Glycosylated Hemoglobin Measurement in Dogs and Cats: Implications for its Utility in Diabetic Monitoring

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SUMMARY
The measurement of glycosylated hemoglobin (HbA1c) levels in humans is used to indicate the degree of long-term diabetic control. Using a commercially available kit for human HbA1c, values were obtained for normal and diabetic dogs and cats. The normal range established in dogs was broad and overlapped considerably with the range in diabetics. Under the assay conditions and with a limited number of diabetic animals, the test was not found to be of value for dogs or cats.

RÉSUMÉ
L’utilité de la détermination de la concentration de l’hémoglobine A1c, pour une vérification suivie du diabète sucré, chez les chiens et les chats
La détermination de la concentration de l’hémoglobine A1c sert à effectuer une vérification suivie du diabète, en médecine humaine. En utilisant une trousse disponible sur le marché, pour la détermination de cette hémoglobine, les auteurs obtiennent des valeurs pour des chiens et des chats, tant normaux que diabétiques. Les valeurs obtenues chez les chiens normaux affichaient un large éventail et se confondaient en grande partie avec celles des sujets diabétiques. En tenant compte des conditions de leur expérience et du nombre relativement restreint d’animaux diabétiques qu’ils utilisèrent, les auteurs concluent à l’inutilité de ce test, pour les chiens et les chats.

INTRODUCTION
Diabetes mellitus is a common endocrinopathy of small animals, as well as of man. Clinically diagnosed diabetic dogs have been estimated as comprising 0.5% of the population, which appears to be four times the incidence of the disease in cats (1). The availability of insulin and the technical ease of therapy provide the means for increasing the life expectancy of affected animals. However, frequent evaluation of glucose status is required and such testing can present an economic hurdle to the client and an interpretative problem to the clinician. A reliable means of monitoring long-term control of blood glucose in diabetics has long been sought. A method which would also eliminate confounding factors such as age, diet, exercise, patient excitability, type and duration of therapy and concomitant disorders at the time of determination would be advantageous.

Hemoglobins in mammals can be separated into various major and minor components. One of these, hemoglobin A1c (HbA1c), is a glycosylated fraction which represents 75-80% of the total minor hemoglobin components in man (2). In humans, glucose regulation has been successfully and accurately monitored using the measurement of concentration of HbA1c (3). This particular hemoglobin was first isolated from the blood of normal persons and described in 1958 (4). A decade later it was reported as occurring in increased concentrations in diabetic patients (5,6). Since that time, the characterization of this unique glycoprotein and the refinement of measurement techniques have been the subjects of numerous reports (7-14).

Recently, the feasibility of monitoring canine diabetics by measuring HbA1c levels has been proposed (15,16,17). However, to be clinically practical and cost-effective, such determination would need to be available through a commercial laboratory. The aims of the present study were to establish normal glycosylated hemoglobin values of nondiabetic dogs and cats, and to compare these with those obtained from some diabetic animals. In order to provide data that was of use to the veterinary practitioner, the assays were performed by a commercial laboratory.

MATERIALS AND METHODS
Blood samples were obtained from clinically normal and diabetic dogs and cats by venipuncture. Between 1 and 2 mL of blood from each animal was added to 3 mL vacutubes containing 6.0 mg potassium oxalate and 7.5 mg sodium fluoride. \(^1\) Within one hour of collection, the plasma was harvested and within eight hours the plasma glucose levels were determined by using the standardized glucose hexokinase assay\(^2\) (18). A similar amount of blood was placed in 3 mL vacutubes holding 7.2 mg of tripotassium ethylene diamine tetraacetic acid (EDTA). \(^1\) These samples were used for the determination of the complete blood counts. Within one hour of collection these blood samples were stored at 4°C and within one week glycosylated and total hemoglobin determinations were performed in the

\(^1\) Vacutainer, Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey.

\(^2\) Glucose HK, Diagnostics Division, Fisher Scientific Limited, 184 Railside Road, Don Mills, Ontario.
Department of Pathology laboratory, University of Saskatchewan Hospital, using a commercial kit. In this method, hemoglobin \( A_1 \) was assayed utilizing a column cation exchange procedure on red cell hemolysates. The relative concentrations were determined spectrophotometrically at 415 nm and total glycosylated hemoglobin values were calculated as a percentage of the total hemoglobin level.

The animals sampled were 20 normal and five diabetic dogs (Table I) and 22 normal and three diabetic cats (Table II). The breed, sex, age and clinical status of these animals are presented in Tables I and II.

RESULTS

Dogs

The clinically normal dogs had blood glucose values ranging from 4.39 to 5.72 mmol/L (79 to 103 mg/dL), with a mean of 5.09 mmol/L (91.6 mg/dL) (Table I). Nineteen of the animals demonstrated \( A_1 \) values from 6.2 to 18.6%, with a mean of 10.5% (SD 3.7%). The oldest dog (ten years old) had a \( A_1 \) value of 28.2%, with a blood glucose level of 4.61 mmol/L (83 mg/dL). Fourteen of the dogs had \( A_1 \) values below 12%. There were no trends with respect to age, breed, or sex, and blood glucose concentration did not correlate with the percentage of glycosylated hemoglobin.

