Comparison of measured oxyhemoglobin saturation and oxygen content with analyzer-calculated values and hand-calculated values obtained in unsedated healthy dogs

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Objective—To compare direct measurements of canine oxyhemoglobin (HbO\(_2\)) saturation and blood oxygen content (ContO\(_2\)) in healthy dogs with analyzer-calculated values derived by use of a human HbO\(_2\) relationship and with hand-calculated values derived by use of a canine HbO\(_2\) relationship.

Animals—17 healthy dogs.

Procedure—3-mL samples of heparinized arterial and jugular venous blood were collected from each dog. The pH, PCO\(_2\), PO\(_2\), hemoglobin, HbO\(_2\), carboxyhemoglobin, methemoglobin, and ContO\(_2\) were measured; HbO\(_2\) and ContO\(_2\) were calculated automatically by analyzers and also hand-calculated. Blood gas analyzer-calculated and hand-calculated HbO\(_2\) values were compared with co-oximeter–measured HbO\(_2\) values. Analyzer-calculated and hand-calculated ContO\(_2\) values were compared with oxygen content analyzer-measured values.

Results—Hand-calculated HbO\(_2\) values for arterial and jugular venous samples were slightly but significantly lower than those calculated by a blood gas analyzer or obtained from a co-oximeter. Hand-calculated and analyzer-calculated arterial and venous ContO\(_2\) were similar to measured values.

Conclusions and Clinical Relevance—Although certain HbO\(_2\) and ContO\(_2\) values generated by use of the different methods were significantly different, these differences are unlikely to be clinically important in healthy dogs. (Am J Vet Res 2005;66:1273–1277)

The amount of oxygen in blood is an important index of lung function. There are 3 ways to quantify the amount of oxygen in the blood: the oxygen tension in plasma (Po\(_2\)), the percentage of hemoglobin that is oxyhemoglobin (HbO\(_2\)), and the content (concentration) of oxygen (ContO\(_2\)) in the blood. Most commonly, Po\(_2\) is measured with a blood gas analyzer, and HbO\(_2\) and ContO\(_2\) are calculated from the measured Po\(_2\).

Analyzer algorithms that calculate HbO\(_2\) use a normal human HbO\(_2\) dissociation curve. The canine HbO\(_2\) dissociation curve is similar to that of humans. The canine hemoglobin (Hb) Po\(_2\) (the Po\(_2\) at which the Hb is 50% saturated with oxygen) has been reported\(^5\) to be from 26 to 31.5 mm Hg, whereas that of humans has been reported\(^6,10\) to be from 26 to 29 mm Hg. The assumption has been that algorithms based on the human HbO\(_2\) dissociation curve are sufficient for predicting the canine HbO\(_2\) dissociation curve.

The HbO\(_2\) and ContO\(_2\) can be directly measured in canine blood. The purpose of this study was to compare these direct measurements with analyzer-calculated values derived by use of a human HbO\(_2\) dissociation curve and with hand-calculated values derived by use of a canine HbO\(_2\) dissociation curve in healthy dogs.

Materials and Methods

Seventeen unsedated healthy dogs were studied. Dogs were owned by University of California, Davis, veterinary technicians and students who provided informed consent for the involvement of their dogs in this study. Breed, age, sex, body weight, and rectal temperature were recorded for each dog. The dead space and needle hub of 3-mL syringes were filled with liquid sodium heparin (1,000 U/mL). Paired, simultaneous 3-mL blood samples were collected from the dorsal metatarsal artery and jugular vein of each dog. Air was immediately evacuated from the syringe, which was capped with an airtight seal. Samples were stored in ice water.

