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Enhanced nonenzymatic glucosylation of human serum albumin in diabetes mellitus

(hyperglycemia/hemoglobin A_{Ic}/glycohemoglobins/glucose)

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ABSTRACT **Use of an ion exchange chromatographic method and a colorimetric method with thiobarbituric acid showed that levels of nonenzymatically glucosylated serum albumin were increased in patients with poorly controlled diabetes mellitus compared to controls. The two methods correlated well ($r = 0.99$) and clearly discriminated between normal and poorly controlled diabetic populations. The levels of glycosylated hemoglobin were also measured in both populations. Several patients apparently in good control based on glycosylated hemoglobin measurements were found to have increased levels of glucosylated albumin. Because albumin has a shorter circulating half-life than does the human erythrocyte, the plasma concentration of glucosylated albumin should be expected to reflect short-term control of hyperglycemia in diabetes. The studies reported here suggest that the level of glucosylated albumin may indeed be a sensitive indicator of moderate hyperglycemia and of early glucose intolerance.**

Recent studies showed that plasma proteins are glucosylated nonenzymatically by glucose *in vitro* (1). Glucosylated albumin was isolated from normal human serum by chromatography on CM-cellulose and accounted for about 8% of the total circulating albumin in man. The nonenzymatic glucosylation of albumin appears to proceed through Schiff base formation between the aldehyde form of the sugar and free amino groups in protein, followed by Amadori rearrangement to a ketoamine derivative (Fig. 1) (2). Cyclization of the ketoamine to the hemiketal structure probably contributes to the stability of the glucosyl–protein adduct under physiological conditions (2). The glucosylated albumin yields a positive reaction in the thiobarbituric acid assay for ketoamine derivatives of protein (3) and is glucosylated primarily at ε-amino groups of lysine residues (refs. 1 and 4, unpublished data).

Levels of nonenzymatically glycosylated hemoglobins, HbA_I, are known to be increased during chronic hyperglycemia in patients with poorly controlled or untreated diabetes (5), and the percentage of HbA_I correlates well with mean and fasting blood glucose concentrations (6). Levels of HbA_I may provide an index of long-term blood glucose control in patients with diabetes.

Because the levels of glucosylated albumin might also be of value as indicators of the degree of hyperglycemia in diabetes, studies were undertaken to compare the plasma concentration of glucosylated albumin in patients with diabetes and in normal individuals. These studies show that the levels of glucosylated albumin are increased in patients with poorly controlled diabetes mellitus but do not correlate well with levels of HbA_I in the same patient group, perhaps because different aspects of glucose intolerance are reflected by the two different glycosylated proteins.

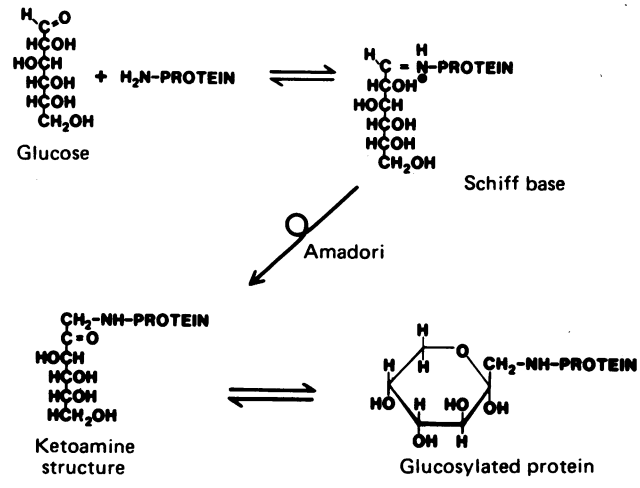

FIG. 1. Proposed reaction scheme for nonenzymatic glucosylation of albumin.

MATERIALS AND METHODS

Patients of both sexes with diabetes mellitus ranged in age from 10 to 74 years, could be classified as either juvenile- or adult-onset diabetics, and were being treated with insulin, oral agents, or only diet manipulation. The duration of their diabetes ranged from 3 to 20 years. In all cases, blood sugar control was poor as judged by records of urinary glucose or serum sugar levels or both. Control patients had no symptoms of uncontrolled diabetes mellitus and their fasting or 2-hr-postprandial blood sugar values were normal. Several control patients did have mildly abnormal results on glucose tolerance tests or had glucosuria during pregnancy, but there were no clinical data to suggest sustained hyperglycemia.

