# RELATIONSHIP BETWEEN GLYCOSYLATED HEMOGLOBINS AND DIABETES MELLITUS IN THE DOG

by

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# LITERATURE REVIEW

## Introduction

Diabetes mellitus commonly affects humans and dogs. Deficient insulin control of lipid, protein and carbohydrate metabolism results in a multiorgan disease. One common characteristic, hyperglycemia, is controlled by exogenous insulin or other hypoglycemic agents. Therapy is often monitored by measuring blood and urine glucose concentrations. Many factors acutely affect blood and urine glucose including insulin action peak and duration times, exercise, diet and other stresses.[1] Only the time the blood or urine sample was obtained is represented by the glucose concentration. A monitoring technique reflecting the time average glucose for a period of weeks should be more useful. Recently the glycosylated hemoglobin, hemoglobin $A_{1c}$ ($HbA_{1c}$) concentration has been used to monitor human diabetics, because it represents the previous few weeks' glucose status.[2]

## History

Dr. Samuel Rahbar accidently discovered the relationship between $HbA_{1c}$ and diabetes mellitus. While screening patients in the late 1960s for abnormal hemoglobins, he noticed an unusual hemoglobin fraction.[3] It migrated in approximately the same position as fetal hemoglobin, hemoglobin F (HbF), when electrophoresed on agar gel in 0.05m citrate buffer, pH6.2. In other respects it was unlike HbF. It was neither resistant to denaturation by alkali, nor distinguishable from normal adult

hemoglobin, hemoglobin A(HbA), when electrophoresed on starch gel at alkaline pH. The samples containing this fraction were from diabetics. He first thought "diabetic hemoglobin" was HbA bound to oxidized glutathione. After synthesizing the compound, it did not exhibit the mobility of diabetic hemoglobin. When hemolysates from diabetics were column chromatographed, "diabetic hemoglobin" corresponded to the $HbA_{1c}$ previously reported in normal individuals.[4] The quantitatively higher concentration found in uncontrolled diabetics was the difference.

Glycosylation of Hemoglobins

The glycosylated hemoglobins (glycohemoglobins) are a group consisting of HbA with various attached carbohydrates. The proposed structures are found in table 1.[5] Glycohemoglobins represent normal HbA glycosylated by a slow, irreversible, nonenzymatic, post translational modification. All minor components are found in normal human erythrocytes. Normal human erythrocyte concentrations of glycohemoglobins appear low in the youngest erythrocytes and high in the oldest erythrocytes.[6] This is due to the slow reaction described over the erythrocyte lifespan. In diabetics, the reaction rate appears to increase due to elevated glucose in erythrocytes, hence elevating $HbA_{1c}$ by mass action. The other glycohemoglobins are only elevated slightly.[5] The glycosylation of HbA occurs mostly at the N-terminal of the $\beta$ chains (figure 1).[5] Recently, other glycosylation locations have been identified on the hemoglobin molecule. These include glycosylation of the N-terminal of the $\alpha$ chains and on lysine residues. These are relatively minor HbA alterations.[7]

| Minor Hemoglobin | Abbreviation | Attached Carbohydrate | Human Levels % Normal | Diabetic |
|---|---|---|---|---|
| Hemoglobin $A_{1a_1}$ | Hb $A_{1a_1}$ | Fructose 1,6-diphosphate? | | |
| Hemoglobin $A_{1a_2}$ | Hb $A_{1a_2}$ | Glucose 6-phosphate | 1.0-2.0 | 2.0-3.0 |
| Hemoglobin $A_{1b}$ | Hb $A_{1b}$ | unknown | | |
| Hemoglobin $A_{1c}$ | Hb $A_{1c}$ | Glucose | 3.3-3.5 | 6.0-10 |

Table 1. The different glycosylated hemoglobins found in erythrocytes.

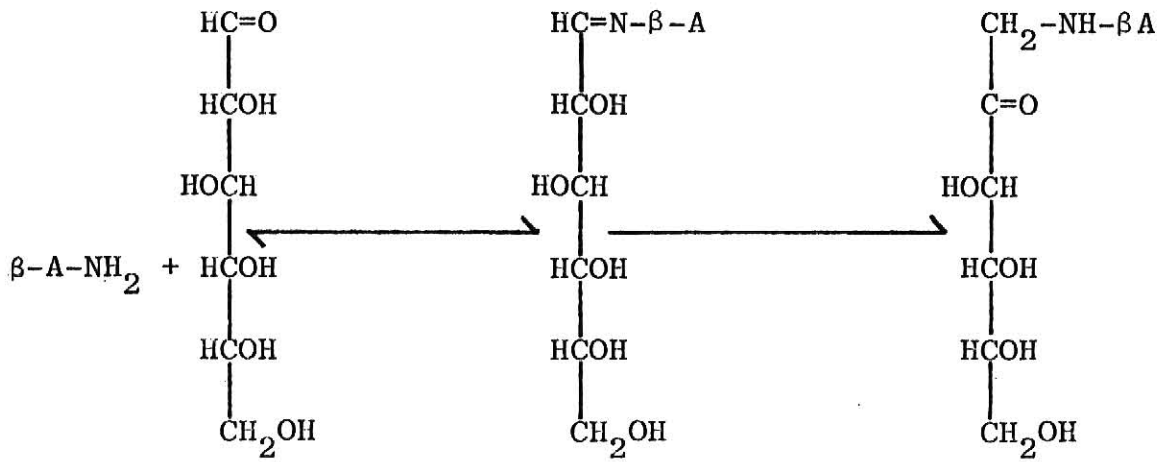


Figure 1. Glycosylation of a β chain of HbA by glucose.

