

# Hemoglobin A<sub>1c</sub> as an Indicator of the Degree of Glucose Intolerance in Diabetes

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## SUMMARY

Hemoglobin A<sub>1c</sub> concentration, fasting blood sugar, response to an oral glucose tolerance test, and skeletal muscle capillary basement membrane thickness were measured in diabetic patients. Hemoglobin A<sub>1c</sub> concentration correlates with both response to a glucose tolerance test ( $r = 0.82$ ,  $p < 0.001$ ) and fasting blood sugar ( $r = 0.62$ ,  $p < 0.001$ ). The correlation of hemoglobin A<sub>1c</sub> concentration with glucose tolerance is independent of fasting blood sugar concentration (partial  $r = 0.61$ ,  $p < 0.005$ ), whereas that of hemoglobin A<sub>1c</sub> with fasting blood sugar probably reflects the relationship between fasting blood sugar levels and glucose tolerance (partial  $r = 0.22$ ,  $p > 0.05$ ). Hemoglobin A<sub>1c</sub> levels do not correlate with basement membrane thickness ( $r = 0.15$ ,  $p > 0.05$ ). *DIABETES* 25:230-32, March, 1976.

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Although the abnormalities of glucose metabolism are well defined in diabetes mellitus, it has been difficult to correlate these abnormalities with the major secondary sequelae of the disease, including micro- and macroangiopathy. We have recently initiated studies of the glycohemoglobin, hemoglobin A<sub>1c</sub>, in hopes of gaining insight into the pathologic sequelae of diabetes mellitus.

Hemoglobin A<sub>1c</sub> (Hb A<sub>1c</sub>), which comprises about 3-6 per cent of normal human hemoglobin, is elevated to 6-12 per cent in diabetes.<sup>1</sup> Hemoglobin A<sub>1c</sub> differs from hemoglobin A in that an unknown carbohydrate is attached to the amino-terminal valine of the  $\beta$ -chain.<sup>2</sup> Studies in the diabetic mouse<sup>3,4</sup> have shown a similarly elevated hemoglobin that is made as

a postsynthetic modification of hemoglobin A at a constant rate throughout the life of the red cell. The rate of synthesis of hemoglobin A<sub>1c</sub> appears to be dependent on the presence of an unknown plasma factor present in elevated amounts in mice with either genetic or chemically induced diabetes after they become hyperglycemic. Hemoglobin A<sub>1c</sub> production is an example of the modification of a naturally occurring protein by the addition of a carbohydrate moiety that results in altered characteristics—e.g., electrophoretic mobility<sup>5</sup> and oxygen affinity.<sup>6</sup> Similar glycosylation reactions might explain many of the secondary abnormalities in diabetes, including white cell dysfunction,<sup>7</sup> platelet dysfunction,<sup>8</sup> and basement membrane thickening,<sup>9</sup> as well as abnormal structure or function of other specific proteins—for example, lens<sup>10</sup> or peripheral nerves.<sup>11</sup>

In an attempt to correlate hemoglobin A<sub>1c</sub> with the microangiopathy of diabetes, hemoglobin A<sub>1c</sub> was measured in normal subjects and in diabetics, with and without basement-membrane thickening. Glucose tolerance tests and measurements of skeletal muscle capillary basement-membrane thickness, in addition to fasting blood-sugar determinations, were performed in subgroups of those subjects.

## MATERIALS AND METHODS

A total of 38 diabetic patients, ranging in age from 16 to 82 years, were studied. Seventeen were maintained on insulin therapy, 12 on oral hypoglycemic agents, and nine on diet alone. Thirteen patients were females; 25 were males.

