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

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SUMMARY

The measurement of glycosylated hemoglobin (HbA_1) levels in humans is used to indicate the degree of long-term diabetic control. Using a commercially available kit for human HbA_1, 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 A_1, pour une vérification suivie du diabète sucré, chez les chiens et les chats

La détermination de la concentration de l'hémoglobine A_1 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 obtinrent 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 experiénce et du nombre relativement restreint d'animaux diabétiques qu'ils utilisèrent, les auteurs conclurent 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 A_{1c} (HbA_{1c}), 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 the concentration of HbA_{1c} (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 HbA_{1c} 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 3mL 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

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[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.[3] 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 HbA_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 HbA_1 value of 28.2%, with a blood glucose level of 4.61 mmol/L (83 mg/dL). Fourteen of the dogs had HbA_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 HbA_1 determinations from 9.8 to 14.6% (Table I). There was no correlation between blood glucose level and HbA_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 64.8 and 97.0%. No trends were discernible (see Table II).

DISCUSSION

The hemoglobin molecule consists of four polypeptide subunits, which in man are two α chains and two β chains. Hemoglobin A_{1c} is hemoglobin with a glucose moiety attached at the aminoterminus of the β 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 (19). 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 HbA_{1c} provides a time-averaged estimate of blood glucose levels (2,3,20). In human diabetics, the concentration of HbA_{1c} has been found to be approximately twice that in normal individuals. Since other minor components, namely HbA_{1a} and HbA_{1b}, are also elevated in diabetics and may be precursors to HbA_{1c} (12,13), determination of total HbA_1 may, clinically, be a more useful measurement (2,3). Total HbA_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 HbA_1 levels for our diabetic dogs were all within this normal range. One study (15) used a conventional macrocolumn ion exchange chromatographic procedure to measure HbA_1 components in seven normal and seven diabetic dogs. They found that mean normal HbA_{1c} concentrations constituted approximately 3% of total hemoglobin concentra-

TABLE I
SUMMARY OF DATA FROM CLINICALLY NORMAL AND DIABETIC DOGS

Breed	Sex[a]	Age (years)	Clinical Status[b]	Plasma Glucose (mmol/L)	HbA_1 (%)
Labrador Retriever	M	6	N	5.61	6.2
German Shepherd cross	F	0.5	N	4.94	6.8
Mixed Breed	F	1	N	4.72	7.0
Old English Sheepdog	M	7	N	4.83	7.2
Chihuahua cross	F	1	N	5.33	7.6
Collie	M	4	N	5.56	7.8
Chihuahua x Terrier	F	3	N	5.61	8.2
Terrier x Poodle	F	2	N	5.67	8.5
German Shepherd	F	6	N	4.77	9.1
Cockapoo	M	2	N	4.39	9.8
Springer Spaniel	F	3	N	5.17	10.0
Airedale	F	1	N	4.83	10.5
Collie	FS	4	N	4.44	10.8
Boxer	F	1	N	5.17	11.5
Terrier cross	M	1	N	5.33	13.4
German Shepherd x Husky	F	0.5	N	5.72	14.0
Poodle x Terrier	F	2	N	5.61	15.2
Terrier cross	F	1.5	N	4.67	17.8
German Shepherd	F	2	N	4.77	18.6
Chihuahua x Pomeranian	M	10	N	4.61	28.2
Mean				5.09	11.4
SD				0.44	5.3
Terrier cross	FS	5.5	D ++	9.89	14.6
Terrier x Labrador	M	12	D ++++	24.22	14.2
Terrier x Chihuahua	F	9	D +	12.89	9.8
Irish Terrier	M	10	D ++++	26.00	14.3
Poodle	M	2	D ++	6.17	13.8

[a]M = male, F = female, FS = female spayed.
[b]N = normal, D = diabetic, + = iatrogenic hyperadrenocorticism, ++ = moderately well controlled, ++++ = uncontrolled.

[3]Bio-Rad Hemoglobin A_1 by Column Test, Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario.

tions, whereas this value was significantly elevated in diabetics, approximately 5%. When examined on the basis of total glycosylated hemoglobins (HbA_1), 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 approximately 13% for diabetic dogs. The same investigators compared (22) their chromatographic procedure with a colorimetric method[4] for the measurement of canine glycosylated hemoglobin levels. The values obtained from both methods were significantly correlated. Another study (17), in an attempt to find a more practical assay method, used a commercially available kit,[5] in their veterinary hospital laboratory, to measure total HbA_1. These workers determined HbA_1 value for 56 nondiabetic and 16 diabetic dogs. The mean HbA_1 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 diabetic HbA_1 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).