The diabetic dogs had blood glucose values from 6.17 to 26.00 mmol/L (111 to 468 mg/dL) and \( A_1 \) determinations from 9.8 to 14.6% (Table I). There was no correlation between blood glucose level and \( A_1 \).

Cats

The clinically normal cats had blood glucose values ranging from 4.11 to 12.09 mmol/L (74 to 216 mg/dL), with a mean of 5.91 mmol/L (106.4 mg/dL) (Table II). Values obtained for glycosylated hemoglobin ranged from 81.6 to 99.2%, with a mean of 90.9% (SD 5.4%). As with the dogs, no trends with respect to age, breed or sex could be established, nor was there any correlation between blood glucose and glycosylated hemoglobin levels.

The diabetic cats had blood glucose levels between 9.89 and 21.94 mmol/L (178 and 395 mg/dL) and glycosylated hemoglobin determinations between 60.8 and 97.0%. No trends were discernible (see Table II).

DISCUSSION

The hemoglobin molecule consists of four polypeptide subunits, which in man are two \( \alpha \) chains and two \( \beta \) chains. Hemoglobin \( A_1c \) is hemoglobin with a glucose moiety attached at the aminoterminus of the \( \beta \) chain. It is formed through an essentially irreversible, nonenzymatic process, which is dependent upon blood glucose concentration. This occurs continuously throughout the life of the erythrocyte. Since the red cell is not insulin-dependent, the extent of glycosylation is directly related to the average blood glucose concentration over an extended period of time. Thus, the measurement of \( A_1c \) provides a time-averaged estimate of blood glucose levels (2,3,20). In human diabetics, the concentration of \( A_1c \) has been found to be approximately twice that in normal individuals. Since other minor components, namely \( A_{1d} \) and \( A_{1e} \), are also elevated in diabetics and may be precursors to \( A_{1c} \) (12,13), determination of total \( A_1 \) may, clinically, be a more useful measurement (2,3). Total \( A_1 \) was the parameter measured in this study.

Our observations regarding normal and diabetic dogs are not consistent with previous reports (15,17,21,22). In particular, the total glycosylated hemoglobin values obtained for our normal dogs was 6.2-28.2%, which is a considerably broader range than that of earlier studies. The \( A_1c \) levels for our diabetic dogs were all within this normal range. One study (15) used a conventional macrocolumn ion exchange chromatographic procedure to measure \( A_1c \) components in seven normal and seven diabetic dogs. They found that mean normal \( A_1c \) concentrations constituted approximately 3% of total hemoglobin concentra-

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex*</th>
<th>Age (years)</th>
<th>Clinical Statusb</th>
<th>Plasma Glucose (mmol/L)</th>
<th>( A_1c )%</th>
</tr>
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<tbody>
<tr>
<td>Labrador Retriever</td>
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<td>6</td>
<td>N</td>
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<td>N</td>
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<td>N</td>
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</tr>
<tr>
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<td>3</td>
<td>N</td>
<td>5.61</td>
<td>8.2</td>
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<td>N</td>
<td>5.67</td>
<td>8.5</td>
</tr>
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<td>N</td>
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<td>9.1</td>
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<td>N</td>
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<td>M</td>
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<td>N</td>
<td>4.61</td>
<td>28.2</td>
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</tbody>
</table>

# = male, F = female, FS = female spayed.

* Normal, ** diabetic, + = iatrogenic hyperadrenocorticism, ++ = moderately well controlled, 
**** = uncontrolled.

tions, whereas this value was signifi-
cantly elevated in diabetics, approxi-
mately 5%. When examined on the
basis of total glycosylated hemoglobins
(HbA1), their data indicate mean
values of approximately 5% and 7%,
respectively. These may be contrasted
to our mean values of approximately
11% for normal dogs and approxi-
mately 13% for diabetic dogs. The
same investigators compared (22) their
chromatographic procedure with a
colorimetric method4 for the meas-
urement of canine glycosylated
hemoglobin levels. The values
obtained from both methods were sig-
ificantly correlated. Another study
(17), in an attempt to find a more prac-
tical assay method, used a com-
commercially available kit; in their veterinary
hospital laboratory, to measure total
HbA1. These workers determined
HbA1 value for 56 nondiabetic and 16
diabetic dogs. The mean HbA1 value
for 40 nondiabetic hospitalized dogs
was 6.43% (range 4.90-9.03%), and for

16 laboratory colony dogs it was
5.62% (range 4.26-7.22%). Their dia-
etic HbA1 values had a mean of
9.63%, with a range of 6.24 to 13.33%.
In both studies there is considerable
overlap of diabetic values within their
given normal ranges (Figure 1).