The blood samples were analyzed by a blood gas and electrolyte analyzer\(^7\) (analizer A) that measures pH, PCO\(_2\), Po\(_2\), sodium, potassium, chloride, calcium, glucose, and lactate concentrations and calculates bicarbonate, standard base excess, HbO\(_2\) saturation, and ContO\(_2\); a co-oximeter\(^4\) (analyzer B) that measures Hb, HbO\(_2\), carboxyhemoglobin (COHb), and methemoglobin (metHb) by 6-wavelength spectrophotometry by use of canine Hb absorption coefficients and calculates ContO\(_2\) and functional HbO\(_2\); and an oxygen content analyzer\(^2\) (analyzer C) that measures total ContO\(_2\). All analyses were performed in duplicate. If a value differed by >10%, a third analysis was performed, and the discordant value was discarded. Blood gas values were measured at 37ºC and were temperature-corrected to that of the dog at the time of sampling. All samples were processed through analyzers A and B within 30 minutes of sample collection. All samples were processed through analyzer C within 120 minutes of sample collection and within 90 minutes of processing through analyzers A and B. Analyzer C measurements were adjusted for in vitro storage changes in mea-
sured ContO2 over time (+0.05 mL/dL/h); time of analyzer A and B measurements was considered time 0.

The hand-calculated HbO2 was calculated from PO2 by the 3-coefficient formula proposed by Reeves et al4 as follows:

\[
\text{HbO2} = \left(\frac{38,848}{(202 \times \text{PO2}) + (1.17 \times \text{PO2}^2) + \text{PO2}^3}\right) + 1\times 100.
\]

The Bohr coefficient corrections suggested by Reeves et al4 were applied to the measured PO2 to generate an apparent PO2 for inclusion into the first formula as follows:

\[
\text{PO2}_{\text{app}} = \left(\frac{200}{\text{Pb} - 50}\right) + 1\times 10.
\]

Arterial and jugular venous blood oxygen contents (ContO2 and ContO2, respectively) were hand-calculated by use of the following formula:

\[
\text{ContO2} = (1.39 \times \text{Hb} \times \text{HbO2}) + (0.003 \times \text{PO2}),
\]

where HbO2 was the hand-calculated HbO2 value and Hb was the analyzer-calculated Hb concentration. Arterial-venous oxygen content difference (a-v ContO2) was calculated by subtracting the analyzer A–measured PO2 from the calculated PAO2. The hand-calculated PO2 was calculated from PO2 by use of the following formula:

\[
\text{PO2} = \left(200 - 3.9\times(\text{Pb} - 50)\times0.21\right) - 3.1\text{PO2}(1.1),
\]

where Pb is barometric pressure (millimeters of mercury); 50 is the partial pressure of water vapor (millimeters of mercury) at 38.5°C; 0.21 is the fraction of atmospheric oxygen; and 1.1 is 1/RQ, where RQ is assumed to equal 0.9. The alveolar-arterial PO2 difference (A-a gradient) was calculated by subtracting the analyzer A–measured PO2 from the calculated PO2. The barometric pressure on the day of this study was 763 mm Hg.

For calculating venous admixture (Qs/Qt), end-pulmonary capillary PO2 was assumed to equal PO2. End-pulmonary capillary HbO2 was calculated from end-pulmonary capillary PO2 with the formula of Reeves et al4, as described. End-pulmonary capillary ContO2 (ContO2) was calculated by use of the content formula, as described. Venous admixture was calculated by use of the following formula:

\[
(\text{ContO2} - \text{ContO2})(\text{ContO2} - \text{ContO2}).
\]

Statistical analyses—Means and SDs were determined for the arterial and venous sets of data. The hand-calculated and analyzer-calculated HbO2 were compared with the HbO2 obtained from analyzer B for each arterial and jugular venous sample by ANOVA for repeated measures.5 The hand-calculated and analyzer-calculated ContO2 values were compared with the measured ContO2 values obtained from analyzer C. When a significant effect was found, the Bonferroni (all pairwise) multiple comparison test was used to determine significant differences; differences were considered significant at P < 0.05.

