Circulating 1,5-Anhydroglucitol Levels in Adult Patients With Diabetes Reflect Longitudinal Changes of Glycemia

A U.S. trial of the GlycoMark assay

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OBJECTIVE — 1,5-Anhydroglucitol (1,5AG) is a major circulating polyol arising primarily from ingestion and excreted competitively with glucose. Japanese studies have demonstrated reduced concentrations of 1,5AG in serum in hyperglycemic patients in comparison with euglycemic subjects and a gradual normalization of 1,5AG values for patients responding to antihyperglycemic therapies. In this first U.S. study, we assessed the ability of 1,5AG measurements to monitor glycemic control in a cohort of 77 patients with diabetes (22 with type 1 diabetes, 55 with type 2 diabetes) who presented with suboptimal glycemic control at baseline (defined as HbA1c ≥7%).

RESEARCH DESIGN AND METHODS — Each patient received therapies consisting of combinations of diabetes education, nutritional counseling, and addition or dose adjustment of various insulins or oral antihyperglycemic medications. Therapy was targeted to reduce mean HbA1c by ≥1.0% over the monitoring period. 1,5AG, fructosamine, and random glucose measurements were performed at baseline and at 2, 4, and 8 weeks after the initiation of therapy.

RESULTS — 1,5AG, fructosamine, and glucose values progressed significantly toward euglycemia by week 2 of monitoring (Wilcoxon’s signed-rank test, P < 0.05), with median changes of 93, −7, and −13% for 1,5AG, fructosamine, and glucose, respectively. In contrast, HbA1c values did not respond significantly to therapy until week 4. On an individual patient basis, 89.6% of patients displayed longitudinal changes of 1,5AG from baseline to week 8 in concordance with HbA1c. 1,5AG was also highly correlated with HbA1c and fructosamine (Spearman r = −0.6459 and −0.6751, respectively; both P < 0.0001).

CONCLUSIONS — We conclude that 1,5AG responds sensitively and rapidly to changes in glycemia and monitors glycemic control in accordance with established markers.

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The incidence of diabetes is clearly a serious health concern. During the period 1991–2000, the incidence in the U.S. increased by 49% (4.9–7.3% of the total population), and this was correlated with a 61% increase in obesity during the same period (1,2). For those afflicted with diabetes, control of their glycemia is of paramount importance because good metabolic control can reduce the incidence of micro- and macrovascular complications (2–5).

In recent years, various biochemical markers have been cleared by the U.S. Food and Drug Administration (FDA) for assessment of glycemia. These include self-monitored blood glucose methods and assays for HbA1c and fructosamine. These tests differ in the time period in which measured values reflect glycemia. Random glucose measurements convey a “snapshot” of ambient circulating glucose but do not report the consistency of glycemic control or excursion thereof. In contrast, HbA1c and fructosamine measurements reflect time-averaged glycemia in the past 2–3 months and in the past 2–3 weeks, respectively. Of these two measures, HbA1c has been identified in multiple studies as the most valid predictor of risk of complications (2–6). However, despite the demonstrated use of HbA1c measurements, the slow rate of change may contribute to delays in modification of therapy. Thus, a marker that responds rapidly and significantly to changes in glycemia, that is metabolically stable, that demonstrates low biological variability, and that can be easily measured would be useful in management of patients with diabetes.

1,5-Anhydroglucitol (1,5AG) has been recently proposed as a marker conforming to these criteria (7). 1,5AG was first discovered in the plant family Polygala senega in 1888. The structure was identified in 1943, and the presence of the compound in human blood (8) and cerebrospinal fluid (9) was established in 1972 and 1973, respectively. Research studies have shown that 1,5AG originates mostly from foods with a mean intake of ~4.4 mg/day, that the closed pyran ring structure confers metabolic stability, that the rate of intake is matched by the daily excretion rate, and that a bodily pool of ~500–1,000 mg of 1,5AG is constantly

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maintained (10). The body pool may originate from an accumulation of small amounts of retained dietary 1,5AG or from biosynthesis. There is some evidence to support de novo biosynthesis of 1,5AG in the amount of ~0.5 mg/day (10), but this has neither been extensively investigated nor confirmed.

