low P50 in Greyhounds suggests that unknown factors have selected for a very specific Hb oxygen affinity in this breed. Since previous reports show that 2,3-DPG concentrations in Greyhounds are not different from those in other breeds, we suggest that Hb in Greyhounds may be more sensitive to pH changes, causing the ODC to shift to the left. As discussed above, during a race, lactate concentration and temperature in Greyhounds increase, and pH decreases markedly (even when compared to humans and horses performing sprint exercise). The change in ODC may be an adaptation to these intra- and post-racing homeostatic changes.

Although arterial samples are 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. In addition, there should be a minimal variation in P50 among venous and arterial samples, and from a practical standpoint, venous samples are more commonly obtained and convenient to evaluate in the clinical setting.

As mentioned previously, O₂Ct and O₂Cap are calculated using the formulas is 1.39 (Hb x SO₂%) + 0.003 x PO₂ and 1.39 x (O₂Hb%+HHb%)/100 x [tHb], respectively. In this study, both O₂Ct and O₂Cap were significantly higher in Greyhounds compared to non-Greyhounds. This is directly caused by the higher Hb content found in RRGs, since
both formulas contain this parameter. These increases could also be consequence of the high affinity Hb and stronger binding between Hb and O₂.

It is unclear how an athletic breed like the Greyhound benefits from a low $P_{50}$. Recent studies on hemoglobin-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 pre-capillary sphincter, rather than in the capillary bed. 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 (i.e. through arteriolar circulation), and should therefore be released at a deeper tissue level (i.e. capillaries), where oxygen tension is lower. This may allow delivery of oxygen to the tissues which need it most (i.e. muscles); this should be beneficial during strenuous exercise. Although counterintuitive to traditional wisdom, these mechanisms could explain the benefits of having a high-affinity Hb in an athletic breed like Greyhounds.

This study has some limitations. Sample handling could have influenced venous SO₂%, $P_{CO₂}$ and $P_{O₂}$. However, all samples were handled and processed by the same operator, and SO₂% and $P_{O₂}$ in Greyhounds are still significantly higher than in non-Greyhounds. The reason for the high SO₂% may be that the high oxygen affinity Hb (low $P_{50}$) found in Greyhounds allows oxygen to remain bound to oxygen their Hb. This higher SO₂% could also be due to the collection method, and the delay in processing the sample, that would result in equilibration with atmospheric air, and therefore an increase in $O₂$ and decrease in $CO₂$ levels. It is also important to note that, although the blood gas
analyzer and cooximeter are widely used in hospitals and emergency practices.\textsuperscript{43-45} Validation for their use in dogs has not been reported. To our knowledge, the only parameter that has been validated in vitro is oxygen saturation (SO\textsubscript{2}%).\textsuperscript{46}

The blood gas and cooximetry results in Greyhounds, in combination with the previous findings of decreased cooperative binding of Hb,\textsuperscript{8} indicate that Greyhounds may have a unique structural variation in the Hb molecule that makes oxygen transport more effective for a breed that requires maximum muscle contraction in a short period of time.
Table 1: Venous cooximetry and blood gas values (mean ± SD) in Greyhounds and non-Greyhounds.

<table>
<thead>
<tr>
<th></th>
<th>GREYHOUNDS (G)</th>
<th>NON-GREYHOUNDS (NG)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hct (%)</strong></td>
<td>51.70 (3.91)</td>
<td>46.13 (2.78)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.414 (0.033)</td>
<td>7.400 (0.032)</td>
<td>P=0.0344</td>
</tr>
<tr>
<td><strong>P O₂ (mmHg)</strong></td>
<td>60.29 (12.01)</td>
<td>52.07 (8.74)</td>
<td>P=0.0014</td>
</tr>
<tr>
<td><strong>P CO₂ (mmHg)</strong></td>
<td>32.75 (3.56)</td>
<td>34.51 (4.92)</td>
<td></td>
</tr>
<tr>
<td><strong>SO₂ (%)</strong></td>
<td>89.18 (5.29)</td>
<td>77.05 (11.35)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>tHb (g/dL)</strong></td>
<td>21.53 (1.72)</td>
<td>18.16 (1.56)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>O₂ Hb (%)</strong></td>
<td>86.51 (5.45)</td>
<td>75.41 (10.35)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>COHb (%)</strong></td>
<td>2.40 (0.77)</td>
<td>2.47 (1.03)</td>
<td></td>
</tr>
<tr>
<td><strong>MetHb (%)</strong></td>
<td>0.43 (0.4)</td>
<td>0.53 (0.49)</td>
<td></td>
</tr>
<tr>
<td><strong>HHb (%)</strong></td>
<td>10.77 (5.19)</td>
<td>21.36 (9.3)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>P 50 (mmHg)</strong></td>
<td>26.54 (0.39)</td>
<td>29.89 (4.27)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>O₂ Ct (mL/dL)</strong></td>
<td>25.86 (3.00)</td>
<td>18.96 (2.82)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>O₂ Cap (mL/dL)</strong></td>
<td>28.96 (2.55)</td>
<td>24.35 (2.08)</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

*Data not normally distributed, presented as medians (interquartile range)
Figure 2: Hemoglobin P50 values in healthy Greyhounds (G) and non-Greyhounds (NG).

