Clinical Validation of a Second-Generation Fructosamine Assay
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The serum fructosamine assay, used to monitor short-term clinical glycemic control, reportedly has several technical drawbacks. However, technical improvements have resulted in a new second-generation assay of fructosamine. We evaluated this second-generation assay (from Roche Diagnostics) in 529 nondiabetic and diabetic patients and found a highly significant correlation with results of the first-generation assay \( r = 0.91, P < 0.001 \). Use of the second-generation assay with samples from patients classified on the basis of glycemic control according to their glycohemoglobin (GHb) values, enabled us to discriminate between the nondiabetics, diabetics with “good/moderate” control (i.e., GHb <10%), and diabetics with “poor” control (GHb ≥10%). We evaluated the validity of the second-generation assay to assess short-term glycemic control in 23 non-insulin-dependent diabetic patients who participated for 10 weeks in an intensive intervention program designed to rapidly normalize the clinical glycemic profile. Results correlated significantly with the one-week average capillary blood glucose concentration (CBG) and with the three-week average CBG in all 23 patients. In addition, the second-generation fructosamine assay results demonstrated a significant decrease at each week of study, as did the average CBG. Results of the first- and second-generation assays correlated significantly at each week of study. GHb correlated significantly with both the second- \( r = 0.78, P < 0.001 \) and first-generation fructosamine assay results \( r = 0.77, P < 0.001 \) for the baseline blood samples of the intervention study, but this correlation decreased (to \( r = 0.35, P = 0.09 \) and \( r = 0.34, P = 0.09 \), respectively) by the conclusion of the study.

Additional Keyphrases: glycohemoglobin · diabetes mellitus · glucose · intermethod comparison

The ability to objectively monitor clinical glucose control in diabetic outpatients has been facilitated by use of the nonenzymatic glycoanalysis (i.e., glycation) reaction. In this reaction, glucose is nonenzymatically attached to serum and tissue proteins, subject to two main clinical determinants: the half-life of the protein in the circulation or tissue, and the duration and degree of the hyperglycemia \( (1, 2) \). Because the half-life of an erythrocyte is 60 to 90 days, glycated hemoglobin (“glycohemoglobin”) is a valid measure of glycemic control over the previous two to three months; this measure has been used as the “gold standard” clinically for well over 10 years \( (3, 4) \). However, interest has been intense in evaluating other glycated serum proteins with shorter half-lives (i.e., albumin, with a half-life of 14–20 days), which reflect glycemic control over the previous two to four weeks \( (5–8) \).

Determining glycated serum proteins by use of nitroblue tetrazolium (NBT) colorimetric methods—the serum fructosamine assay—was first described by Johnson et al. \( (9) \) in 1983. Several clinical studies have shown the fructosamine assay to be a valid marker of short-term glycemic control \( (10–13) \). However, despite the clinical validity reported for the fructosamine assay, the NBT method has some drawbacks. In particular, the nonspecific nature of the reaction allows interference by uric acid, bilirubin, and lipids \( (14, 15) \); other major concerns are the effects of albumin concentration on the measurement \( (16, 17) \) and assay calibration \( (18) \). Recently, a new, second-generation fructosamine assay was introduced that addressed the above concerns and had an improved analytical performance \( (19) \). The technical improvements consisted of incorporating uricase in the reagent to eliminate interference with uric acid and using nonionic surfactants to eliminate the protein-matrix effect and interference from lipemia \( (19) \). In addition, the second-generation assay is standardized against glycated polylsine, the reaction behavior of which is closer to that of the physiologically occurring fructosamines, instead of a synthetic standard, deoxymorpholinofructose, as was used in the first-generation assay \( (19, 20) \).

To clinically validate this second-generation fructosamine assay, we compared the results of the first-generation assay with those of the second-generation assay for accurately monitoring an adult diabetic population during a 10-week intervention study. In addition, we performed a cross-sectional assessment of the utility of this assay in insulin-dependent, non-insulin-dependent, and nondiabetic subjects, to establish reference ranges for this assay and to evaluate correlations with glycated protein variables currently used in assessing glycemic control.