The time average glucose represented by $HbA_{1c}$ concentrations is debatable. Time periods ranging from 3-4 weeks[8] to 2-3 months[7] have been suggested. Factors affecting $HbA_{1c}$ concentrations must be considered. Obviously, plasma glucose and erythrocyte lifespan are important. With these factors in mind, patients with hemolytic disease and reticulocytosis have abnormally low glycohemoglobin concentrations. These factors also explain the elevation of $HbA_{1c}$ in poorly controlled diabetics without hemolytic disease. The erythrocyte is freely permeable to glucose and hexokinase is saturated at normal physiological glucose concentrations, so the intraerythrocyte glucose rises in diabetics.[10] Due to the rise in erythrocyte glucose, hemoglobin glycosylation by glucose increases, elevating $HbA_{1c}$.[5] The fates of glycohemoglobins during the erythrocyte lifespan are poorly understood. Although the generally accepted reactions described for the formation appear simple, explanation of concentrations observed is unclear. The glycohemoglobin levels may reflect not only the synthetic rate, but also degradation or conversion rate. Suggestive, but not convincing, evidence proposes that $HbA_{1b}$ forms from $HbA_{1c}$.[11]

Glycosylated Hemoglobin Analysis

Physical separation and quantification of glycohemoglobins is accomplished by several techniques including column chromatography, electrophoresis, and isoelectric focusing. Because glycohemoglobins are more negatively charged than HbA, they are separable. Hemoglobin $A_{1a}$, $HbA_{1b}$, $HbA_{1c}$, and HbA represent order from most negative to least negative as first separated chromatographically.[4]

Column chromatography is the most widely used technique. Columns of a cation exchange resin separate glycohemoglobins in order of most negative to least negative. Columns of various sizes have been used, made from pasteur pipets[12] and syringe barrels[13] to commercial glass columns.[14] The most commonly-used cation exchange resin is Bio Rex 70. Sodium phosphate-potassium-cyanide buffers elute the hemoglobins through the columns.[14] The refinement of the technique determines the number of glycohemoglobin fractions separated. After separation, fractions are measured spectrophotometrically at 415 nm. Results consist of expressing each hemoglobin fraction as a percentage of the total hemoglobin sample eluted.[14] Commercial column kits are available, but most only separate the glycohemoglobins from HbA.[15] This still reflects $HbA_{1c}$ concentration even though collected as a group. These are used in human clinical medicine. Another column chromatography technique developed for speed of analysis is high pressure liquid chromatography (HPLC).[16]

Column chromatography has presented some problems in producing consistent, accurate results. Temperature fluctuations severely affect separation of fractions.[15] When the temperature is increased the column resin becomes more acidic. By lowering pH the cation exchange resin becomes more positive resulting in less HbA remaining on the column for separation. As a result, a false increase in glycohemoglobin is measured. In addition, the effect also is dependent upon the relative amounts of glycohemoglobins analyzed. Another problem recently

discovered was rapid fluctuations in glycohemoglobin concentration.[17] This opposes the usefulness of glycohemoglobin determinations, because the concentrations supposedly were unaffected by rapid glucose fluctuations. The discrepancy appears when nondialyzed samples were analyzed on small columns or HPLC. Apparently, acute elevation of plasma glucose caused rapid formation of glycohemoglobins. Because these rapidly formed glycohemoglobins decreased after dialyzation, they appear to be still in a reversible stage of formation. The glycosylation is probably at the reversible aldimine linkage and could be dialyzed off. The problem is easily resolved by hemolysate dialyzation before use on small columns or HPLC. Another problem with columns is that HbF separates like glycohemoglobins.[5] Patients require screening for HbF levels for accurate glycohemoglobin interpretation. Regardless of the problems, column chromatography presently is the most widely used clinical technique of measuring glycohemoglobins.

Electrophoresis also separates glycohemoglobins for quantitation. Dr. Rahbar used this technique as described earlier. Clinical electrophoresis kits are available. Isoelectric focusing also separates glycohemoglobins based on the different charge and isoelectric properties.[18] Both techniques, though usable, rate low in popularity.

Currently, colorimetric techniques are being developed for measuring glycohemoglobins. Many advantages would be gained over columns. Those include shorter time requirement, decreased problems with temperature fluctuations and less cost. One such technique involves forming a hydroxymethylfurfural

derivative.[19,20] Its production results from incubating the hemolysate in oxalic acid hydrolyzing the ketoamine linkage to 5-hydroxymethylfurfural. The remainder of the HbA β chain is precipitated out with trichloroacetic acid. The 5-hydroxymethylfurfural reacts with 2-thiobarbituric acid to form a chromophore (figure 2). The chromophore concentration is read at 443 nm. The chromophore concentration represents the proportional glycohemoglobin level. Although this test is not sensitive to fluctuating temperatures and can be performed in less time than columns, it requires a relatively large blood sample and it cannot be automated readily.