Table 2: Reference intervals for venous cooximetry and blood gas analysis for Greyhounds and non-Greyhounds.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Greyhounds</th>
<th>Non-Greyhounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>43.9 – 59.5</td>
<td>40.6 – 51.7</td>
</tr>
<tr>
<td>PO2 (mmHg)*</td>
<td>36.3 – 84.3</td>
<td>34.6 – 69.6</td>
</tr>
<tr>
<td>PCO2 (mmHg)</td>
<td>25.6 – 39.9</td>
<td>24.7 – 44.4</td>
</tr>
<tr>
<td>SO2 (%)</td>
<td>78.6 – 99.8</td>
<td>54.4 – 99.8</td>
</tr>
<tr>
<td>tHb (g/dL)*</td>
<td>18.1 – 25.0</td>
<td>15.0 – 21.3</td>
</tr>
<tr>
<td>O2Hb (%)</td>
<td>75.6 – 97.4</td>
<td>54.7 – 96.1</td>
</tr>
<tr>
<td>COHb (%)</td>
<td>0.9 – 3.9</td>
<td>0.4 – 4.5</td>
</tr>
<tr>
<td>MetHb (%)</td>
<td>0.0 – 2.2§</td>
<td>0.1 – 2.8§</td>
</tr>
<tr>
<td>HHb (%)</td>
<td>0.4 – 21.2</td>
<td>2.7 – 40.0</td>
</tr>
<tr>
<td>P50 (mmHg)*</td>
<td>26.0 – 28.4§</td>
<td>21.4 – 38.4</td>
</tr>
<tr>
<td>O2Ct (mL/dL)*</td>
<td>19.7 – 32.0</td>
<td>13.3 – 24.6</td>
</tr>
<tr>
<td>O2Cap (mL/dL)*</td>
<td>23.8 – 34.1</td>
<td>20.2 – 28.5</td>
</tr>
</tbody>
</table>

*Parameters showing significant differences between the two groups (Greyhound and non-Greyhound dogs).
§ Data non-normally distributed. Reference intervals expressed as observed ranges.
CHAPTER 3: COMPLEMENTARY EXPERIMENTS

3.1. Effects Of Blood Viscosity On $P_{50}$ Cooximetry Results

We recently determined reference intervals for blood gases and cooximetry in RRGs [Chapter 2]. The $P_{50}$ value was within a narrow range, from 26.42 to 26.65 mmHg in RRGs, and had low coefficient of variation (CV) compared to other breeds (28.3 to 31.49 mmHg). Due to the Greyhounds’ high mean Hct (51.70 ± 3.92%), we postulated that the associate increase in blood viscosity could have affected the cooximetry results by impairing a constant blood flow through the analyzer. This study was designed to determine the effect of blood viscosity on the $P_{50}$ determinations.

Jugular venous samples from 13 RRGs were obtained following the same protocol used for previous study [Chapter 2]. Samples were divided into two heparinized tubes, and PBS was added to one of the aliquots, diluting the sample by 20%, in order to obtain a hematocrit (Hct) within the reference range for non-Greyhound dogs (after dilution, 39.77 ± 2.68 %). Both undiluted and diluted samples were analyzed at the same time using the Nova CCX. The $P_{50}$ was also calculated manually, using the formula provided in the user’s manual. Differences between Greyhound (G), and Greyhound diluted (Gd) groups, and between manual and instrument calculations of $P_{50}$ were assessed using paired T-test.
The mean Hct decreased after dilution (51.97% versus 39.77%). There were no significant differences between G (26.51 ± 0.16) and Gd (26.34 ± 0.16). Calculated P_{50} was lower (p<0.0001) than measured P_{50}, in both undiluted (mean, 23.55mmHg) and diluted (mean, 23.22mmHg) samples, and had higher CV.

We concluded that the results obtained in the previous study were not affected by the high viscosity of the sample. Unexpectedly, when calculated manually, the distribution and values of P_{50} were significantly different from instrument calculated P_{50}.

