

GLYCOSYLATED HEMOGLOBIN AND THE RISK OF MICROALBUMINURIA IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS

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Abstract Background. The risk of microalbuminuria in patients with insulin-dependent diabetes mellitus (IDDM) is thought to depend on the degree of hyperglycemia, but the relation between the degree of hyperglycemia and urinary albumin excretion has not been defined.

Methods. We measured urinary albumin excretion in three random urine samples obtained at least one month apart from 1613 patients with IDDM. Microalbuminuria or overt albuminuria was considered to be present if the ratio of albumin (in micrograms) to creatinine (in milligrams) was 17 to 299 or ≥ 300 , respectively, for men and 25 to 299 or ≥ 300 , respectively, for women. Measurements of glycosylated hemoglobin (hemoglobin A_{1c}) obtained up to four years before the urine testing were used as an index of hyperglycemia. Twelve percent of the patients had overt albuminuria and were excluded from subsequent analyses.

Results. The prevalence of microalbuminuria was 18

percent in patients with IDDM. It increased with increasing postpubertal duration of diabetes and, within each six-year interval of disease duration, it increased nonlinearly with the hemoglobin A_{1c} value. For hemoglobin A_{1c} values below 10.1 percent, the slope of the relation was almost flat, whereas for values above 10.1 percent, the prevalence of microalbuminuria rose steeply ($P < 0.001$). For example, as the hemoglobin A_{1c} value increased from 8.1 to 10.1 percent, the odds of microalbuminuria increased by a factor of 1.3, but as the value increased from 10.1 to 12.1 percent, the odds were increased by a factor of 2.4.

Conclusions. The risk of microalbuminuria in patients with IDDM increases abruptly above a hemoglobin A_{1c} value of 10.1 percent (equivalent to a hemoglobin A_{1c} value of 8.1 percent), suggesting that efforts to reduce the frequency of diabetic nephropathy should be focused on reducing hemoglobin A_{1c} values that are above this threshold. (N Engl J Med 1995;332:1251-5.)

DIABETIC nephropathy is the chief cause of morbidity and premature mortality in patients with insulin-dependent diabetes mellitus (IDDM).^{1,2} This complication is first manifested as an increase in urinary albumin excretion (microalbuminuria), which progresses to overt albuminuria and then to renal failure.³ Improved glycemic control seems to delay or prevent the onset of microalbuminuria, but the development of cost-effective preventive strategies requires knowledge of the increase in the risk of nephropathy associated with each increase in the degree of hyperglycemia.^{4,6} The recently published results of the Diabetes Control and Complications Trial (DCCT) showed that intensive treatment of diabetes reduces the risk of diabetic complications, including microalbuminuria, but the relation between the degree of hyperglycemia and the risk of microalbuminuria was not examined.⁷ In this study, we examined the relation between the degree and duration of hyperglycemia and the prevalence of microalbuminuria in a large cohort of patients with IDDM.

METHODS

Characteristics and Evaluation of the Study Subjects

Between January 1, 1991, and March 31, 1992, every other diabetic patient between the ages of 15 and 44 years who visited the internal medicine or pediatrics clinic of the Joslin Diabetes Center was screened for microalbuminuria. Most of the patients were referred to the center soon after the diagnosis of diabetes and had received most of their care at the center since that time. Before this study, no sys-

tematic screening for microalbuminuria had been conducted at the center. Patients who visited the pregnancy clinic or who had given birth within the preceding six weeks were excluded. Additional eligibility requirements included an onset of diabetes before the age of 41 years, Massachusetts residency, and registration at the center before 1991. The screening protocol was approved by the committee on human subjects at the center.

By March 31, 1992, a total of 1795 patients had been screened at least once for microalbuminuria. The urine samples were collected randomly at the time of the clinic visit, with no advance instructions concerning fluid intake or urination. The laboratory continued to save urine samples from these patients whenever they returned to the clinic. This analysis is based on results available by December 31, 1993.