Recently, another colorimetric test has been developed and patented, the Glycospec™* test. It is based on the spectra changes in hemoglobin when it interacts with small molecules. Certain organic phosphates such as phytic acid and 2,3-diphosphoglycerate (DPG) bind to the β chains of hemoglobin.[21] That binding is accompanied by a decreased bichromatic absorbance of hemoglobin at 560 nm - 663 nm. When hemoglobin is glycosylated, organic phosphate binding is prevented and the change (decrease) in absorbance is less. The difference can be quantitated using three glycohemoglobin standards supplied by the manufacturer. Although this test can be automated readily, is insensitive to room temperature and is inexpensive, its usefulness has not been determined because of its recent introduction on the market (October, 1980).

*Abbott Laboratories, N Chicago IL, 60064

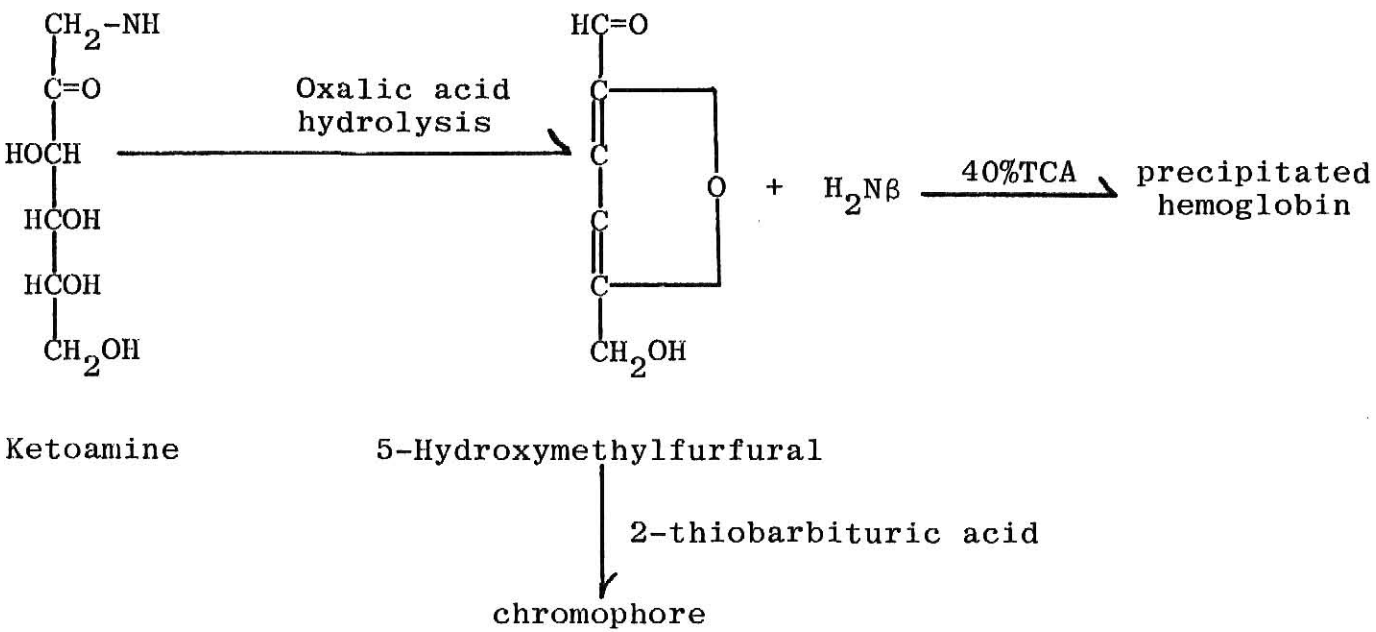


Figure 2. Hydroxymethylfurfural formation from $HbA_{1c}$

## Glycosylated Hemoglobin Model of Other Glycoproteins

Human erythrocytes normally contain approximately 3% of its hemoglobin as $HbA_{1c}$.[14] Human diabetic's erythrocytes contain 6-10% $HbA_{1c}$.[14] The $HbA_{1c}$ level represents the previous few weeks glucose concentrations. Hemoglobin $A_{1c}$ may not be the only protein glycosylated excessively in diabetes. For example, glycosylation of basement membranes may be responsible for microangiopathies seen in long term diabetics.[22] This hypothesis has not been accepted by all investigators.[22] The hope is that improved therapy due to improved monitoring of $HbA_{1c}$ will prevent other long term pathology such as microangioapathy. Better understanding of glycohemoglobins should clarify the possible role of glycosylation of other proteins.