Muscle capillary basement-membrane thickness was determined in 21 of these patients as described previously.<sup>12</sup> Hemoglobin A<sub>1c</sub> was isolated in dupli-

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cate by the column chromatographic method of Trivelli et al.<sup>1</sup> and quantified by the method of Drabkin.<sup>13</sup>

Fasting blood sugars were measured on at least two occasions in 33 patients. Oral glucose tolerance tests were performed by giving a 75-gm. load of glucose after an overnight fast. Glucose concentrations were determined by an AutoAnalyzer. Ten nondiabetic controls, as judged by normal responses to the oral glucose load,<sup>14</sup> were also studied. The response to the glucose challenge was determined by measuring the area under the curve by planimetry of a three-hour glucose tolerance test. These studies were approved by the Clinical Review Boards of the appropriate institutions.

RESULTS

Glucose tolerance tests were performed on 22 diabetic patients. In this group, hemoglobin A<sub>1c</sub> levels varied from 4.49 per cent to 11.20 per cent. The peak glucose response to the glucose tolerance test ranged from 184 mg./dl. to 880 mg./dl. The correlation coefficient between these two variables is highly significant ( $r = 0.82, p < 0.001$ ) (figure 1). The results are unaltered by utilizing the areas under the glucose tolerance curves ( $r = 0.76, p < 0.001$ ) instead of the peak values. A significant but less impressive correlation was also found between fasting blood sugar and hemoglobin A<sub>1c</sub> levels in the 33 diabetics studied ( $r = 0.62, p < 0.001$ ) (figure 2). Because fasting blood sugar concentrations and response to a glucose

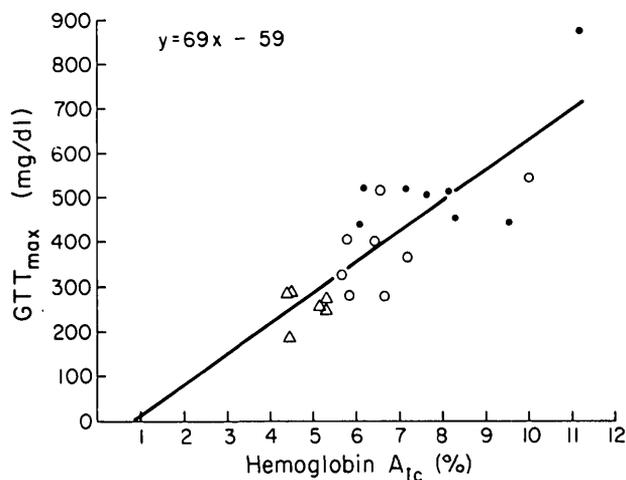


FIG. 1. Correlation between hemoglobin A<sub>1c</sub> concentration and maximal response to an oral glucose tolerance test in 22 diabetics (● patients treated with insulin, ○ patients treated with oral hypoglycemic agents, Δ patients treated with diet alone).

tolerance test are interdependent variables that correlate well with each other ( $r = 0.77, p < 0.001$ ), their respective correlations with hemoglobin A<sub>1c</sub> concentration might simply reflect their interdependence. Partial correlation coefficients,<sup>15</sup> which take into account the interdependence of response to a glucose tolerance test and fasting blood sugar, revealed that hemoglobin A<sub>1c</sub> concentration and response to a glucose tolerance test are indeed correlated (partial  $r = 0.61, p < 0.005$ ); whereas the correlation between fasting blood sugar and hemoglobin A<sub>1c</sub> concentrations loses its significance (partial  $r = 0.22, p > 0.05$ ). The ten nondiabetic controls had hemoglobin A<sub>1c</sub> concentrations ranging from 3.97 per cent to 5.04 per cent, and peak values of 73 mg./dl. to 216 mg./dl. during the oral glucose tolerance test. There was no correlation between hemoglobin A<sub>1c</sub> and glucose tolerance in this population ( $r = -0.43, p > 0.05$ ).