3.2. Effects of storage on blood gases (and concretely P_{50} value) in RRGs

This study was design in order to evaluate the effect of storage on blood gas and cooximetry analysis, specifically on the P_{50} value. Two venous blood samples were drawn from each Greyhound (n=19), using a plastic syringe, and immediately placing the blood into a heparinized tube. One of the tubes was analyzed immediately (within 5-20 minutes) and the other was stored at 4°C for 3 weeks prior analysis. Then, results obtained from the analyzer before and after storage were compared statistically using a paired T-test.

Overall, the samples experienced the expected changes after being stored. The effects of storage on blood gas and cooximetry values are shown in Table 3. There was a significant decrease in pH, PO_{2}, SO_{2}, O_{2}Hb, COHb and O_{2}Ct, and a significant increase in HHct, PCO_{2} and HHb. After 3 weeks of storage, the mean P_{50} decreased (mean ± SD before storage 26.54 ± 0.22 mmHg; mean ± SD after storage 22.83 ± 4.67 mmHg).
However, since the population was not normally distributed, it is better assessed by the median (interquartile range, IQR), that surprisingly was slightly higher [26.6 (8.4)]. The distribution of the values followed two trends, as can be observed in figure 3; nine of the samples registered the same value before and after 3 weeks of storage. When the $P_{50}$ was calculated manually using the formula provided by the manufacturer, the mean $P_{50}$ depletion was more pronounced, decreasing from $24.15 \pm 1.97$ mmHg [mean $\pm$ SD], to $12.62$ (0.68) mmHg [median, IQR], with a different distribution of the data and a CV (Figure 4).

The Nova CCX analyzer reflected the expected changes in blood values after storage, mostly caused by RBC membrane alteration and RBC metabolism. However, in half of our sampling population the $P_{50}$ remained unchanged from baseline, which is physiologically inexplicable. Since 2,3-DPG decreases quickly during storage, $P_{50}$ should decrease accordingly (although the anticoagulant used was different than that used for blood storage). In our study, the mean $P_{50}$ decreased, but when the data were plotted, more than half of the values remained unchanged after three weeks (Figure 3). On the other hand, when the $P_{50}$ was calculated manually, all the values decreased to almost half of the original value.
Figure 3: Nova CCX $P_{50}$ results before (T0) and after (post 3W) 3 weeks of storage.

Figure 4: Manually calculated $P_{50}$ values before (T0) and after (post 3W) 3 weeks of storage.
Table 3. Blood gases and cooximetry values immediately after sampling and 3 weeks after storage, expressed as mean ± SD in retired racing Greyhounds.

<table>
<thead>
<tr>
<th></th>
<th>PRE-STORAGE</th>
<th>POST-STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%) *</td>
<td>51.00 ± 3.65</td>
<td>58.89 ± 4.24*</td>
</tr>
<tr>
<td>pH *</td>
<td>7.401 ± 0.025</td>
<td>7.132 ± 0.035*</td>
</tr>
<tr>
<td>PCO₂ (mmHg) *</td>
<td>34.43 ± 4.04</td>
<td>61.24 ± 6.97*</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>64.96 ± 15.45</td>
<td>42.80 (10.1)§</td>
</tr>
<tr>
<td>tHb (g/dL)</td>
<td>21.56 ± 1.66</td>
<td>21.40 ± 1.38</td>
</tr>
<tr>
<td>SO₂ (%) *</td>
<td>91.94 ± 4.64</td>
<td>83.91 ± 8.06*</td>
</tr>
<tr>
<td>O₂Hb (%) *</td>
<td>90.31 ± 4.82</td>
<td>82.76 ± 7.73*</td>
</tr>
<tr>
<td>COHb (%) *</td>
<td>1.45 ± 0.482</td>
<td>1.94 ± 0.63*</td>
</tr>
<tr>
<td>MetHb (%)</td>
<td>0.35 ± 0.16</td>
<td>0.30 (0.45)$</td>
</tr>
<tr>
<td>HHb (%) *</td>
<td>7.89 ± 4.52</td>
<td>14.88 ± 7.24*</td>
</tr>
<tr>
<td>P₅₀ (mmHg) *</td>
<td>26.54 ± 0.22</td>
<td>26.6 (8.4)$</td>
</tr>
<tr>
<td>P₅₀ calculated (mmHg) *</td>
<td>24.15 ± 1.97</td>
<td>12.62 (0.68)$</td>
</tr>
<tr>
<td>O₂Ct (mL/dL) *</td>
<td>27.06 ± 2.13</td>
<td>24.39 ± 2.55*</td>
</tr>
<tr>
<td>O₂Cap (mL/dL) *</td>
<td>29.10 (3.8)$</td>
<td>29.09 ± 1.80</td>
</tr>
</tbody>
</table>

* Significant differences (p<0.05) from pre-storage values.

§ Data non normally distributed, expressed as median (interquartile range).