## Canine Glycosylated Hemoglobin Study

Glycosylated hemoglobin study in dogs would be of great value. Clinical management of diabetic dogs could improve with a better monitoring technique such as $HbA_{1c}$. The dog has been widely used as a model for human diabetes.[23] It is especially applicable for study of glycohemoglobins. Dogs and humans have similar erythrocyte lifespan (120 days), one normal adult hemoglobin (HbA), and the same normal and diabetic plasma glucose concentrations. Additionally, dogs experience many of the long term effects of diabetes, such as cataracts, glomerulosclerosis, and retinopathies.[23] Study of long term effects requires long term monitoring of glucose. Thus, $HbA_{1c}$ concentrations in dogs should be useful for these studies also. Dogs are easy to handle and relatively large blood samples obtainable,

which also makes the dog a good source of glycohemoglobins for further study of altered glycoprotein biosynthesis in diabetics.

## Rationale for Thesis

The purpose of this study was three fold. First, glycohemoglobins, especially $HbA_{1c}$, were identified, and quantified in the dog. Second, elevation of $HbA_{1c}$ in diabetic dogs was established. Third, the glycosylation rate after induced diabetes was determined to better interpret $HbA_{1c}$ values.

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# PAPER I. GLYCOSYLATED HEMOGLOBIN AND CANINE DIABETES MELLITUS

Diabetes mellitus occurs commonly in dogs. In Sweden, the disease was found in 167 of 10,993 (1.5%) dogs necropsied during a 27-year period.[1] After initial clinical diagnosis and treatment, diabetic patients often remain on long-term insulin therapy. They require monitoring and periodic reevaluation of glucose status. Monitoring is generally accomplished by determination of plasma and urine glucose concentrations. Peak time of insulin action, excitability of the patient, diet, and amount of exercise affect results of this test. There would be obvious advantages to a test that, by a single determination, would reflect plasma glucose content over a period of weeks. Such a test would be less likely to yield misleading results caused by the aforementioned factors and would eliminate the necessity for a large number of determinations over an extended period.

The status of human diabetics is monitored by glycosylated hemoglobin determinations that reflect the plasma glucose concentration for the previous month.[2] Glycosylated hemoglobin is hemoglobin with a carbohydrate attached; it appears to form by a nonenzymatic, post-translational modification of the hemoglobin molecule. The glycosylation rate depends on the nature and concentration of the carbohydrate in the RBC over time. Because the RBC is an insulin-independent cell, intracellular glucose increases as plasma glucose rises. Hemoglobin glycosylated by glucose has been designated hemoglobin $A_{1c}$ ($HbA_{1c}$). Present in low concentration in the RBC of clinically normal human beings, it is found in increased amounts in human patients with uncontrolled diabetes.[2]

## Materials and Methods

Blood samples were obtained in EDTA from thirteen adult, clinically normal, mixed-breed dogs and from fourteen adult, clinically diagnosed diabetic dogs. Plasma glucose determinations were done by the o-toluidine blue method[3] or by use of glucose oxidase. The RBC were separated by centrifugation and washed three times in physiologic saline solution. Hemolysates were prepared by vigorously mixing 1 ml of packed RBC (centrifuged at 935 g for 5 minutes) with 1 ml of demineralized water and 0.5 ml of carbon tetrachloride, then centrifuging again at 935 g for 5 minutes.[4] One milliliter of hemolysate was then chromatographed on Bio Rex 70 (200-400 mesh) column (1 cm X 30 cm).

The first eluting buffer was sodium phosphate (0.042 M) and potassium cyanide (0.01 M), pH 6.68.[5] From 40 to 70 ml of the first buffer was used to separate the two minor hemoglobin fractions. The major hemoglobin fraction then was eluted with 75 to 150 ml of a high molarity (0.15 M) sodium phosphate buffer, pH 6.55.[4] Approximately 1-ml samples were collected by an automatic fraction collector at 25 C. Each blood sample was chromatographed in duplicate, and the results were recorded as the average of the two values. The hemoglobin concentration was measured at 415 nm, using a microsample spectrophotometer.

## Results

Two minor hemoglobin fractions were separated as hemoglobin $A_{1a+b}$ and next as $HbA_{1c}$.[4] Hydroxymethylfurfurol derivatives were measured to confirm that the second minor fraction of canine hemoglobin was analogous to human $HbA_{1c}$.[6] Results were

calculated by taking the leading and trailing edges of each fraction represented by the peaks in absorbance of the samples measured, then dividing the sum of the absorbances between those two points by the sum of all absorbances at the points of collection. The two minor fractions were expressed as a percentage of the total hemoglobin sample. Hemoglobin $A_{1c}$ was significantly higher ($P<0.001$) in canine diabetics ($t=5.25$).