Urine samples with abnormal sediments on routine urinalysis were discarded. All others were assayed for albumin within seven days after collection (samples not analyzed immediately were refrigerated) with the use of reagent strips (Multistix; Ames Division, Miles Laboratories, Elkhart, Ind.), which were read by an optical scanner. If the reading was strongly positive ($\geq 2+$; albumin concentration, $\geq 1000 \mu\text{g}$ per milliliter), the patient was considered to have overt albuminuria and the sample was not analyzed further. In the remaining samples, the urinary albumin concentration was measured by immunonephelometry with N Albumin kits (Behring, Somerville, N.J.) normally used to measure serum albumin and a manufacturer-supplied protocol specifically designed to detect the low concentrations of albumin in urine.⁸ The intraassay and interassay coefficients of variation were less than 2 percent and less than 4 percent, respectively. Urinary creatinine concentrations were measured by colorimetry (modified Jaffé reaction) on an Astra-7 automated system (Beckman Instruments, Brea, Calif.).

For male patients normoalbuminuria was defined as a ratio of albumin (measured in micrograms) to creatinine (measured in milligrams) of less than 17 and for female patients as a ratio of less than 25. These sex-specific values are equivalent to a urinary albumin excretion rate of 30 μg per minute (unpublished data). A ratio of albumin to creatinine of 300 or higher, regardless of sex, was considered to indicate overt albuminuria. Microalbuminuria was defined as a ratio of albumin to creatinine in the intermediate range: 17 to 299 for male patients and 25 to 299 for female patients. The results of one or two subsequent measurements in 80 percent of the patients one or more months later (median, five) were very similar to the initial results (Spearman correlation, 0.81), confirming that the majority of patients were properly categorized according to urinary albumin excretion in the first sample.

If the results of a second measurement placed the patient in a dif-

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Supported by a grant (RO1-DK41526) from the National Institutes of Health.

ferent category from that based on the first measurement, a third urine sample was obtained to confirm either the first or second measurement. If the results of all three measurements were different, the patient was considered to have microalbuminuria. If a third urine sample was not obtained (as was true for 5 percent of the group), the assignment was based on the geometric mean of the first two measurements. If the results of only one measurement were available (15 percent of the entire group), they were used to make the classification. The 20 percent of patients classified on the basis of one assay or two discordant assays did not differ from the rest with respect to age, sex, duration of diabetes, glycosylated hemoglobin (hemoglobin A_{1c}) value, or urinary albumin excretion. The 24 patients who had received renal transplants were classified as having overt albuminuria regardless of assay results.

We extracted from the records of the clinical laboratory all information on measurements of hemoglobin A_{1c} in these patients during two periods: the 12 months of 1988 and the 24 months of 1990 and 1991. The degree of hyperglycemia in each patient was determined by calculating the geometric mean of all the hemoglobin A_{1c} values obtained in 1988 and the geometric mean of three values (separated by at least a month) obtained in 1990 and 1991 (values for 1989 could not be readily abstracted from the patients' records). Hemoglobin A_{1c} (normal range, 5.4 to 7.4 percent) was measured electrophoretically (Corning Medical and Scientific, Corning, N.Y.).⁹ To facilitate comparisons with the results of the DCCT, 52 blood samples were analyzed in the standard manner by our laboratory and according to the Diamat high-performance liquid chromatographic method (Bio-Rad Laboratories, Hercules, Calif.¹⁰) by the Department of Pathology, University of Missouri School of Medicine, Columbia. The latter assay was calibrated to match the reference system used by the central hemoglobin A_{1c} laboratory of the DCCT.¹¹ Hemoglobin A_{1c}, a component of hemoglobin A₁, is the most chemically specific glycated hemoglobin that can be measured.¹⁰ The correlation between the two sets of results was 0.98, and linear regression analysis was used to determine a conversion formula that would yield hemoglobin A_{1c} values that corresponded approximately to the hemoglobin A_{1c} values obtained in our laboratory (hemoglobin A_{1c} = [hemoglobin A_{1c} - 0.14]/1.23).

The date of diagnosis of diabetes was abstracted from the medical records or obtained from the patients. All cases of diabetes diagnosed in patients less than 21 years old were classified as insulin-dependent. If diabetes was diagnosed between the ages of 21 and 40 years, the medical record was reviewed by a physician to classify the type of diabetes. Patients who began insulin therapy within two years after the diagnosis of diabetes and continued to receive it were considered to have IDDM. An age of less than 41 years at diagnosis and a need for continuous treatment with insulin were found to be accurate criteria for an operational definition of IDDM in other studies.^{12,15}

Of 1795 patients screened, 177 (10 percent) had non-insulin-dependent diabetes and were excluded from the analysis. Another five patients were excluded because they had nondiabetic renal disease predating the diagnosis of diabetes. Ninety-two percent of the remaining 1613 patients reported their racial or ethnic origin as non-Hispanic white. The remaining 8 percent of the group was composed of nearly equal proportions of blacks, Hispanics, and Asians; all patients in these groups were considered together for this analysis.