1,5AG is well absorbed in the intestine and distributes to all organs and tissues (11). Renal reabsorption of 1,5AG is 99.9% and is competitively inhibited by excess excretion of urinary glucose (glucosuria). With this finding, Japanese research groups demonstrated reduced concentrations of 1,5AG in serum of hyperglycemic patients in comparison with euglycemic subjects (12,13). Additionally, a gradual normalization of 1,5AG values for patients responding to antidiabetic therapies (14) has been demonstrated, and studies have shown that 1,5AG measurements reflect glycemic status over the previous 48 h to 2 weeks. Dietary variation does not appreciably affect the efficacy of such measurements because the content of 1,5AG is similar in various starches (mean, 2.5 ± 1.1 μg/g), meats and seafood (0.9 ± 0.6 μg/g), vegetables (0.4 ± 0.2 μg/g), fruits (0.7 ± 0.6 μg/g), and beverages (0.8 ± 0.7 μg/g) (10). Only raw soybeans have been demonstrated to have significantly enriched levels of 1,5AG, although processed soybeans (e.g., tofu, soy sauce) have 1,5AG content essentially equivalent to all other starches (10). Based on these early analytical and clinical findings, an automated assay using an enzymatic methodology was developed and has been commercially available in Japan since 1991 (15). A domestic version of this assay (GlycoMark) has been under evaluation in clinical trials in the U.S. and has recently been cleared for marketing by the FDA as a tool for intermediate-term monitoring of glycemia.

It is the objective of this study to present the results of the first U.S. clinical study evaluating the ability of the GlycoMark assay to respond to and reflect changes in glycemia in a cohort of type 1 and type 2 diabetic patients with suboptimal glycemic control who are being managed aggressively with antihyperglycemic treatments. Specific attention is given to addressing the question of whether the assay can reflect changes in accordance with the established method of choice, HbA1c.

**RESEARCH DESIGN AND METHODS** — The experimental protocol for this study was approved by the Washington University Medical Center Human Studies Committee. Written informed consent was obtained from all patients, and all participants were either previously or newly diagnosed with either type 1 or type 2 diabetes as defined by the American Diabetes Association criteria (16). Patients who were pregnant or lactating or had a history of severe hypoglycemia or liver dysfunction, various hematological abnormalities (including significant anemia), unstable or advanced renal disease or significant proteinuria, unstable retinopathy, or recent retinal procedure were excluded. Hypoprothrombinemia was not an exclusion criterion. Patients were recruited from the outpatient clinic population of the investigator, clinics associated with the principal investigator's hospital or institution, or from a volunteer database. Each patient presented at baseline with suboptimal glycemic control who are being managed aggressively with antihyperglycemic treatments, including diabetes and nutritional education, various insulin therapies, thiazolidinediones, sulfonylureas, glinides, α-glucosidase inhibitors, and metformin. Therapy was targeted toward reducing HbA1c by at least 1.0% on average over the monitoring period. Blood was drawn for biochemical measurements at baseline and at 2, 4, and 8 weeks. At these time points, individual treatment regimens were adjusted as necessary to achieve progression toward euglycemia. Serum 1,5AG values were not used in patient management.

**Biochemical measurements**

Serum 1,5AG was measured with the GlycoMark assay (Toenol America, New York, NY) as automated on a Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN). The method uses pyranose oxidase (PROD) as an oxidase of 1,5AG to oxidize the second position hydroxyl group of 1,5AG and to detect the generated hydrogen peroxide by colorimetry. Because PROD also reacts with glucose, the sample is pretreated by enzyme reaction using glucokinase, and glucose is changed into a nonreactive substance by phosphorylation. This makes the reaction specific for 1,5AG. The reactions are performed at 37°C. At time zero, 4 μl of standard (50 μg/ml 1,5AG), control, reagent blank, or sample is added to 120 μl of the glucokinase-containing pretreatment reagent. After a 5-min incubation, 50 μl of coloring reagent (containing PROD) is added, and the absorbance is measured at 546 and 700 nm. Absorbance at both wavelengths is again measured after a 10-min incubation. The absorbance at 700 nm is subtracted from that at 546 nm to correct for background. The kinetic difference in corrected absorbance at 546 nm is then calculated. Concentrations of 1,5AG in the reagent blank, control, or sample are determined by comparison with a two-point calibration based on the corrected kinetic absorbance of the reagent blank and standard.