## Discussion

Human beings have a mean normal $HbA_{1c}$ concentration of approximately 3%[7] of total hemoglobin concentration, as was found in the normal dogs of our study. However, human diabetics have $HbA_{1c}$ values ranging from 6% to 10%,[7] which compares with 3% to 7% for the diabetic dogs of our study. The period of hyperglycemia required to elevate $HbA_{1c}$ in dogs is unknown, but it would be expected to be approximately the same as for human beings, because dogs have a similar RBC life span (approx 120 days). If the times are similar, measured $HbA_{1c}$ would reflect plasma glucose status for the preceding few weeks, which would be a more useful monitor of insulin therapy than the plasma glucose concentration. This is exemplified by diabetic dog 14 (Table 2), which was in only for a routine checkup. The clinician believed this patient was under fair insulin control. The $HbA_{1c}$ concentration was in the normal dogs' range, reflecting adequate control. Dog 13 was very hyperglycemic for 5 days, then glucose concentrations dropped slowly to normal, with only a mild elevation of $HbA_{1c}$. The first sample from Dog 5 was taken when diabetes was first diagnosed. After starting on insulin and increasing the dosage within the past couple months,

DATA FOR NORMAL DOGS

Table I

| No. | Plasma glucose (mg/dl) | $HbA_{1a+b}$ (%) | $HbA_{1c}$ (%) |
|---|---|---|---|
| 1 | 74 | 1.56 | 2.56 |
| 2 | 94 | 1.61 | 2.63 |
| 3 | 113 | 2.30 | 3.30 |
| 4 | 75 | 2.10 | 2.30 |
| 5 | 111 | 2.34 | 3.07 |
| 6 | 110 | 2.60 | 3.25 |
| 7 | 90 | 2.82 | 2.79 |
| 8 | 86 | 1.53 | 3.00 |
| 9 | 93 | 1.69 | 2.57 |
| 10 | 108 | 2.30 | 3.25 |
| 11 | 107 | 1.62 | 2.29 |
| 12 | 102 | 1.70 | 2.62 |
| 13 | 84 | 1.65 | 2.92 |
| Mean | 95 | 1.99 | 2.81 |
| SD | 13 | .44 | .35 |

DATA FROM DIABETIC DOGS

Table II

| No. | Clinical Notes | Plasma glucose (mg/dl) | $HbA_{1a+b}$ (%) | $HbA_{1c}$ (%) |
|---|---|---|---|---|
| 1 | Polydipsia, polyuria, ketoacidosis, pancreatitis (1973), urinary incontinence | 534 | 3.36 | 7.36 |
| 2 | Bilateral cataracts, cushingoid, acute pancreatitis, plasma glucose concentration of 1,280 mg/dl at 2 days after admission | 633 | 3.35 | 6.75 |
| 3 | Poorly regulated diabetic for 2 years | 220 | 2.27 | 5.76 |
| 4 | Ketoacidosis, glucose data not available | --- | 2.21 | 5.61 |
| 5 | Bilateral cataracts, polyuria, polydipsia, weight loss past month | 419 | 1.78 | 5.06 |
| | In for boarding 4 months later, increased insulin. Glucose not done | --- | 1.71 | 3.21 |
| 6 | Cataracts, on daily insulin | 104 | 2.45 | 4.64 |
| 7 | Diabetic 6 months, developing bilateral cataracts, poorly regulated, on daily insulin | 114 | 1.96 | 4.57 |
| | One month later, in for cataract surgery, still appeared poorly regulated, glucose not done | --- | 2.20 | 5.86 |
| 8 | Bilateral cataracts, daily insulin, in for boarding | 63 | 1.97 | 4.41 |
| | In for boarding again, 9 months later, glucose not done | --- | 2.22 | 4.14 |
| 9 | Vomiting, on insulin since 1976 | 270 | 2.08 | 4.22 |
| 10 | Vomiting, diarrhea, anorexia | 648 | 1.90 | 3.90 |
| 11 | Polydipsia, polyuria, cataracts | 532 | 1.66 | 3.82 |

DATA FROM DIABETIC DOGS (cont.)

| No. | Clinical Notes | Plasma glucose (mg/dl) | $HbA_{1a+b}$ (%) | $HbA_{1c}$ (%) |
|---|---|---|---|---|
| 12 | In due to hypoglycemia, been on insulin 2 months | --- | 2.06 | 3.70 |
| 13 | Iatrogenic cushingoid, gluco-corticoids past 11 yrs., acute pancreatitis 14 days ago | 191 | 1.43 | 3.29 |
| 14 | Fair control with NPH insulin daily, bilateral cataracts, in for check-up | 209 | 1.90 | 3.16 |
| Mean | | | 2.14 | 4.67 |
| SD | | | 0.52 | 1.23 |

clinically control appeared good, and $HbA_{1c}$ was lower, four months later. The other diabetic dogs (1-4, 6-12) were examined because of acute illness and variable periods of previous hyperglycemia, but all had $HbA_{1c}$ values higher than the normal range. The difference between the highest value in the normal dogs' range (3.34%) and the lowest value of the acutely ill diabetic dogs' range (3.70%) was small (difference of 0.36%), as would be expected. This small difference also was found in clinical reports of human patients,[8] which reflected the variable periods of hyperglycemia.

The use of $HbA_{1c}$ valued in monitoring canine diabetes mellitus seems feasible. Commercial kits for use in man are available, and some human reference laboratories also are measuring human glycosylated hemoglobins; however, caution is advised if either procedure is used. Different techniques, especially for different species, may give different results than those reported here.