Venous blood samples were stored at 4°C and the plasma was separated from the formed elements within 24 hr and usually within 1 hr. The plasma was stored at −20°C until glucosylated albumin was determined; anticoagulated whole blood was stored at 4°C until glycosylated hemoglobin was determined. HbA_I was measured after cation exchange chromatography by determining the absorbance of the two hemoglobin components at 420 nm by a modification of the method of Schnek and

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Table 1. Glucosylated hemoglobin and albumin in diabetic patients and controls

Patient	% HbA_1	% glucosylated albumin: CM-cellulose method	% glucosylated albumin: Thiobarbituric acid method
1	15.1	17.8	25.9
2	11.1	19.2	24.3
3	14.7	22.5	25.5
4	18.3	21.7	25.1
5	11.8	24.3	26.9
6	8.6	26.0	30.5
7	13.2	18.1	20.0
8	17.4	24.6	29.7
9	14.6	22.6	26.0
10	11.8	20.8	23.2
11	8.4	16.3	19.0
12	10.9	20.3	23.6
13	13.6	22.1	25.1
14	16.4	19.5	22.0
15	10.4	17.9	21.0
16	7.6	16.0	18.5
17	11.5	19.8	23.4
18	11.6	24.8	28.2
19	19.2	23.8	27.2
20	9.2	17.4	20.5
21	15.4	22.7	25.8
22	7.6	12.8	14.8
Controls*	7.5 ± 1.5	7.0 ± 1.9	8.3 ± 2.2

* Shown as mean ± SD; for both methods for glucosylated albumin, $n = 25$. The value for % HbA_1 is based on laboratory experience at Duke University Medical Center.

Schroeder (7). The range of HbA_1 values was 6–9% for controls and 6–22% for patients with diabetes.

Serum albumin was purified by affinity chromatography on Affi-Gel Blue (Bio-Rad) (8), and glucosylated albumin was determined by CM-cellulose chromatography or by chemical assay with thiobarbituric acid as described (1, 3). In the chromatographic assay, percentage glucosylated albumin was calculated by dividing the A_{280} in the glucosylated peak by the sum of A_{280} under both the unglucosylated and glucosylated peaks. Based on A_{280} measurements, recovery of protein applied to the columns was quantitative. In the thiobarbituric acid procedure, accurately weighed albumin samples were assayed for glucosylated albumin with hydroxymethylfurfural as a standard; percentage glucosylated albumin was determined as (mol of hydroxymethylfurfural/mol of albumin) × 100.

RESULTS

The percentages of glycosylated hemoglobin and albumin in the blood of patients and controls are listed in Table 1. The normal ranges (mean ± SD) of percentage HbA_1 in the control population were based on laboratory experience at Duke University Medical Center. Tentative ranges for percentage glucosylated albumin by the chromatographic (CM-cellulose) and chemical (thiobarbituric acid) methods were based on the limited sample pool of 25 normal patients. Patients with increased HbA_1 also had increased glucosylated albumin by both methods. Four patients with normal HbA_1 were found to have increased glucosylated albumin.

Measurement of glucosylated albumin by the chromatographic procedure is a complex and tedious procedure, requiring purification of total albumin by affinity chromatography followed by dialysis and gradient elution from CM-cellulose (1). It was desirable, therefore, to establish that direct