The assay for 1,5AG displayed an analytical sensitivity of 0.2 μg/ml, was linear to 113 μg/ml, and demonstrated intra- and interassay coefficients of variation of <4%. Hemoglobin, triglycerides, and bilirubin did not interfere with the assay at concentrations of up to 125 mg/dl, 1,153 mg/dl, and 53 mg/dl, respectively. Assay results were also unaffected by glucose concentrations up to 1,000 mg/dl. The sex-specific 5th to 95th percentile reference ranges established for the assay, based on analysis of samples from 224 healthy individuals, were 10.7–32.0 μg/ml (men) and 6.8–29.3 μg/ml (women). The reference ranges and associated interindividual biological variation parameters are essentially equivalent to those established for the assay in Japan and reflect an insensitivity to cultural dietary differences.

HbA1c was determined from whole-blood samples via turbidimetric inhibition immunoassay (Tina-Quant; Roche Diagnostics) as automated on the Hitachi
Conversely, decreases in 1,5AG values corresponded to decreases of HbA1c or, alternatively, increased 1,5AG values were the most frequently used and were receiving insulin therapy at baseline, the insulin regimen was modified by changing the dosage of insulin, changing the type of insulin, changing the schedule of insulin delivery, or initiating or changing the combination of insulins. Some patients with type 2 diabetes who were not previously receiving insulin initiated insulin therapy under the study protocol. Oral antihyperglycemic regimens were added or modified for patients with type 2 diabetes. Throughout the monitoring period, these regimens were continually optimized to achieve the average HbA1c target decrease of $\geq 1\%$. Of the oral antihyperglycemic medications, metformin and various glitazones and sulfonylureas, in combination with insulin therapies, were the most frequently used and were observed to best induce progression toward euglycemia.

Distribution of biochemical marker values, correlation, and longitudinal changes

The distribution of values for 1,5AG, HbA1c, fructosamine, and random glucose measurements was found to be non-gaussian, thus supporting the need for nonparametric analyses. Correlations between variables were assessed. 1,5AG was most closely associated with HbA1c and fructosamine, with Spearman $\rho$ values of $-0.6459$ and $-0.6751$, respectively (both $P < 0.0001$). HbA1c and fructosamine were also highly associated ($\rho = 0.6955$). Correlations of glucose with 1,5AG ($\rho = -0.3358$), HbA1c ($\rho = 0.3334$), and fructosamine ($\rho = 0.3529$) were lower due to the diurnal and situational variability of random glucose measurements.

Longitudinal changes in 1,5AG, HbA1c, fructosamine, and glucose values were assessed (Table 1). Whole-blood HbA1c was reduced by 1.3% from baseline to week 8, indicating that the therapeutic regimens used had achieved the study design target. However, mean and median absolute percent changes versus baseline were markedly higher for 1,5AG than for HbA1c, fructosamine, and glucose (Fig. 1). By week 2 of treatment, 1,5AG values had already increased by 57.9%, reflecting the sensitive and rapid response of 1,5AG to changes in blood glucose. For HbA1c, significant changes were not observed until 4 weeks. By week 8, 1,5AG mean values had increased by $\sim 160\%$ versus diminution of values of 12.1–35.3% for the established markers of glycemia.

Concordance of longitudinal changes

Longitudinal changes in glycemic control were evaluated on an individual patient basis (Fig. 2). HbA1c and 1,5AG values at baseline and week 8 were assessed individually for indications of progression toward, or away from, euglycemia. The concordance of these indications was then tabulated. In the cohort of 77 patients, the longitudinal changes of 1,5AG were in concordance with changes of HbA1c in 69 (89.6%) of 77 patients. Seventy-five of the 77 patients were classified as responders to therapy based on diminution of HbA1c values with time. The two patients classified as nonresponders were both men with type 2 diabetes. In these patients, 1,5AG values decreased with time and HbA1c values increased, thus indicating 100% concordance in these patients.

Concordance between longitudinal changes of 1,5AG and HbA1c in the intermediate time points of the study was also assessed. Concordance ranged from 58.4 to 76.6%, with earlier time points displaying lower concordance. Concordance...
from baseline to week 8 between 1,5AG and fructosamine (67 of 77 patients, 87.0%) and HbA1c and fructosamine (75 of 77 patients, 97.4%) was also assessed. Those patients demonstrating discordance between 1,5AG and HbA1c displayed concordance between HbA1c and fructosamine and vice versa (i.e., the two patients found to be discordant by assessments of HbA1c and fructosamine were concordant when comparing changes of 1,5AG with changes of HbA1c).