Age, the duration of diabetes, and the duration of follow-up at the Joslin Diabetes Center were calculated as of the date the second urine sample was obtained. For patients given a diagnosis of diabetes before the age of 10 years, the duration of the disease after puberty was calculated beginning after the 10th birthday, since the years of diabetes before puberty do not contribute to the development of diabetic nephropathy.^{14,15}

Statistical Analysis

Analysis of variance and analysis of covariance were used to compare the subgroups of patients with different degrees of albuminuria; Tukey's studentized range test was used to assess statistical significance. The relations between the prevalence of microalbuminuria and the hemoglobin A_{1c} value and the postpubertal duration of diabetes were evaluated by calculations of odds ratios.¹⁶ The statistical significance of these associations, after adjustment for covariates, was evaluated with logistic-regression models in which hemoglobin A_{1c} was treated as a grouped variable or expressed as a logarithm.^{17,18}

To evaluate the relation between the hemoglobin A_{1c} level and the

Table 1. Clinical Characteristics of Patients with IDDM, According to the Degree of Albuminuria.*

| CHARACTERISTIC | NORMO-ALBUMINURIA (N = 1117) | MICRO-ALBUMINURIA (N = 295) | OVERT ALBUMINURIA (N = 201) | P VALUE |
|---|------------------------------|-----------------------------|-----------------------------|---------|
| Male sex (%) | 46 | 51 | 52 | >0.10 |
| Age (yr) | 28±8 | 30±8 | 33±5 | † |
| Duration of IDDM (yr) | 12±8 | 17±8 | 22±6 | <0.001 |
| Duration of IDDM at time of first visit to Joslin Center (yr) | 3±5 | 3±5 | 5±7 | † |
| Age at diagnosis of IDDM (yr) | 16±9 | 12±8 | 10±6 | † |
| Hemoglobin A _{1c} (%) | | | | |
| In 1988 | 10.3±2.0 | 11.3±2.3 | 11.7±2.3 | <0.001‡ |
| In 1990–1991 | 10.3±1.9 | 11.3±2.0 | 11.6±1.9 | <0.001‡ |
| Correlation between 1988 value and 1990–1991 value§ | 0.60 | 0.60 | 0.34 | |

*Plus-minus values are means ±SD.

†The differences between the groups do not reflect an independent effect of this variable but are indirect associations due to the correlation of this variable with a long duration of IDDM.

‡The values in the groups with microalbuminuria and overt albuminuria were not significantly different from each other but were significantly higher than the values in the group with normoalbuminuria. The P values shown are based on comparisons of the log-transformed hemoglobin A_{1c} values with the use of Tukey's studentized range test.

§All correlations were statistically significant (P<0.001).

risk of microalbuminuria, three alternative models were examined: a simple exponential,¹⁸ a threshold,¹⁹ and a changepoint²⁰ model.

RESULTS

Among the 1613 patients (duration of IDDM, 1 to 39 years) who were screened for microalbuminuria during the 36-month study period, 295 (18 percent) had microalbuminuria and 201 (12 percent) had overt albuminuria. The characteristics of the patients are summarized in Table 1 according to the degree of albuminuria. Both the patients with microalbuminuria and those with overt albuminuria had had diabetes longer and had higher hemoglobin A_{1c} values in both 1988 and 1990 to 1991 than the patients with normoalbuminuria. The hemoglobin A_{1c} values in the group with microalbuminuria and the group with overt albuminuria were similar during both periods. The correlation between the hemoglobin A_{1c} values in 1988 and those in 1990 to 1991 was high in the patients with normoalbuminuria (r=0.60) and in the patients with microalbuminuria (r=0.60), but not in those with overt albuminuria (r=0.34), indicating considerable stability in the degree of hyperglycemia in the first two groups. By contrast, the low correlation in the patients with overt albuminuria suggests greater variation in the degree of hyperglycemia, as a result of intensified efforts to control glycemia prompted by the clinical diagnosis or deteriorating renal function. For this reason, the patients with overt albuminuria were excluded from further analyses.