Results

Arterial and venous blood gas and Hb variables obtained from the 3 analyzers for 17 adult healthy dogs were tabulated (Table 1). All variables other than Hb were significantly different between arterial and jugular venous samples. Significant differences were detected among arterial HbO2 values that were hand-calculated, calculated by the factory-programmed algorithm of analyzer A, and obtained from analyzer B (Table 2). The hand-calculated HbO2 values were significantly lower than those calculated by analyzer A or analyzer B for arterial and jugular venous samples.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Variable</th>
<th>Arterial blood</th>
<th>Jugular venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>pH</td>
<td>7.41 ± 0.02*</td>
<td>7.38 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>PO2 (mm Hg)</td>
<td>34.8 ± 2.7</td>
<td>40.0 ± 2.9*</td>
</tr>
<tr>
<td></td>
<td>PO2 (mm Hg)</td>
<td>101.1 ± 6.2</td>
<td>43.0 ± 8.3*</td>
</tr>
<tr>
<td>B</td>
<td>Hb (g/dL)</td>
<td>16.1 ± 1.5</td>
<td>16.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>HbO2 (%)</td>
<td>98.2 ± 0.6</td>
<td>72.1 ± 11.2*</td>
</tr>
<tr>
<td></td>
<td>COHb (%)</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>metHb (%)</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.1*</td>
</tr>
<tr>
<td>C</td>
<td>ContO2 (mL/dL)</td>
<td>21.9 ± 2.3</td>
<td>15.9 ± 3.5*</td>
</tr>
</tbody>
</table>

*Significantly different from arterial blood (P < 0.05). Reported value is percentage saturation of functional or available Hb; analyzer B measures percentage saturation of total Hb and subtracts carboxyhemoglobin (COHb) and methemoglobin (metHb) values to obtain this value.

HbO2 = Hb saturated by O2.

**Within a column, values with different superscript letters differ significantly (P = 0.01).

See Table 1 for remainder of key

Table 1—Mean ± SD values for pH, blood gases, total hemoglobin (Hb) concentration, Hb species, and oxygen content (ContO2) in arterial and venous blood of 17 healthy dogs as determined by use of 3 analyzers.

Table 2—Mean ± SD ContO2 (mL/dL) of arterial and jugular venous blood in 17 healthy dogs as determined by use of 4 methods.

Table 3—Mean ± SD PO2 (mm Hg) and PO2 in 17 healthy dogs as determined by use of 4 methods.

Table 4—Mean ± SD HbO2 (%) in 17 healthy dogs as determined by use of a factory-programmed algorithm in analyzer A, a cooximeter (analyzer B), or hand-calculated by use of a formula with Bohr coefficient corrections.

Discussion

Arterial and jugular venous blood gases were comparable to reported12-25 blood gas values in healthy dogs. Few reports of metHb and COHb values for dogs exist; the results from our study were similar to published values for dogs26 and humans.27

Each of the methods for determining arterial HbO2 yielded values that were significantly different; however, all values were within reference ranges, and differences were not considered clinically important in the healthy dogs studied. The hand-calculated method
yielded the lowest mean HbO2 value for arterial and jugular venous blood. The differences between hand-calculated values and HbO2 values obtained by use of the other 2 methods were more pronounced in the jugular venous samples than in the arterial samples. The relationship between PO2 and HbO2 in venous blood falls on the steep portion of the sigmoidal HbO2 dissociation curve, so small differences in PO2 equate to large differences in HbO2. This relationship also explains the greater intragroup variability detected in jugular venous samples, compared with arterial samples.