**CONCLUSIONS** — The present study has examined the ability of the GlycoMark assay for 1,5AG to reflect changes of glycemia in a cohort of patients with type 1 and type 2 diabetes who are being treated aggressively with various regimens and who are being monitored with established biochemical markers of glycemia. Over the 8-week monitoring period, values for 1,5AG, HbA1c, and fructosamine were highly correlated, and the results were in good agreement with previous Japanese studies (17,18). Furthermore, 1,5AG responded rapidly and significantly to population-based changes in glycemia, with the first significant change appearing at 2 weeks of treatment. In contrast, HbA1c responded more slowly, and both HbA1c and fructosamine displayed more modest changes in value. These results are in agreement with the literature. For instance, in a 1996 study by Yamanouchi et al. (17), 56 patients newly diagnosed with type 2 diabetes were monitored for 4 weeks after initiation of oral antihyperglycemic medications. At the end of the 4-week period, one-half of the patients continued on treatment while the rest discontinued treatment. The results showed that 1,5AG

**Table 1—Longitudinal changes of biochemical variables**

<table>
<thead>
<tr>
<th>Time point/statistic</th>
<th>1,5AG (μg/ml)</th>
<th>HbA1c (%)</th>
<th>Fructosamine (μmol/l)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.9 ± 1.9</td>
<td>9.5 ± 1.7</td>
<td>410.6 ± 108.6</td>
<td>225.0 ± 105.6</td>
</tr>
<tr>
<td>Median</td>
<td>1.4</td>
<td>9.1</td>
<td>375.0</td>
<td>218.0</td>
</tr>
<tr>
<td>Minimum to maximum</td>
<td>0.0–10.7</td>
<td>7.0–14.2</td>
<td>269.0–908.0</td>
<td>64.0–574.0</td>
</tr>
<tr>
<td><strong>Week 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.0 ± 2.2†</td>
<td>9.1 ± 1.5</td>
<td>362.4 ± 76.5†</td>
<td>187.4 ± 91.0†</td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
<td>8.7</td>
<td>348.5</td>
<td>190.0</td>
</tr>
<tr>
<td>Minimum to maximum</td>
<td>0.0–12.6</td>
<td>6.8–14.0</td>
<td>232.0–572.0</td>
<td>27.0–448.0</td>
</tr>
<tr>
<td>Mean percent change vs. baseline</td>
<td>57.9</td>
<td>4.2</td>
<td>11.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Median percent change vs. baseline</td>
<td>92.9</td>
<td>4.4</td>
<td>7.1</td>
<td>12.8</td>
</tr>
<tr>
<td><strong>Week 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.7 ± 2.5†</td>
<td>8.8 ± 1.4†</td>
<td>340.0 ± 79.1†</td>
<td>181.4 ± 102.4†</td>
</tr>
<tr>
<td>Median</td>
<td>3.5</td>
<td>8.7</td>
<td>321.0</td>
<td>157.0</td>
</tr>
<tr>
<td>Minimum to maximum</td>
<td>0.0–13.3</td>
<td>6.6–13.2</td>
<td>206.0–559.0</td>
<td>29.0–701.0</td>
</tr>
<tr>
<td>Mean percent change vs. baseline</td>
<td>94.7</td>
<td>7.4†</td>
<td>17.2</td>
<td>19.4</td>
</tr>
<tr>
<td>Median percent change vs. baseline</td>
<td>150.0</td>
<td>4.4</td>
<td>14.4</td>
<td>28.0</td>
</tr>
<tr>
<td><strong>Week 8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.0 ± 3.6†</td>
<td>8.2 ± 1.2†</td>
<td>317.5 ± 75.4†</td>
<td>172.6 ± 100.5†</td>
</tr>
<tr>
<td>Median</td>
<td>4.8</td>
<td>8.0</td>
<td>295.0</td>
<td>141.0</td>
</tr>
<tr>
<td>Minimum to maximum</td>
<td>0.0–15.7</td>
<td>6.3–12.3</td>
<td>197.0–575.0</td>
<td>16.0–545.0</td>
</tr>
<tr>
<td>Mean percent change vs. baseline</td>
<td>163.2</td>
<td>13.7</td>
<td>22.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Median percent change vs. baseline</td>
<td>242.9</td>
<td>12.1</td>
<td>21.3</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Reference ranges: 1,5AG, 10.7–32.0 μg/ml (men) and 6.8–29.3 μg/ml (women); HbA1c, <6.0%; fructosamine, 205–285 μmol/l; glucose, 55–115 mg/dl. *One male patient did not provide a blood sample at week 2, thus the n for this time point is 76. †P < 0.05 vs. baseline.