To examine the association between microalbuminuria and the degree of hyperglycemia, the patients with normoalbuminuria and microalbuminuria were subdivided into quintiles based on the distribution of the hemoglobin A_{1c} values in 1990 to 1991 (5.9 to 8.8 percent, 8.9 to 9.8 percent, 9.9 to 10.7 percent, 10.8 to 11.9 percent, and 12.0 to 21.3 percent). The prevalence of mi-

Table 2. Odds Ratios for the Effect of Variations in Hemoglobin A₁ Values on the Development of Microalbuminuria, According to the Postpubertal Duration of IDDM.*

| DURATION OF IDDM (YR) | HEMOGLOBIN A ₁ VALUES IN 1990-1991 | | | | |
|-----------------------|---|-----------|-----------|------------|------------|
| | 5.9-8.8% | 8.9-9.8% | 9.9-10.7% | 10.8-11.9% | 12.0-21.3% |
| | <i>odds ratio (total no. of patients)</i> | | | | |
| 1-6 | 1.0 (104)† | 1.6 (65) | 2.6 (64) | 2.2 (49) | 5.8 (74) |
| 7-12 | 2.4 (58) | 2.3 (82) | 2.4 (81) | 6.8 (94) | 13.2 (107) |
| 13-18 | 2.3 (47) | 4.7 (69) | 3.9 (52) | 7.5 (52) | 28.8 (43) |
| 19-24 | 11.3 (45) | 15.0 (40) | 14.3 (44) | 12.1 (52) | 23.6 (35) |
| 25-32 | 7.1 (27) | 7.9 (25) | 13.0 (35) | 19.0 (37) | 12.5 (21) |

*The geometric mean ratios of albumin to creatinine in the patients with microalbuminuria were 67.6, 66.1, 56.2, 73.4, and 56.5 for those with hemoglobin A₁ values ranging from 5.9 to 8.8 percent, 8.9 to 9.8 percent, 9.9 to 10.7 percent, 10.8 to 11.9 percent, and 12.0 to 21.3 percent, respectively. Hemoglobin A₁ was not measured in 10 patients.

†The prevalence of microalbuminuria was 3.8 percent in the reference group.

croalbuminuria was 11.7 percent in the lowest quintile of hemoglobin A₁ and increased progressively with each quintile to 15.3 percent, 17.4 percent, 24.5 percent, and 36.1 percent. To control for the effect of the duration of diabetes, the prevalence of microalbuminuria in each of these quintiles was examined according to the postpubertal duration of diabetes (Table 2). Among patients who had had diabetes six years or less, the prevalence of microalbuminuria in the lowest quintile of hemoglobin A₁ was 3.8 percent. This prevalence, which is much higher than the prevalence of 0.9 percent reported in normal subjects (unpublished data), was used as the reference point for calculating the odds ratios for other combinations of hemoglobin A₁ values and durations of diabetes. The pattern of results was similar when the 1988 and 1990 to 1991 hemoglobin A₁ values were combined (data not shown).

The risk of microalbuminuria increased with the duration of IDDM (Table 2) except among patients who had had diabetes for 25 to 32 years. In a multiple logistic-regression model with adjustment for hemoglobin A₁ value, sex, and age at the time of diagnosis, each successive 6-year interval of disease duration through the 19-to-24-year interval was associated with an increase in risk of approximately 80 percent ($P < 0.001$). The absence of any additional increase in risk with a longer duration of disease may be due to the exhaustion of the pool of susceptible patients after 24 years.²¹

The risk of microalbuminuria also increased with the level of hemoglobin A₁ (Table 2). The risk rose moderately between the first and fourth quintiles and then steeply in the fifth quintile. This pattern was almost identical in each category of disease duration except for that spanning 25 to 32 years. The increment in risk between the first and fifth quintiles of hemoglobin A₁ was estimated in a multiple logistic-regression model that adjusted for the duration of diabetes, sex, and age at diagnosis. As compared with the first quintile, the risk rose 34 percent ($P = 0.29$) in the second quintile, 33 percent ($P = 0.31$) in the third quintile, 101 percent ($P = 0.009$) in the fourth quintile, and 412 percent ($P < 0.001$) in the fifth quintile. Hemoglobin A₁ was then modeled as a continuous variable, and the fifth

quintile was treated as an outlier. The relative risk increased by a factor of 1.25 with each increase of 1 percentage point on the hemoglobin A₁ scale ($P < 0.001$), but in the fifth quintile, the relative risk was higher by a factor of 1.67 ($P = 0.04$) than that predicted by the regression line.