Patients and Methods

We recruited 529 patients (ages 18–70 years) for the cross-sectional aspect of study, of whom 230 were nondiabetics. The patients were from the outpatient clinics of the Department of Medicine and from the Diabetes Research Laboratories and Clinics, Department of Medicine, Section on Endocrinology/Metabolism, Bowman Gray School of Medicine, 300 S. Hawthorne Road, Winston-Salem, NC 27103.


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1 Nonstandard abbreviations: NBT, nitroblue tetrazolium; CBG, capillary blood glucose; and FA-1, FA-2, first- and second-generation fructosamine assays, respectively.
Research Clinics of the Bowman Gray School of Medicine. All patients gave informed consent, and the study was approved by the Clinical Research Practices Committee of the Bowman Gray School of Medicine. All patients had fasting blood drawn for assay by the first- and second-generation serum fructosamine tests and for determinations of glycohemoglobin, serum glucose, total protein, and albumin. The first-generation serum fructosamine assay (RoTag; Roche Diagnostics, Nutley, NJ) (interassay CV = 2.2%; intra-assay CV = 2.4%) and the second-generation serum fructosamine assay (interassay CV = 2.1%, intra-assay CV = 2.4%) were performed with a Cobas-Mira Chemistry Analyzer using the Roche reagents, as were glucose, total protein, and albumin assays, also with Roche reagents. Glycohemoglobin was determined by automated affinity HPLC (Model CLC-330; Primus Corp., Kansas City, MO), for which interassay CV = 1.2%, intra-assay CV = 2.1%.

To evaluate the validity of the second-generation assay for accurately reflecting recent glycemnic excursions, we recruited 23 non-insulin-dependent diabetic patients for an intensive outpatient monitoring and intervention study. Because this aspect of the study attempted to control an above-normal glycemic state over a short interval, all patients were required to have an initial glycohemoglobin value of >10%. In addition, all patients, if treated with insulin, were required to be taking <35 units (total) daily. Any previous history of ketosis excluded patients from the study. All patients were assessed initially with complete blood counts, liver-function tests, measurements of electrolytes and glycohemoglobin, and first- (FA-1) and second-generation (FA-2) fructosamine determinations. In addition, all patients, before entering the study, obtained 24-h urine collections for determination of albumin excretion and creatinine clearance to check for normal renal function.

At the initial exam, all patients were placed on a computer-based home glucose monitoring system, which involves electronic log books used with the Accu-check II glucose meter and the Merlin Diabetes Data Management System (Boehringer Mannheim Diagnostics, Indianapolis, IN). Patients were instructed to determine capillary blood glucose (CBG) concentrations before meals and at bedtime. After samples for baseline blood analysis were obtained, all anti-diabetic medication (oral sulfonylureas and insulin) was stopped and blood was taken weekly for the next two weeks to evaluate the response of fructosamine and average weekly CBG during the "off medication" interval. At the end of this two-week period, patients were placed on a calorie-restricted diet (as recommended by the American Diabetes Association) or a calorie-restricted diet plus insulin (0.4 units/kg of body weight, given as a split/mixed neutral protamine Hagedorn (NPH)/regular human insulin regimen). Patients returned to the clinic weekly to supply samples of fasting blood and to download their electronic log books, so that computer-derived averages for daily and weekly CBG could be obtained. Insulin and dietary manipulations were made to decrease the average weekly glucose value toward a final goal of 8.3 mmol/L (150 g/L), established as the upper limit of normal for a weekly average of pre-prandial and bedtime outpatient glucose values for our population. This value was derived by extrapolation of outpatient CBG values obtained in our previous outpatient monitoring studies that corresponded to the upper limit of normal as established for the first-generation fructosamine assay (2.8 mmol/L) (10, 11, 23). Glycohemoglobin was measured at baseline and at weeks 2, 6, and 10 of the study. Patients were required to maintain daily food records so that weekly analysis could assess compliance of the dietary aspect of intervention.

Data were analyzed with Pearson correlation coefficients and Student's *t*-test where appropriate.