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# PAPER II. IN VIVO BIOSYNTHESIS OF GLYCOSYLATED HEMOGLOBINS IN DOGS AFTER INDUCING DIABETES MELLITUS

Diabetes mellitus commonly affects humans and dogs. Clinical manifestations of diabetes, both acute and chronic, depend upon efficacy of metabolic control. While treatment with exogenous insulin greatly benefits diabetics, many basic problems remain. Accurate monitoring of metabolic control is a major problem. Recently, hemoglobin $A_{1c}$ ($HbA_{1c}$) concentrations have been introduced to monitor human diabetics.[1] This measurement reflects glucose control over the past few weeks. Hemoglobin $A_{1c}$ is one of three minor hemoglobins found in erythrocytes ($HbA_{1a}$, $HbA_{1b}$, $HbA_{1c}$) and elevates in poorly controlled diabetics.[2] It appears to form by a slow, irreversible, nonenzymatic, post-translational modification of normal adult hemoglobin, hemoglobin A (HbA).[2] The glycosylation to produce $HbA_{1c}$ appears to take place by the attachment of glucose to the N-terminal of the hemoglobin beta chains. Apparently, the rate depends upon the plasma glucose concentrations over the lifespan of the erythrocyte.[2] Thus, measurement of $HbA_{1c}$ reflects control better than does a single plasma or urine glucose concentration.

Humans and dogs share many normal and diabetic physiological characteristics. Both have approximately 120-day erythrocyte lifespans, the same quantitative plasma glucose fluctuations and normally one type of adult hemoglobin, HbA. These factors make the dog a useful model for comparative studies of diabetes mellitus, including glycosylated hemoglobin studies. Recently, $HbA_{1c}$ concentrations were found elevated in a group of clinically diabetic dogs.[3] Concentrations reported were similar to levels reported in humans. The study presented here was

designed to determine the rate of $HbA_{1c}$ elevation after induced diabetes mellitus. Obviously this type of experiment could not be done in humans, but due to the important similarities, these data may reflect approximately the in vivo glycosylation rate of human HbA. The data reflected a glycosylation rate of 0.45% increase per week after diabetes induction. Additionally, $HbA_{1c}$ concentrations elevated higher than the normal range after two weeks of hyperglycemia.

Materials and Methods

The mixed-breed dogs used in the study were initially clinically normal. After three random blood samples were drawn to establish baseline data, diabetes mellitus was induced by partial pancreatectomy and/or chemically induced by a combination low dose of alloxan (50mg/kg) and streptozotocin (30mg/kg).[4] Blood samples were drawn weekly and plasma separated immediately for glucose determinations by glucose oxidase.

Glycosylated hemoglobins were determined on EDTA blood samples within 10 days of phlebotomy. The erythrocytes were washed three times with physiologic saline. Hemolysates were prepared by vigorously mixing 1ml demineralized water, 1ml of packed erythrocytes and 0.5ml carbon tetrachloride, and centrifuged for 10 minutes at 935g. Next, 0.5ml of hemolysate was put on a Bio-Rex 70 (200-400 mesh) column (1x30cm) pre-equilibrated with Developer 6 (0.04 molar sodium phosphate-0.01 molar potassium cyanide-buffer pH 6.68).[3] One ml samples were collected at 25°C eluted by Developer 6. After the two fast fractions were collected the major hemoglobin fraction (HbA) was collected in a 100ml graduated cylinder by using a high molarity

sodium phosphate buffer (0.15 molar pH 6.55). Each sample was then diluted with demineralized water and read at 415 nm in a micro-sample spectrophotometer. Minor hemoglobin fractions percent were calculated by the sum of absorbances representing their peaks and dividing by the sum of all the absorbances. Each analysis was done in duplicate and reported as the mean. Data was determined in 13 normal dogs and 5 dogs with induced diabetes mellitus. The diabetic dogs were accustomed to phlebotomy and maintained in a consistent environment. The data were evaluated by analysis of variance using one-way classification with linear regression.[5]

Results

Data of 13 normal dogs established values for $HbA_{1c}$ as (2.81% $\pm$ 0.35), $HbA_{1a+b}$ (1.99% $\pm$ 0.44), and plasma glucose (96 mg/dl $\pm$ 13). The normal range was set equal to the mean $\pm$ 2 standard deviations, representing 2.11% - 3.51% $HbA_{1c}$. After induction of diabetes the weekly $HbA_{1c}$, $HbA_{1a+b}$ and plasma glucose concentrations were determined. The mean values and standard errors calculated from the five dogs are plotted in figure 1; for individual data see appendix. The $HbA_{1c}$ values increased linearly ($p<0.001$). The regression line increased 0.45% per week and had a Y intercept 2.77%. The elevation rate of $HbA_{1a+b}$ was also linear ($p<0.05$) but much slower, 0.047% per week and a Y intercept of 1.75%. Elevation of the mean $HbA_{1c}$ above the normal range occurred after 2 weeks of hyperglycemia.