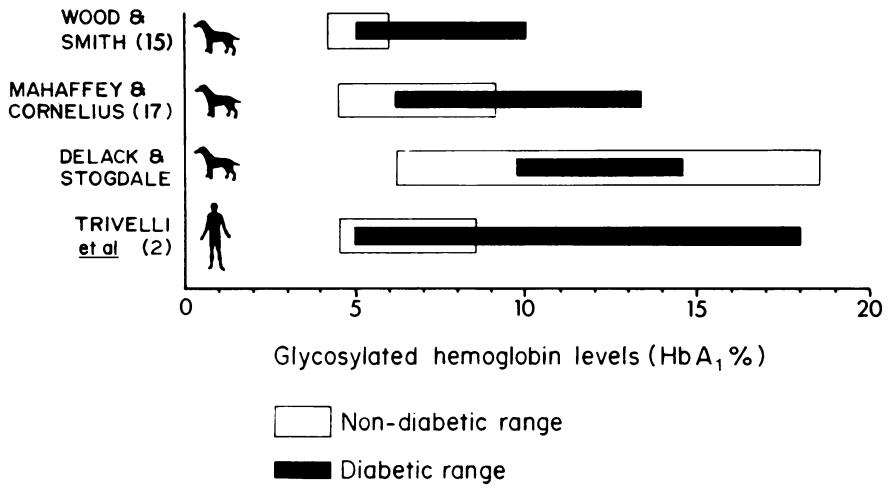

FIGURE 1. Comparison of HbA, ranges in non-diabetic and diabetic dogs. Representative human data are included for comparative purposes.

In our study, all diabetic dogs' HbA_1 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,[6] and the phosphate 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 human diabetic studies (14). In essence, until the technique within and between laboratories can be standardized, comparison of results must be made with care. It must be noted that neither this nor the previous studies have compared the HbA_1 values within individual animals over extended periods of time. Nor have HbA_1 levels been followed sequentially in diabetic dogs with respect to the degree of diabetic control.

The HbA_1 values obtained for normal and diabetic cats were very high and no discernable trend was detectable. There exist no published reports on glycosylated hemoglobin measurement 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 aminoterminal sequences of the α chains are identical to those in other mammals,

TABLE II
SUMMARY OF DATA FROM CLINICALLY NORMAL AND DIABETIC CATS

Breed[c]	Sex[a]	Age (years)	Clinical Status[b]	Plasma Glucose (mmol/L)	HbA_1 (%)
DSH	F	0.5	N	4.11	81.6
DSH	M	0.5	N	8.50	81.8
DLH	M	0.5	N	11.22	82.4
DSH	F	1	N	4.61	83.8
DSH	M	0.5	N	5.50	85.5
DSH	M	0.5	N	9.50	86.0
DSH	M	2	N	5.06	88.2
DSH	F	1.5	N	5.83	89.2
DSH	F	2	N	4.83	89.4
DSH	F	0.5	N	5.17	91.7
DSH	M	2	N	4.67	91.8
DSH	F	1	N	4.94	92.4
DSH	F	1	N	4.56	92.6
DSH	M	1	N	12.00	93.7
DSH	F	0.5	N	4.17	93.9
DSH	M	0.5	N	4.39	94.0
DSH	F	5	N	4.39	94.8
DSH	M	0.5	N	6.44	95.4
Himalayan	F	1	N	4.44	96.2
DSH	M	0.5	N	6.39	97.6
DSH	F	4	N	4.50	97.7
DSH	F	2	N	4.83	99.2
Mean				5.91	90.9
SD				2.29	5.4
Siamese	MC	12	D ++	9.89	64.8
DSH	FS	10	D +++	20.89	97.0
Siamese	FS	6	D +++	21.94	89.0

[a]M = male, F = female, MC = male castrated, FS = female spayed.
[b]N = normal, D = diabetic, ++ = moderately well controlled, +++ = poorly controlled.
[c]DSH = domestic short hair cat, DLH = domestic long hair cat.

[4]Glycospec, Abbott Laboratories, North Chicago, Illinois.
[5]Fast Hemoglobin Test, Isolab, Akron, Ohio.
[6]Bio-Rad Laboratories, Richmond, California.

but the β chain aminotermini are structurally different from other mammalian hemoglobins and 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 aminoterminus of the γ chain in a minor component of human fetal hemoglobin, HbF_1. 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 (pH_I) of 6.75, which is lower than that of canine HbA (pH_I 7.0) and of human hemoglobin (pH_I 6.95) (H.F. Bunn, personal communication). Consequently, cat HbA would elute from the column rapidly and cochromatograph with HbA_1, similarly to human HbF (14), giving falsely elevated values for glycosylated hemoglobin. Moreover, feline HbB, like human HbF_1, will elute rapidly due to its essential nonreactivity with the column resins, contributing to a falsely elevated value for HbA_1. 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 pH_I of both major fractions and the basic nonreactivity 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 HbA_1 values for normal and diabetic patients overlap substantially.

Until a valid, repeatable, interpretable assay method for HbA_1 levels in dogs becomes commercially available, alternative methods of evaluating diabetic control must be used. The assessment of long-term diabetic control in dogs may be accomplished by monitoring serum alkaline phosphatase levels (J.W. Kramer, personal communication). This will only be valid in the absence of other disease processes affecting liver or bone. Concentrations of this enzyme are elevated in diabetics when there is inadequate insulin supplementation, which results in increased fat deposition in hepatocytes. In cats, the most practical means by which to monitor diabetic control is by occasional measurement of blood glucose and by weekly measurement of water intake and body weight.

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