In our study, all diabetic dogs' HbA1
values lay within the normal range.
Such differences may be attributable
to the chromatographic procedures
employed. The resin columns in all
three studies were supplied by the
same manufacturer, and the phos-
phate eluting buffers were of similar
pH, namely 6.7. However, differences
in technique between laboratories may
account for the three varying ranges
reported above; such problems of reproducibility have been reported in
diabetic studies (14). In essence,
until the technique within and
between laboratories can be standar-
dized, comparison of results must be
made with care. It must be noted that
neither this nor the previous studies
have compared the HbA1 values
within individual animals over
extended periods of time. Nor have
HbA1 levels been followed sequen-
tially in diabetic dogs with respect to
the degree of diabetic control.

The HbA1 values obtained for
normal and diabetic cats were very high
and no discernable trend was detect-
able. There exist no published reports
on glycosylated hemoglobin measure-
ment in this species.

Feline red cells contain two major
hemoglobin components, HbA and
HbB, each of which consists of two
unique α and β chains. The aminoter-
minal sequences of the α chains are
identical to those in other mammals,

TABLE II
SUMMARY OF DATA FROM CLINICALLY NORMAL AND DIABETIC CATS

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical Status</th>
<th>Plasma Glucose (mmol/L)</th>
<th>HbA1 (%)</th>
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<tbody>
<tr>
<td>DSH</td>
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<td>N</td>
<td>4.11</td>
<td>81.6</td>
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<td>N</td>
<td>4.61</td>
<td>83.8</td>
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<tr>
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<td>85.5</td>
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<td>N</td>
<td>5.83</td>
<td>89.2</td>
</tr>
<tr>
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<td>N</td>
<td>4.83</td>
<td>90.4</td>
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<td>91.8</td>
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<td>N</td>
<td>4.94</td>
<td>92.4</td>
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<tr>
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<td>N</td>
<td>4.56</td>
<td>92.6</td>
</tr>
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</tr>
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<td>N</td>
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<td>94.8</td>
</tr>
<tr>
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<td>N</td>
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<td>96.2</td>
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<tr>
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<td>N</td>
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<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical Status</th>
<th>Plasma Glucose (mmol/L)</th>
<th>HbA1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSH</td>
<td>MC</td>
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<td>D ++</td>
<td>9.89</td>
<td>64.8</td>
</tr>
<tr>
<td>DSH</td>
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<td>97.0</td>
</tr>
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<td>D +++</td>
<td>21.94</td>
<td>89.0</td>
</tr>
</tbody>
</table>

- M = male, F = female, MC = male castrated, FS = female spayed.
- N = normal, D = diabetic, ++ = moderately well controlled, +++ = poorly controlled.
- DSH = domestic short hair cat, DLH = domestic long hair cat.
- Fast Hemoglobin Test, Isolab, Akron, Ohio.
- Bio-Rad Laboratories, Richmond, California.

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but the β chain aminoterminal are structurally different from each other (23). Hemoglobin A-β has a free aminoterminal glycine and is identical in this respect to human fetal hemoglobin (HbF). Hemoglobin B-β has its aminoterminal serine blocked by an acetyl group, thus resembling the aminoterminal of the γ chain in a minor component of human fetal hemoglobin, HbF. Feline HbB-β is more negatively charged than HbA-β (24,25). More negatively charged compounds are associated with a lower isoelectric pH.

The resin columns employed to obtain the glycosylated hemoglobins are very sensitive to pH. Feline HbA has an isoelectric pH (pH1) of 6.75, which is lower than that of canine HbA (pH1 7.0) and of human hemoglobin (pH1 6.95) (H.F. Bunn, personal communication). Consequently, cat HbA would elute from the column rapidly and cochromatograph with HbA1, similarly to human HbF (14), giving falsely elevated values for glycosylated hemoglobin. Moreover, feline HbB, like human HbF, will elute rapidly due to its essential non-reactivity with the column resins, contributing to a falsely elevated value for HbA1. These facts are sufficient to explain the unusually high percentages obtained for feline glycosylated hemoglobin in the present study. The techniques used in human reference laboratories are not designed to compensate for the above characteristics of feline hemoglobin, namely the lower pH1 of both major fractions and the basic non-reactivity of HbB with resins in current use. Thus, the use of a cation exchange procedure designed for measurement of human glycosylated hemoglobin cannot be valid for determining the degree of glycosylation of feline hemoglobin.

In conclusion, the validity of the use of glycosylated hemoglobin levels for the assessment of long-term diabetic control in dogs awaits further definition. Currently, the commercially available technique employing a cation resin exchange column requires exacting attention to procedural detail. It is evident that, even when using similar assay techniques, the normal canine ranges for glycosylated hemoglobin vary considerably. Even within a single laboratory, the ranges of HbA1c values for normal and diabetic patients overlap substantially.

References