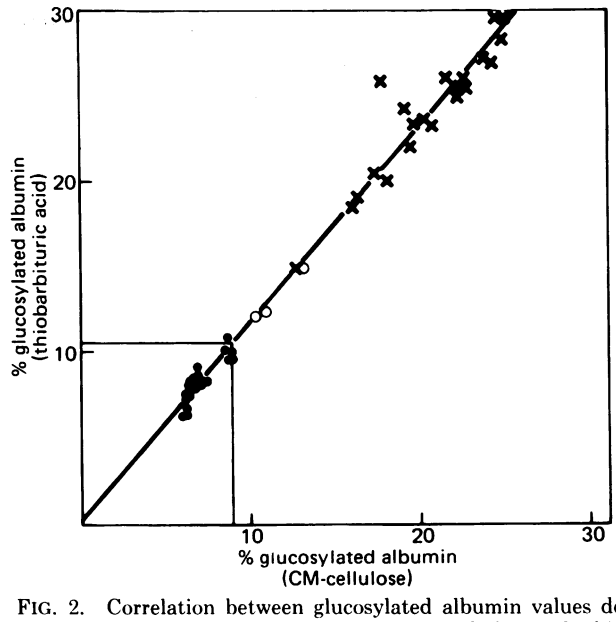

FIG. 2. Correlation between glucosylated albumin values determined by chromatographic (CM-cellulose) and chemical (thiobarbituric acid) methods. Sample population included 25 controls and 22 diabetic patients. Upper limits of normal are indicated by the rectangle in the lower left corner. ●, Normal; x, diabetic; o, glucose intolerant. $y = 0.16 + 1.16\,x$; $r = 0.993$.

application of the thiobarbituric acid assay to purified, unfractionated albumin could be used for estimation of percentage glucosylated albumin. The correlation between determinations of percentage glucosylated albumin by the chemical and chromatographic procedures was excellent ($r = 0.99$; y-intercept, <0.5%) (Fig. 2). However, the slope of the correlation line, $m = 1.16$, indicated that the chemical assay measured about 16% more glucosylated albumin than the chromatographic assay (see *Discussion*). The reproducibility of the two tests was comparable. Coefficient of variation was <5% throughout the range 5–30% glucosylated albumin by either method. It is apparent from Fig. 2 that both the CM-cellulose and the thiobarbituric acid assays are useful for discriminating between controls and patients with poorly controlled diabetes. Three control patients shown in Fig. 2 (open circles) were classified as glucose intolerant but not diabetic, on the basis of glucose tolerance tests (see *Discussion*); levels of glucosylated albumin for these individuals, determined by either test, were clearly outside of the normal range, at the low end of the group with diabetes.

Correlations of percentage glucosylated albumin with percentage HbA_1 are shown in Fig. 3. The upper limit of normal for these tests (see Table 1) is defined by the box at the lower left. It is clear that the normal and diabetic populations occupy distinctly different regions of this graph. However, several patients who appear to be in good control based on HbA_1 levels are clearly abnormal in their levels of glucosylated albumin. The correlation between glycosylated hemoglobin and albumin appears to be complex, and a linear least-squares regression analysis does not seem appropriate.

DISCUSSION

These studies were undertaken to determine if the blood glucosylated albumin level is increased in patients with poorly controlled diabetes. The experimental results clearly document that an increase can be detected by either chromatographic or chemical assay of glucosylated albumin. The tentative normal ranges (mean ± 2 SD) for glucosylation of albumin are ap-

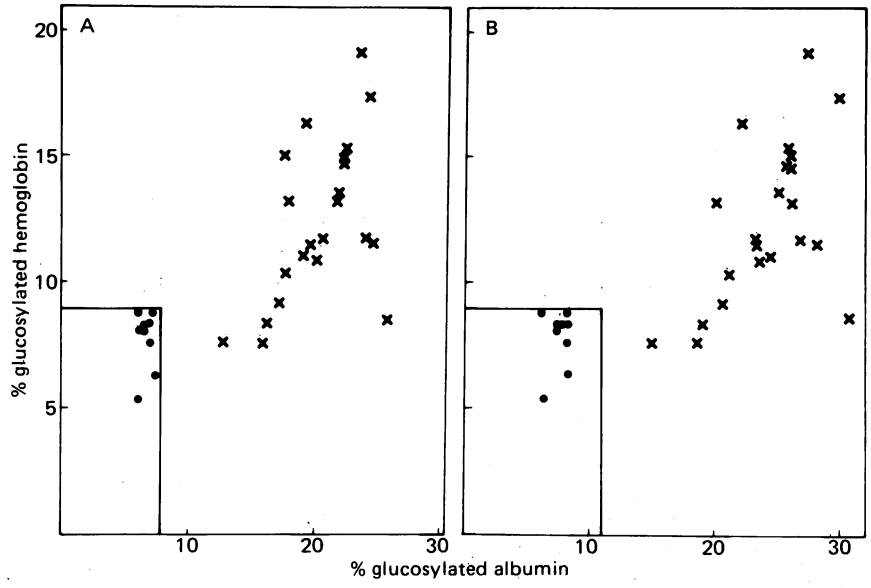

FIG. 3. Correlations between percentage HbA_I and percentage glucosylated albumin in normal controls (●) and diabetic patients (x). (*A*) Glucosylated albumin by CM-cellulose. (*B*) Glucosylated albumin by thiobarbituric acid. Upper limits of normal are indicated by the rectangles in the lower left corners.

proximately 7.0 ± 1.9% and 8.3 ± 2.2% by the CM-cellulose and thiobarbituric acid assays, respectively; levels as high as 30% are likely in poorly controlled diabetics.