Discussion

Studies on $HbA_{1c}$ elevation in induced diabetic dogs has not been previously reported. The diabetic dog appears to be a

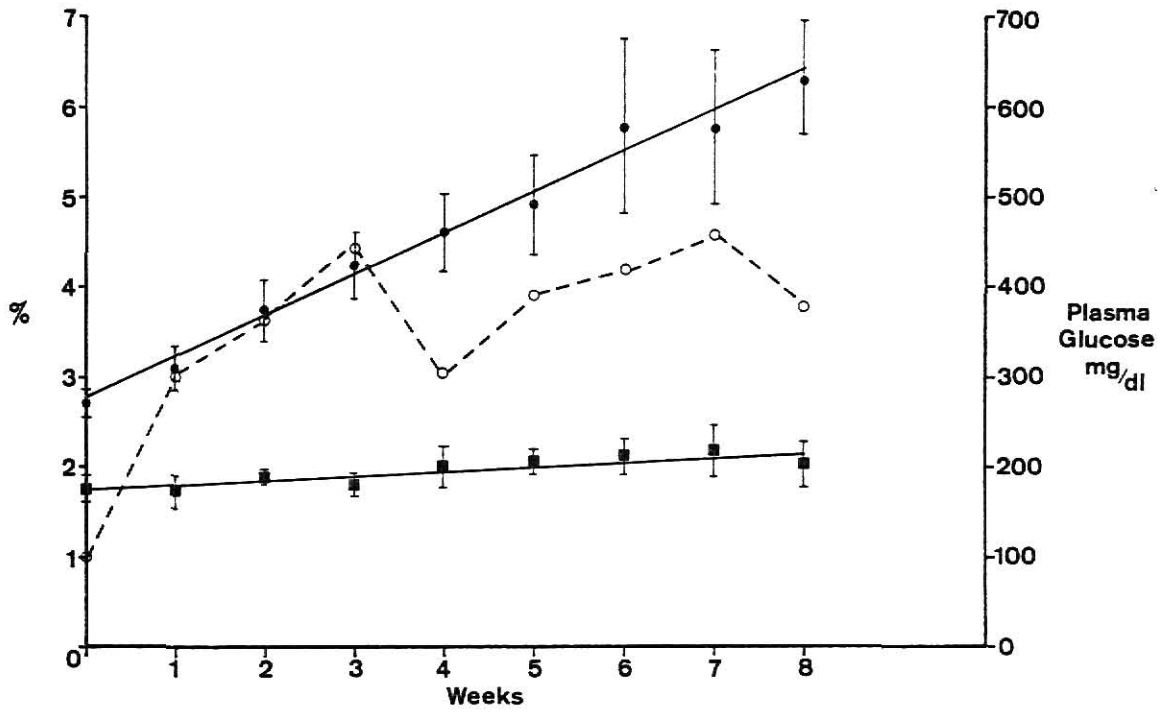


Figure 1. $HbA_{1c}$ ●, $HbA_{1a+b}$ ■, and plasma glucose ○ after inducing diabetes mellitus. Weeks 6 and 7 data were on 4 dogs. Bars represent SEM.

good animal model of human diabetes mellitus, especially for glycosylated hemoglobin studies.

In vivo biosynthesis of $HbA_{1c}$ in the dog increases linearly at 0.45% increase per week after diabetes induction. No correlation is drawn between the mean glucose and $HbA_{1c}$ values determined weekly (figure 1). The glucoses determined whether or not the dogs remained hyperglycemic after inducing diabetes. These values represent the glucose status only at the time of sampling. After two weeks of hyperglycemia the dog $HbA_{1c}$ values elevated higher than the normal dogs' range.

Improving interpretation of $HbA_{1c}$ values in human diabetics requires knowing the approximate time-average glucose represented. Conflicting reports include ranges from 3-4 weeks[6] to 2-3 months (8-12 weeks).[7] The shorter range was determined by improving glucose regulation in hospitalized diabetic patients and decreasing their $HbA_{1c}$ values in 3-4 weeks. The time reflected appears to be 3-4 weeks as glucose levels decline.[6] The 2-3 month range was determined by radioactive labeling of human hemoglobin and following the $HbA_{1c}$ biosynthesis in the nondiabetic state.[7] The rate of $HbA_{1c}$ biosynthesis increases in acutely induced or poorly regulated patients. The mass action nature of the glycosylation process would reflect a shorter time to drastically elevate $HbA_{1c}$ than in the non-diabetic glycosylation rate. The 2-3 month range appears to be too long when glucose concentrations are acutely decreasing or increasing.

The time required to elevate $HbA_{1c}$ may not be equal to the established time required to depress levels after improving

control. One reason is the fate of $HbA_{1c}$ during erythrocyte lifespan remains unknown. Suggestive evidence proposes that another yet unidentified minor hemoglobin fraction, $HbA_{1b}$, may be formed from $HbA_{1c}$.[8] This implies the $HbA_{1c}$ concentration is determined not only by the irreversible glycosylation rate, but also rates of interconversion or degradation.

We conclude from this study in dogs that the $HbA_{1c}$ concentration represents approximately 2 weeks time-average as plasma glucose acutely rises. This represents the similar time of 3-4 weeks in humans with declining glucose concentrations. Also, these data imply that degradation or interconversion may not be important in determining $HbA_{1c}$ concentrations.