Muscle capillary basement-membrane thickness varied from  $612 \pm 141$  to  $2,975 \pm 830$  Å in the 21 diabetics studied. The hemoglobin A<sub>1c</sub> concentrations of these same patients ranged from 4.49 per cent to 10.84 per cent. The correlation coefficient obtained

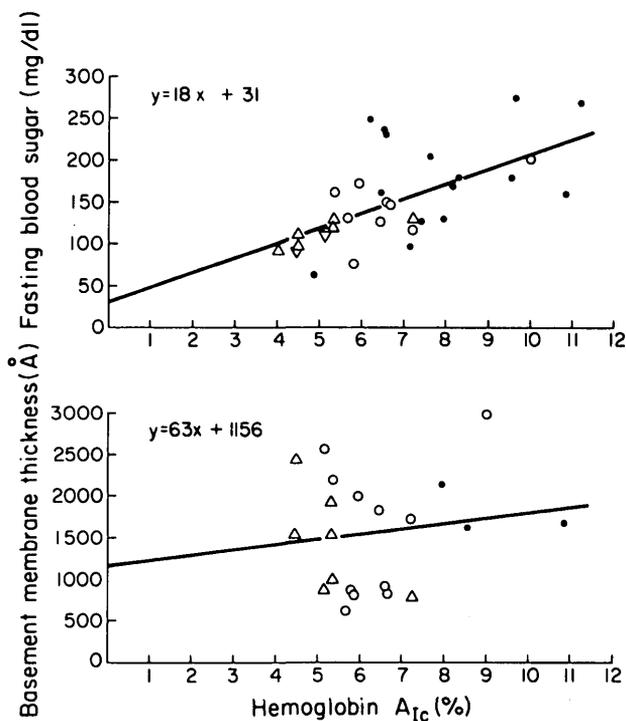


FIG. 2. Correlations between hemoglobin A<sub>1c</sub> levels and both fasting blood sugar (top) and skeletal muscle capillary basement membrane thickness (bottom) in diabetic patients (● patients treated with insulin, ○ patients treated with oral hypoglycemic agents, Δ patients treated with diet alone).

between these two parameters was not statistically significant ( $r = 0.15$ ,  $p > 0.05$ ) (figure 2).

## DISCUSSION

Although a previous study<sup>1</sup> failed to demonstrate a correlation between the degree of elevation of "fast" hemoglobins (which include Hb A<sub>1c</sub>) and clinical parameters of diabetes, we report a highly significant correlation ( $r = 0.82$ ,  $p < 0.001$ ) between Hb A<sub>1c</sub> concentration and response to an oral glucose tolerance test in diabetic patients. This correlation is independent of fasting blood sugar levels, as determined by the partial correlation coefficient (partial  $r = 0.61$ ,  $p < 0.005$ ). Although a significant correlation was found between hemoglobin A<sub>1c</sub> and fasting blood sugar concentrations ( $r = 0.62$ ,  $p < 0.001$ ), this correlation probably reflects the interdependence of fasting blood sugar and glucose tolerance, since the partial correlation coefficient, which takes this interdependence into account, revealed no correlation between fasting blood sugar and Hb A<sub>1c</sub> concentrations (partial  $r = 0.22$ ,  $p > 0.05$ ). Hemoglobin A<sub>1c</sub> levels probably reflect, better than fasting blood sugar concentrations or glucose tolerance test results, the mean daily blood sugar concentration and the degree of carbohydrate imbalance, and may provide a better index of control of the diabetic patient without resorting to a glucose loading procedure.

The lack of correlation of hemoglobin A<sub>1c</sub> levels with muscle capillary basement-membrane thickness is disappointing, since the thickening of basement membrane is believed to be intimately related to the microangiopathic sequelae of diabetes. This lack of correlation may be due either to the fact that basement-membrane thickening is probably the result of kinetic and chemical factors, some of which are unrelated to events involved in Hb A<sub>1c</sub> synthesis, or to the fact that the hemoglobin A<sub>1c</sub> levels measured reflect the degree of control over only the previous few months, whereas several years are required for the development of detectable basement-membrane thickening.

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