We examined the evidence of a nonlinear relation between the risk of microalbuminuria and the hemoglobin A₁ value more closely by grouping the hemoglobin A₁ values in small intervals of equal width (0.45 percent) (Fig. 1). The intervals in the tails of the distribution were combined as necessary to maintain the sample size. The hemoglobin A₁ groups were modeled with indicator variables in a logistic-regression model of the prevalence of microalbuminuria that included covariates to adjust for age at onset of diabetes, the duration of diabetes, and sex. As before, patients who had had diabetes for at least 25 years were excluded. The reference group for the adjusted relative odds was the group of patients with the lowest hemoglobin A₁ values (range, 5.9 to 7.9 percent; mean, 7.3 percent). To find

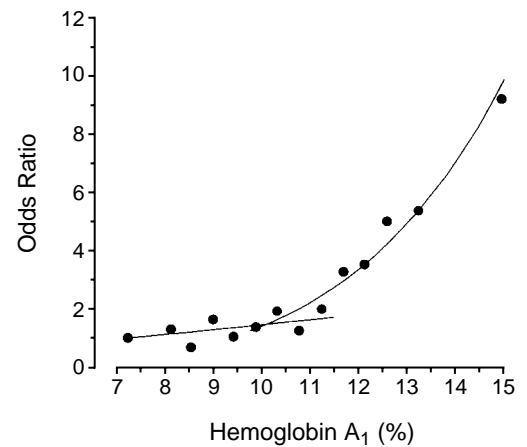


Figure 1. Relation between Mean Hemoglobin A₁ Values and the Risk of Microalbuminuria in Patients with IDDM.

Hemoglobin A₁ values were grouped into small intervals of equal width (0.45 percent, or 0.90 percent in the tails) and modeled with indicator variables in a logistic-regression model of the prevalence of microalbuminuria with covariates to adjust for the age at onset of diabetes, the duration of diabetes, and sex. The reference group for the adjusted relative odds (circles) was the group of patients with hemoglobin A₁ values ranging from 5.9 to 7.9 percent. A changepoint model, including the same covariates, was fitted to the logarithm of hemoglobin A₁ as a continuous variable to estimate the location of the changepoint and the regression slopes below and above the changepoint (continuous line). The horizontal axis shows hemoglobin A₁ values as well as the equivalent values of hemoglobin A_{1c} (see the Methods section) and the blood glucose profile.¹¹ In the DCCT, the blood glucose profile was determined by measuring the capillary-blood glucose concentration seven times in a 24-hour period (before and 90 minutes after each of the three major meals and before bedtime). The results of quarterly determinations of the blood glucose profile over a one-year period were averaged, and the line of regression was determined on the basis of the average of quarterly determinations of hemoglobin A_{1c} during the same year. To convert values for blood glucose to millimoles per liter, multiply by 0.05551.

a suitable model for the relation between the risk of microalbuminuria and the degree of hyperglycemia, we tested three alternative logistic-regression models: a simple exponential,¹⁸ a threshold,¹⁹ and a change-point²⁰ model. The results were similar. The simple exponential model confirmed the earlier indication of nonlinearity because the resulting curve bent sharply upward, in a manner consistent with the occurrence of a threshold. The results of the threshold test were significant ($P=0.03$), with an estimated threshold value for hemoglobin A₁ of 9.9 percent. The change-point model estimated the threshold at 10.1 percent and allowed a non-zero slope for the line below the threshold — a model that seemed to represent the data most closely (Fig. 1).

For hemoglobin A₁ values below 10.1 percent, the slope of the relation was almost flat ($P=0.25$), whereas for values above 10.1 percent the prevalence of microalbuminuria rose steeply ($P<0.001$). For example, as the hemoglobin A₁ value increased from 8.1 to 10.1 percent, the odds of microalbuminuria were increased by a factor of 1.3 (95 percent confidence interval, 0.8 to 2.0), but as the value increased from 10.1 to 12.1 percent, the odds were increased by a factor of 2.4 (95 percent confidence interval, 1.9 to 3.0).