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# APPENDIX

| | DOG #1 | | | DOG #2 | | | DOG #3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Week | Glucose | $HbA_{1a+b}$ | $HbA_{1c}$ | Glucose | $HbA_{1a+b}$ | $HbA_{1c}$ | Glucose | $HbA_{1a+b}$ | $HbA_{1c}$ |
| 0 | 108 | 2.30 | 3.25 | 107 | 1.62 | 2.29 | 93 | 1.69 | 2.57 |
| 1 | 285 | 2.26 | 3.34 | 325 | 1.22 | 2.79 | 387 | 1.77 | 3.90 |
| 2 | 244 | 2.08 | 4.36 | 400 | 1.65 | 3.55 | 745 | 2.10 | 4.62 |
| 3 | 389 | 2.23 | 4.28 | 313 | 1.64 | 4.12 | 687 | 1.91 | 5.50 |
| 4 | 527 | 2.60 | 4.30 | 342 | 1.45 | 4.21 | 344 | 2.38 | 6.26 |
| 5 | 522 | 1.92 | 3.65 | 402 | 2.10 | 4.42 | 480 | 2.53 | 6.88 |
| 6 | 422 | 2.12 | 7.02 | 302 | 1.63 | 3.88 | 491 | 2.62 | 7.81 |
| 7 | 490 | 2.54 | 6.20 | 630 | 1.61 | 3.93 | 544 | 2.70 | 7.89 |
| 8 | 470 | 2.57 | 7.86 | 562 | 1.53 | 5.04 | 358 | 2.60 | 7.76 |

Table 1. Weekly Data From Dogs With Induced Diabetes Mellitus

| | DOG #4 | | | DOG #5 | | | WEEKLY TOTALS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | Glucose | $HbA_{1a+b}$ | $HbA_{1c}$ | Glucose | $HbA_{1a+b}$ | $HbA_{1c}$ | Glucose | $HbA_{1a+b}$ | SE | $HbA_{1c}$ | SE |
| 0 | 84 | 1.65 | 2.92 | 102 | 1.70 | 2.62 | 99 | 1.79 | .16 | 2.73 | .13 |
| 1 | 156 | 1.61 | 2.90 | 374 | 1.76 | 2.56 | 305 | 1.71 | .24 | 3.10 | .17 |
| 2 | 209 | 1.84 | 3.23 | 209 | 1.79 | 2.97 | 361 | 1.89 | .32 | 3.74 | .085 |
| 3 | 674 | 1.50 | 3.64 | 148 | 1.75 | 3.63 | 442 | 1.80 | .34 | 4.23 | .13 |
| 4 | 206 | 1.50 | 4.12 | 84 | 2.11 | 4.12 | 301 | 2.01 | .42 | 4.60 | .23 |
| 5 | 325 | 1.79 | 5.25 | 220 | 1.91 | 4.40 | 390 | 2.05 | .55 | 4.92 | .13 |
| 6 | --- | ---- | ---- | 454 | 2.04 | 4.42 | 417 | 2.10 | .96 | 5.78 | .20 |
| 7 | --- | ---- | ---- | 160 | 1.90 | 4.97 | 456 | 2.18 | .85 | 5.75 | .26 |
| 8 | 299 | 1.73 | 5.89 | 209 | 1.61 | 4.88 | 379 | 2.01 | .64 | 6.29 | .24 |

Table 1. Continued

# RELATIONSHIP BETWEEN GLYCOSYLATED HEMOGLOBINS AND DIABETES MELLITUS IN THE DOG

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AN ABSTRACT OF A MASTER'S THESIS

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The glycosylated hemoglobin, Hemoglobin $A_{1c}$ ($HbA_{1c}$), has recently been introduced to monitor humans with diabetes mellitus. It forms by a slow, nonenzymatic glycosylation of hemoglobin A by glucose. The concentrations are dependent upon plasma glucose over the lifespan of the erythrocytes. Measurement of $HbA_{1c}$ reflects a long-term average glucose concentration as opposed to short-term concentration when the blood sample was drawn.

Hemoglobin $A_{1c}$ was identified and quantified in normal dogs. A group of clinically diabetic dogs had a significantly higher ($p<.001$) $HbA_{1c}$. The normal $HbA_{1c}$ range was $2.81\% \pm 35$ and diabetic dogs had $HbA_{1c}$ up to approximately 7.5%. The $HbA_{1c}$ was structurally analogous to human $HbA_{1c}$ as determined by column chromatography and hydroxymethylfurfural derivatives.

The glycosylation rate of $HbA_{1c}$ was determined in induced diabetic dogs. After inducing diabetes mellitus, $HbA_{1c}$ increased 0.45% per week linearly. The $HbA_{1c}$ was greater than the normal range after two weeks of hyperglycemia.

The $HbA_{1c}$ data establishes normal and diabetic values and the glycosylation rate in dogs. This information should be useful for monitoring diabetic dogs in clinical veterinary medicine and dog models for comparative studies of diabetes mellitus.