3.3. Hemoglobin structure and oxyhemoglobin dissociation curve

The Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA) is a device that accurately records ODCs during deoxygenation with nitrogen gas, and plots the curve obtained on graph paper. This analyzer detects the oxygen tension by a Clarke electrode, while the oxyhemoglobin fraction (%HbO₂) is evaluated by a dual-wavelength spectrophotometer. This instrument provides a simple, quick and reliable method for recording the ODC, providing information about the delivery of oxygen to the tissues. Although laboratories are usually equipped with blood gas analyzers and cooximeters for
the determination of these values, the advantages of the Hemox-Analyzer are stability of the measurements and the reproducibility of the results.48

Two healthy adult dogs (one Greyhound and one mixed-breed) were used for this study; 6 ml of blood were drawn from the jugular vein in each dog, and immediately placed into two 3 ml heparinized tubes. The samples were drawn at the Ohio State Veterinary Medical Center (OSUVMC), and one sample of each dog was immediately placed in a box with ice packs and transported to Dr. Andre Palmer’s Lab, in the Department of Chemical and Biomolecular Engineering, The Ohio State University. The remaining two samples were submitted to the clinical Laboratory at OSUVMC for blood gas and cooximetry, using a Nova CCX.

The ODC of the Greyhound and the non-Greyhound/mixed breed (figure 4) indicated that the Greyhound has lower \( P_{50} \) than the non-Greyhound. \( P_{50} \) was also determined manually, using the formula given in the manual of the blood gas analyzer (Nova CCX). In table 5, \( P_{50} \) values from the Hemox-Analyzer, the blood gas analyzer (Nova CCX), and the manually calculated \( P_{50} \) using the formula are compared.
Figure 5. Oxyhemoglobin dissociation curve (ODC) determined by the Hemox-Analyzer for venous blood from a Greyhound (blue) and a non-Greyhound (red).

Table 4. Blood gas and cooximetry values obtained from Greyhounds and non-Greyhounds determined by the Clinical Laboratory at the OSUVMC.

<table>
<thead>
<tr>
<th></th>
<th>Greyhounds</th>
<th>non-Greyhounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.432</td>
<td>7.411</td>
</tr>
<tr>
<td>pCO₂</td>
<td>32.4</td>
<td>34.5</td>
</tr>
<tr>
<td>pO₂</td>
<td>43.8</td>
<td>51.4</td>
</tr>
<tr>
<td>Hct</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>tHb</td>
<td>21.7</td>
<td>19.8</td>
</tr>
<tr>
<td>SO₂</td>
<td>84</td>
<td>87.4</td>
</tr>
<tr>
<td>O₂Hb</td>
<td>*</td>
<td>86</td>
</tr>
<tr>
<td>COHb</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>MetHb</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>HHb</td>
<td>15.6</td>
<td>12.4</td>
</tr>
<tr>
<td>P₅₀</td>
<td>26.6</td>
<td>26.6</td>
</tr>
<tr>
<td>O₂Ct</td>
<td>25.5</td>
<td>23.7</td>
</tr>
<tr>
<td>O₂Cap</td>
<td>29.4</td>
<td>27.1</td>
</tr>
</tbody>
</table>

*Data missing
Table 5. Comparison between the $P_{50}$ value obtained from the Hemox-Analyzer, the critical care blood gas analyzer with cooximeter, and the manual calculation, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Greyhound</th>
<th>non-Greyhound</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{50}$ Hemox-Analyzer</td>
<td>20.21</td>
<td>24.82</td>
</tr>
<tr>
<td>$P_{50}$ Nova CCX</td>
<td>26.6</td>
<td>26.6</td>
</tr>
<tr>
<td>$P_{50}$ calculated</td>
<td>23.71</td>
<td>25.1</td>
</tr>
</tbody>
</table>

Although two isolated samples are not representative, we can observe the lower $P_{50}$ in the Greyhound sample (and therefore demonstrate that Greyhound Hb has a higher affinity for the oxygen). The comparison of ODC among Greyhounds and non-Greyhounds was already done by Sullivan et al in 1994 with a similar method, so this higher affinity is already demonstrated. Unexpectedly, in our study we observed that the Nova CCX analysis performed in the same samples does not reflect accurately this oxygen affinity, since we obtained the exact same value in both samples (26.6 mmHg). The formula used for the calculation of the $P_{50}$ value contains the SO$_2$%. According with the manufacturer, this SO$_2$% should not be above 80% in order to calculate $P_{50}$, and in these two samples the SO$_2$% was >80%. This finding, along with low coefficient of
variation previously discussed, supports the notion that the instrument might default $P_{50}$ when it detects any abnormalities in the remaining values (presumably higher $SO_2\%$ than expected)
REFERENCES