Although the differences in HbO2 values were not considered clinically relevant in these dogs, there are several issues relevant to the derivations of these HbO2 values in sick dogs that may be important when these HbO2 values are used to calculate variables such as oxygen content, delivery, and consumption. The lower HbO2 values derived via the hand-calculated method may be largely explained by differences in P50. The canine data provided by Reeves et al28 and used to generate the formula had a P50 of 31.5 mm Hg, whereas the HbO2 value from analyzer A was calculated with the assumption of a human P50 of 26.84 mm Hg.29 This canine HbO2 dissociation curve is therefore shifted to the right of the human curve, which would explain the lower hand-calculated arterial and venous HbO2 values in the dogs reported here. A formula based on data with a P50 of 26.84 mm Hg would have generated higher hand-calculated values that would have been closer to the HbO2 values calculated by analyzer A or measured by analyzer B.

Although the P50 reported by Reeves et al28 was higher than in other reports, that formula was used in the present study because it provided the most complete set of data across the range of PO2 values of interest. Rossing and Cain’s formula,3 a canine nomogram with a P50 of 29.8 mm Hg at a body temperature of 38.7°C, generated a mean calculated jugular venous HbO2 of 71.7% for the dogs reported here. This value was not significantly different from HbO2 values obtained from analyzers A and B; however, Rossing and Cain’s formula3 generated a much lower mean calculated arterial HbO2 (93.9%), compared with those obtained from analyzers A and B (Table 2), and therefore was not used in the present study. A relationship estimated from data of Bartels and Harms,28 with a P50 of 29.1 mm Hg, generated a mean calculated jugular venous HbO2 of 76.8% for the dogs reported here. This value was higher than HbO2 values obtained from analyzers A and B. Additionally, their data did not include PO2 values in the range of those of the arterial blood samples of the present study.

Another reason for differences among values obtained via analyzers A and B and hand-calculated HbO2 values in this study was that they did not represent the same value. One may presume that because analyzer B actually measures HbO2, it would provide the gold standard of these techniques for determining HbO2, but the value displayed by the analyzer is a calculated value for functional Hb rather than the measured fractional HbO2. The co-oximeter (analyzer B) measures total Hb concentration and 3 Hb species (HbO2, metHb, and COHb). The metHb and COHb values are subtracted from the total Hb value, and the HbO2 that is displayed is a percentage of the remaining available Hb; this is functional HbO2.30 Functional Hb is more precisely correlated with PO2 than fractional Hb and provides a better assessment of lung function. The difference between functional and fractional HbO2 could be important in a clinical setting if there are high concentrations of COHb or metHb. Calculation of HbO2 by use of the hand-calculated method makes no such allowance for abnormal Hb molecules. This method calculates HbO2 as a percentage of total Hb; this is fractional Hb. In patients with normal quantities of COHb and metHb, functional HbO2 will be only slightly higher than fractional HbO2 and the difference will be negligible; however, in patients with high concentrations of abnormal Hb, the difference will be proportionately greater. In the present study, the fractional HbO2 of arterial blood as measured (but not displayed) by analyzer B was 96.5 ± 6.6%, a value that is nearly the same as the hand-calculated fractional HbO2 value.

The programmed algorithm of analyzer A assumes fixed fractions of metHb and COHb (0.004 [0.4%] for each) in its calculation of HbO2.29; the result is an approximation of functional HbO2. Blood gas analyzer–calculated HbO2 will underrepresent functional HbO2 when the concentrations of abnormal Hb are higher than the default values and will always overrepresent hand-calculated fractional HbO2 calculations, which do not make adjustments for abnormal Hb molecules. This explains why arterial and jugular venous HbO2 values calculated by analyzer A were lower than the functional HbO2 values reported by analyzer B and greater than the hand-calculated fractional HbO2 values in this study.

Functional and fractional HbO2 are important conceptually because they provide different perspectives of a patient’s oxygenation status. Functional HbO2 is better correlated to PO2 and provides a more relevant measure of pulmonary function and blood oxygenation. Fractional HbO2 is more relevant when calculating ContO2 because this value accounts for metHb and COHb, which do not bind oxygen. The dogs in this study had little metHb or COHb, and under these conditions, there is little difference between fractional and functional HbO2; either value would result in the same clinical assessment. However, abnormal Hb species may be in high concentrations in sick dogs. Under such conditions, if one relied solely on the analyzer B–reported functional HbO2, one could be led to believe that total Hb saturation and ContO2 were higher than they actually were. Similar misinformation could result from analyzer A–calculated HbO2 values derived with a fixed default for metHb or COHb.