DISCUSSION

We found that the risk of microalbuminuria in patients with IDDM is strongly related to both the duration of diabetes and the degree of hyperglycemia, measured as the prevailing level of hemoglobin A₁ during the preceding two to four years. In patients who had had diabetes for less than 25 years and whose hemoglobin A₁ values were below 10.1 percent, the risk of persistent microalbuminuria varied little, although it was higher than in normal subjects. In contrast, in patients with hemoglobin A₁ values above 10.1 percent, the risk of microalbuminuria rose steeply. This nonlinear pattern for the relation between hemoglobin A₁ values and the risk of microalbuminuria was independent of the effect of the duration of diabetes; the latter was an independent risk factor for which no threshold effect could be found.

Our results are consistent with those of the DCCT.⁷ In both the primary- and secondary-prevention components of that trial, the patients who received intensive treatment had a statistically significant reduction in the cumulative incidence of microalbuminuria as compared with the patients who received conventional treatment. The lower risk of microalbuminuria in the former group was attributed to improved glycemic control, but the relation was not further defined. A relation between elevated hemoglobin A₁ values and an increased risk of microalbuminuria has been reported previously, but none of the studies had a sample size sufficient to examine the relation more closely.²²⁻²⁵ Small sample size has not been the only obstacle to the detection of a threshold in the relation. An emphasis on models that smooth the data has been a factor,^{7,26} and there are limitations inherent in the statistical procedures used for this purpose.^{19,27} The finding in the

DCCT of a high risk of hypoglycemia associated with intensive treatment prompted our scrutiny of the evidence of a threshold in the relation between the hemoglobin A₁ value and the risk of microalbuminuria. There seems to be a similar threshold in the relation between the degree of hyperglycemia and the development of diabetic retinopathy.²⁶

The distinctly different risks of microalbuminuria in patients with low hemoglobin A₁ values and those with high values suggest that diabetes damages the kidney through several mechanisms. The mechanisms operating below the threshold hemoglobin A₁ value of less than 10.1 percent (which corresponds to prevailing blood glucose concentrations of less than 200 mg per deciliter [11.1 mmol per liter]) seem to be independent of the level of hyperglycemia and may be influenced by other components of the diabetic milieu — for example, abnormalities in plasma insulin concentrations.^{28,29} At high hemoglobin A₁ values, which are indicative of high blood glucose concentrations, microalbuminuria is most likely caused by the deleterious effects of hyperglycemia on cell functions and extracellular structures such as the basement membrane and mesangial matrix.^{30,31}

Our data have several shortcomings. First, the measurements of hemoglobin A₁ determined before the screening for microalbuminuria can only be considered as an approximation of the degree of hyperglycemia during the interval in which microalbuminuria developed. However, the resulting misclassification of the degree of hyperglycemia most likely increased the random variation of hemoglobin A₁ values, making the detection of a threshold value for hemoglobin A₁ more difficult.³² Second, since measurements of hemoglobin A₁ (and hemoglobin A_{1c}) vary among laboratories, the threshold value reported here cannot be generalized, except to laboratories that have calibrated their values to a reference method such as that used in the DCCT.^{10,11} Third, our findings were obtained in patients whose IDDM had been diagnosed before the age of 41 years. Whether there is a similar relation between the degree of hyperglycemia and the development of microalbuminuria in older patients with IDDM and in patients with non-insulin-dependent diabetes mellitus is not known.

In conclusion, our findings have implications for the care of patients with IDDM. Patients and care providers should give the highest priority to improving glycemic control sufficiently to maintain hemoglobin A₁ values below 10.1 percent (equivalent to hemoglobin A_{1c} values below 8.1 percent). If this can be achieved, the number of patients in whom microalbuminuria develops should decline substantially, which should, in turn, lower the number in whom overt macroalbuminuria and end-stage renal disease develop.

We are indebted to Drs. A.R. Christlieb and K. Quicquel for assistance in starting the study; to the clinic staff for help in implementing the screening; to Dr. J. Robins for comments regarding the threshold model; to Drs. R.R. Little and D.E. Goldstein for assistance in developing a conversion formula for expressing the value for hemoglobin A₁ as an approximate hemoglobin A_{1c} value; and to Mr. G. Gearin,

Mr. F. Denri, Mr. S. Federman, Mr. M. Wantman, and Ms. W. Fisher of the Section on Epidemiology and Genetics, Joslin Diabetes Center, for making the study possible.

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