**Results**

Figure 1 demonstrates the distribution of the FA-1 and FA-2 values in the nondiabetic patients (*n* = 230). As shown, the range for FA-2 results was 137–368 μmol/L (mean 227, SD 35, μmol/L). Using the 95th
percentile as the upper limit, we determined the upper range of normal for this assay to be 289 μmol/L. The range of results for nondiabetics by the FA-1 was 1.5–3.5 mmol/L (mean 2.2, SD 0.28), with the 95th percentile as the upper range of normal determined to be 2.7 mmol/L. On the basis of the values obtained for glycohemoglobin (normal range 4–7%), the diabetics were then classified as being in “good/moderate” control (glycohemoglobin <10%) or in “poor” control (glycohemoglobin ≥10%). Values for FA-1, FA-2, and fasting blood glucose were significantly different between all three groups (Table 1). Figure 2 demonstrates a highly significant correlation between FA-1 and FA-2 for all patients (r = 0.91, P <0.001). In addition, there was a significant correlation between FA-1 and FA-2 values and the fasting blood glucose in all patients (r = 0.65, P <0.001 and r = 0.67, P <0.001, respectively). Glycohemoglobin at the initial blood draw significantly correlated with the FA-1 (r = 0.81, P <0.001) and FA-2 (r = 0.81, P <0.001). There was no significant correlation between albumin concentration and either FA-1 (r = 0.02) or FA-2 (r = 0.04) or between total protein and either FA-1 (r = 0.08) or FA-2 (r = 0.04).

Twenty-three non-insulin-dependent diabetic patients (12 men, 11 women, average age 52, SD 12 years) agreed to participate in the intensive intervention program. All patients had normal results for liver-function tests, total protein, albumin, and serum creatinine. Urinary albumin excretion quantified in 24-h urine collections revealed normal albumin excretion rates (<30 mg/g creatinine) in 21 patients. Two patients had albumin excretion values up to about 520 mg/g creatinine, but normal values for serum creatinine, albumin, and total protein.

Figure 3 demonstrates the average weekly CBG of the 23 non-insulin-dependent diabetic patients in the intensive intervention phase of study. The CBG significantly increased during the two-week "off" medication period (P <0.05). Although the FA-2 result obtained during this period also increased by the end of week 2, the increase obtained was not significant (P = 0.08). After this two weeks, all patients responded to the clinical intervention by demonstrating a significant decrease in the average weekly CBG. At study initiation, the average weekly CBG value was 13.3 (SD 3) mmol/L, which decreased to 7.5 (SD 1.4) mmol/L by study completion (P <0.001). FA-2 results also decreased significantly at each week of study.

Figure 4 depicts the results of FA-1 and FA-2 and the average CBG and glycohemoglobin values during the monitoring study. After clinical intervention was initiated, the results of each fructosamine assay were less at each week of study; by week 5 of intervention, values were in the high normal range established for each assay. In addition, by week 5 the average weekly CBG decreased into the normal range, as established for this study. The glycohemoglobin decreased during the study but was not in the normal range at the completion of the study (12.9% ± 3% vs 9.6% ± 2.6%, mean ± SD, P <0.001). FA-2 results correlated significantly with FA-1 results at each week of study (r = 0.87, P <0.001). In addition, FA-2 results correlated significantly with both the one-week average CBG (r = 0.64, P <0.001, Figure 5A) and the three-week average (r = 0.61, P <0.001,

Table 1. Fructosamine and Glucose Concentrations In Subjects Classified According to Glycemic Control

<table>
<thead>
<tr>
<th>Group*</th>
<th>n</th>
<th>FA-2, μmol/L</th>
<th>FA-1, μmol/L</th>
<th>Fasting glucose, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>230</td>
<td>227 ± 35°</td>
<td>2.2 ± 0.28°</td>
<td>4.7 ± 1.5°</td>
</tr>
<tr>
<td>Good/moderate control</td>
<td>103</td>
<td>299 ± 56</td>
<td>2.6 ± 0.4°</td>
<td>8.6 ± 3.1°</td>
</tr>
<tr>
<td>Poor control</td>
<td>196</td>
<td>406 ± 95°</td>
<td>3.6 ± 0.8°</td>
<td>13.2 ± 5.1°</td>
</tr>
</tbody>
</table>

*Defined according to glycohemoglobin values: 4–7%, <10%, and ≥10% for nondiabetic, good/moderate control, and poor control, respectively.