Figure 1—Percentage change from baseline by time point for serum 1,5AG (●–●), whole-blood HbA1c (■–■), serum fructosamine (○–○), or random serum glucose (△–△). Error bars = SE.
increased rapidly and demonstrated a significant change versus baseline at 2 weeks of monitoring. After discontinuation of treatment, 1,5AG values sharply decreased, and the values at the 6-week time point were significantly different from those in the subgroup who continued on therapy. Results for fructosamine and HbA1c demonstrated a lesser response to therapy in the initial 4 weeks. Furthermore, whereas fructosamine responded to the discontinuation of therapy between weeks 4 and 6, HbA1c did not. Thus, the present study adds to the body of evidence in Japan that demonstrates a rapid and significant response of 1,5AG to antihyperglycemic treatments.

The present study also demonstrates that changes in 1,5AG values reflect changes in glycemic control in good accord with HbA1c on an individual patient basis. Overall, 89.6% displayed concordant changes of 1,5AG and HbA1c from baseline to week 8. Percent concordance was reduced at intermediate time points due to the relative insensitivity of HbA1c to changes in glycemia at time periods <8 weeks. Examination of the results from the eight patients with discordant 1,5AG and HbA1c changes revealed that 1,5AG values either slightly diminished or remained unchanged over the monitoring period, whereas HbA1c values diminished slightly, indicating some minimal improvement in glycemic control. The relative insensitivity of 1,5AG in these patients may be due to excessive depletion of the body pool of 1,5AG. Such depletion has been observed to occur in severe hyperglycemia as a result of persistent glucosuria (19). Additionally, a dynamic mass balance two-compartment

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**Figure 2**—Longitudinal changes in 1,5AG (A), HbA1c (B), fructosamine (FA; C), and glucose (D) in individual patients.
model has been developed for 1,5AG (20), and it demonstrates that the tissue compartment is two to three times the mass of the plasma compartment. Model estimates suggest that accelerated depletion of 1,5AG is associated with slow recovery upon the initial improvement of glycemia, because the body pool of 1,5AG represents ∼5 weeks of normal dietary intake. If the model is accurate, it suggests that the use of 1,5AG is optimal in the range of modest hyperglycemia to near euglycemia. In this range, the GlycoMark assay may represent a valuable means for the maintenance of near-normal glucose levels, as it responds rapidly and sensitively to even transiently ascending glucose concentrations.

Clearly the overall clinical management of diabetes, whether type 1 or type 2 diabetes, requires continual multifactorial evaluation of glycemic status. The GlycoMark assay for 1,5AG may be a useful tool as an adjunct to existing methods. For example, observations of good correlation between circulating 1,5AG and the magnitude of glycemic excursions within a day (21) indicate some potential for 1,5AG to substitute for frequent glucose measurements in type 2 diabetes. Furthermore, circulating 1,5AG concentrations may also reflect transient glycemic excursions due to postprandial hyperglycemia and, as such, may provide significant benefit for improving long-term outcomes. Additionally, 1,5AG as an intermediate-term marker could be evaluated between standard 3-month assessments of HbA1c to indicate worsening of glycemic control and to incite behavioral change before induction of significant vascular damage. There have also been indications of the prognostic capability of 1,5AG measurements. In a study by Sone et al. (22), 1,5AG levels were measured for 5 consecutive days in a cohort of 22 patients with type 2 diabetes. Changes in 1,5AG during the 5 days were significantly correlated (R = 0.70, P < 0.01) with changes in HbA1c over the subsequent 3 months. The authors concluded that 1,5AG could be used to identify patients at high risk for poor glycemc control in the future.

In conclusion, this first U.S. clinical trial of the GlycoMark assay for the metabolically stable analyte, 1,5AG, has demonstrated the capability of this simple, automated method to report changes in glycemia induced by antihyperglycemic treatment regimens. The results are in excellent accord with Japanese studies over the previous two decades and serve to indicate that the ethnically and culturally diverse population of the U.S. does not negatively impact the perceived use of the marker. Furthermore, the recent clearance granted by the FDA makes the GlycoMark assay available for intermediate-term monitoring, which, it is hoped, will allow patients to seek medical intervention in a timely manner, such as when initiating or altering therapy. This may empower patients to achieve and maintain better control of their disease. Of course, further studies will be useful to better clarify the role of 1,5AG measurements in clinical diabetes management.

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