Hand-calculated and analyzer A–calculated ContO2 matched measured values for arterial blood; the calculated ContO2 from analyzer B was slightly but significantly lower. The latter result may be because the programmed algorithm of analyzer B calculates ContO2 by a formula (1.39 × Hb × fractional HbO2) that excludes dissolved oxygen. The calculated blood ContO2 values from analyzers A and B matched the
measured value from analyzer C. The hand-calculated value was slightly but significantly lower, an obligatory effect of the lower hand-calculated jugular venous HbO₂ values. Values ranging from 1.30 to 1.39 mL/L have been used to represent the maximal volume of oxygen that can combine with 1 g of Hb. The factor 1.39 was used in the present study because it provided the best match with analyzer C–measured values. The significant differences between measured and calculated ContO₂ and ContO₂ values for the present study were not considered to be clinically important. Considering that each of these methods used a different formula and different HbO₂ values to calculate ContO₂, the results are remarkably similar.

Combinations of individual variables are often used to calculate other variables that provide important insights into a patient’s condition. For example, a-v ContO₂ difference helps quantify oxygen extraction by the tissues. Differences between calculated and measured ContO₂ values, if they did exist, might not be important if variables calculated from them (such as a-v ContO₂ difference) were consistent between different methods. However, the a-v ContO₂ differences in the present study were significantly different among the various methods of deriving ContO₂, despite our conclusion that the ContO₂ values themselves were not importantly different. The a-v ContO₂ derived from analyzer B–calculated ContO₂ was 5.5 mL/L, compared with 6.5 mL/L for the hand-calculated ContO₂. The second value is 20% higher, and this difference may be clinically important. Sequential measurements on the same patient by use of different analyzers or calculations made by use of different formulas should be interpreted with caution.

The differences discussed here underscore the importance of use of the same method of measurement or calculation when comparing studies, patients, or trends in 1 patient. Similar precaution should be applied to combining values derived by different analyzers and formulas to calculate a third variable (for example, deriving a-v ContO₂ difference from a blood gas analyzer–calculated ContO₂ and a co-oximeter–calculated venous ContO₂). This problem became apparent when an attempt was made to calculate Qs/Qt by use of ContO₂ and ContO₂ values calculated by analyzer A or B or measured by analyzer C in conjunction with a hand-calculated ContO₂ (a value that cannot be measured); ContO₂ values calculated in the present study were lower than analyzer-calculated or analyzer-measured ContO₂. This generated negative numbers for Qs/Qt, a physiologic impossibility. Believable Qs/Qt values were only achieved when ContO₂, ContO₂, and ContO₂ were all calculated by hand with the same formula.

We concluded that although there were significant differences, blood gas analyzer–calculated and hand-calculated HbO₂ were similar to values obtained from a co-oximeter, and differences were partly the result of each method of derivation being fundamentally different from the others. Blood gas analyzer–calculated, hand-calculated, and co-oximeter–calculated ContO₂ values were similar to measured values. Again, although some of the values were significantly different, the differences were not clinically important. No single method for deriving HbO₂ or ContO₂ that was examined in this study was better than the others; however, comparisons between values derived by different methods should be avoided.

The findings of this study apply only to healthy unsedated dogs. Changes in the P₅₀ or the slope of the HbO₂ dissociation curve or increases in concentrations of COHb and metHb would be expected to introduce clinically important differences among results of these methods, and the assumptions in most available formulas would no longer be valid.

References

19. Ilkki JE, Rose RJ, Martin IC. A comparison of simultane-