° P <0.001 vs "good/moderate" control.
FA-2 results correlated significantly with the glycohemoglobin proportion at baseline ($r = 0.78, P < 0.001$), but this correlation had decreased by the end of the intervention study ($r = 0.35, P = 0.09$). Results for FA-1 correlated similarly with glycohemoglobin, being $r = 0.77, P < 0.001$ at baseline, but only $r = 0.34, P = 0.09$ at week 8.

**Discussion**

The above study confirms that the second-generation serum fructosamine assay is a valid short-term index of glycemic control; its results correlated significantly with the outpatient mean glucose concentration in all 23 patients during the 10-week study period. In addition, the FA-2 results correlated well with the values measured by the FA-1 at each week of study. Therefore, this study demonstrates that both assays provide the same clinical information.

Our reference range for FA-2 (based on the 95th percentile) has an upper limit of normal of 289 µmol/L. This value agrees well with the upper limit of normal (285 µmol/L) reported for 556 non-diabetic patients studied at four sites in Europe with the FA-2 (21) and is slightly greater than the value reported in a recent European fructosamine workshop (276 µmol/L) (22). The differences in upper limit for the reference range were probably attributable to the different groups studied (which included hospitalized nondiabetic patients), although the selection criteria for the reference groups were the same (21, 22). In determining the upper limit of normal in our study, we studied only ambulatory outpatients, and not hospitalized patients. Again using the 95th percentile, we report a value of 2.7 mmol/L for FA-1, which agrees with the value of 2.8 mmol/L noted in the package insert.

It is not surprising to find that the serum fructosamine correlates significantly with the glycohemoglobin, because we have previously reported significant correlations between serum fructosamine and glycohemoglobin (Hb A1c, as determined by column chromatography) ranging from $r = 0.7$ to 0.82 in both insulin-dependent and non-insulin-dependent diabetics younger than 65 years and in elderly (older than 65) non-insulin-dependent diabetics (10, 11, 23). Our findings regarding correlation between the two assays agree well with values reported by other investigators (24–27). In contrast, others have reported poor correlations between serum fructosamine and glycohemoglobin in both insulin-treated diabetics ($r = 0.31$) and diabetics treated with oral agents ($r = 0.39$) (28) and in randomly selected populations consisting of both insulin-dependent and non-insulin-dependent diabetics ($r = 0.17$) (29). However, absolute correlation between fructosamine and glycohemoglobin should not be expected because the value expressed for each assay depends on the half-lives of the major proteins measured and reflects the average antecedent glycemic control for different intervals preceding the blood collection. That is, glycohemoglobin measures integrated glucose control over the previous one to three months, whereas serum fructosamine re-
fects average integrated glucose control over the previous two to three weeks, as demonstrated by this and other studies (10–12, 24). Therefore, the correlation between these measures should be better in a diabetic population not subject to recent, intensive intervention, as demonstrated by the significant correlation detected initially in both our cross-section and intervention study patients. In a clinical setting not involving a recent intensive intervention, gradual changes in glycemic control should be reflected in parallel changes in both fructosamine and glycohemoglobin (30). However, once intensive intervention is undertaken, the serum fructosamine will show a rapid decrease over a short-term interval corresponding to a decrease in average glycemia, whereas there will be only a minimal decrease in the glycohemoglobin measured. Thus the correlation between the two will decrease. After the patient has achieved stable, normal concentrations of blood glucose and fructosamine, the glycohemoglobin value will continue to decrease, reflecting the glycemic changes from weeks before. At that time, the correlation between the two assays would be expected to increase. Clearly, changes in serum fructosamine and glycohemoglobin values are not interchangeable; rather, both should be used in a pattern of intensive diabetic monitoring. The decrease in glycated proteins in serum as monitored with the fructosamine assay can predict a drop in glycohemoglobin over time. This indeed was the case in our studies, and suggests strongly that use of both assays is indicated clinically in the management of diabetic patients.

In summary, we have reported on the clinical validity of a second-generation fructosamine assay that has improved technical performance over the first-generation assay. Results of the second-generation assay correlate clinically with those of the first-generation assay, correlate significantly with both one- and three-week outpatient average glucose concentrations, and provide an objective marker of short-term glycemic control. In addition, because both fructosamine assays accurately monitor short-term glycemic excursions, clinical correlation between glycohemoglobin and serum fructosamine should not always be expected; if used together, however, both analytes can provide valuable clinical information on antecedent glycemic control.

References