At this point, quantitation of percentage glucosylated albumin is still a cumbersome procedure, requiring first the purification of total albumin and then estimation of percentage glucosylation. The thiobarbituric acid assay is more rapid and more sensitive than the CM-cellulose assay, and the correlation between the two assays is excellent. The slightly (≈16%) higher estimate of glucosylation in the chemical assay probably results from detection of glucosylation of albumin at sites that do not influence its chromatographic mobility on CM-cellulose. We have, in fact, observed a low level of [6-^{3}H]glucose incorporation and thiobarbituric acid reactivity in the "unglucosylated" fraction isolated from CM-cellulose chromatography (1). Thus, in addition to its speed and sensitivity, the chemical assay probably provides a better estimate of the amount of glucosylated albumin.

Although both glucosylated albumin and HbA_I levels are increased in diabetics, values for the two tests do not correlate well within the normal or diabetic group. The poor correlation suggests that both tests are sensitive to hyperglycemia in diabetes but they are monitoring different aspects of the glucose intolerance. It may be that HbA_I provides an index of relatively long-term glucose control, perhaps during a span of the previous 1–2 months and glucosylated albumin provides a measure of control during a shorter interval, possibly the previous 1–2 weeks. This results from the difference between the life-span of hemoglobin in the blood (≈120 days) (9) and the half-life of circulating albumin (≈17 days) (10). Because of the shorter half-life of albumin, levels of glucosylated albumin should change more rapidly in response to changes in average blood glucose concentration. Measurements of glucosylated albumin may be a better indicator of recent or short-term control and would supplement data obtainable by assays of HbA_I and other indices of blood sugar control. In the four patients with normal HbA_I and increased glucosylated albumin (Fig. 3), it seemed possible that short-term control was worse than long-term, although independent clinical data to rigorously support this argument were not available. Measurements of glucosylated albumin may also be useful for rapidly evaluating alterations in therapeutic regimens because changes in percentage glucosylated albumin should be detectable prior to changes in glycosylated hemoglobin.

It is our impression at this time that the range of increase of glucosylated albumin (up to 30%) is generally greater than the corresponding change in HbA_I (9–20%) in patients with diabetes. The means of normal values for the two determinations are not significantly different; therefore, glucosylated albumin may be a more sensitive indicator of integrated blood glucose levels than is glucosylated hemoglobin. Measurement of glucosylated albumin also may have some inherent advantages over glycohemoglobin measurements in certain clinical situations because the latter are subject to interference by HbF and are sometimes difficult to interpret in hemolytic disorders and pregnancy. No significant differences in percentage glucosylated albumin were observed in samples obtained from several control donors at various times before or during a glucose tolerance test. In addition, because the albumin is purified prior to analysis, the results of the test are not likely to be affected by lipemic or hemolyzed specimens.

Increased glucosylated albumin was detected in three patients with normal glucose tolerance test results by most criteria; however, two had at least one abnormal blood sugar value during the test and one had a family history of diabetes mellitus and was obese. HbA_I was not measured in two of these patients and was normal in the third. If these patients develop overt diabetes in the future, one could argue that glucosylated albumin may be a better indicator of early, significant carbohydrate intolerance than a glucose tolerance test or a fasting or 2-hr-postprandial blood sugar value.

Clearly, a more extensive clinical study needs to be performed to determine if the measurement of glucosylated albumin is useful in assessing carbohydrate intolerance or diabetes control and if there is a correlation between glucosylated plasma proteins and microvascular complications. If measurements of glucosylated albumin prove useful in the detection and management of diabetes, it may be possible to simplify the assay for routine use in the clinical laboratory.

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