Technical performance evaluation of BM/Hitachi 747-200 serum fructosamine assay

Maun-Jan Lin,1* Carolyn Hoke,1 Bruce Ettinger,2 and Robert V. Coyne1

The technical performance of serum fructosamine assay as a short-term index of diabetic control was evaluated by using the BM/Hitachi 747-200 Automatic Analyzer (Boehringer Mannheim Corp). Intra- and interassay precisions (CV) were <2%. Linearity was confirmed up to 1000 μmol/L. Lipemia, produced artificially or from hypertriglyceridemia in vivo, did not interfere with measurement of serum fructosamine concentration. However, hemoglobin (Hb) interfered with measurement substantially at concentrations >15.5 μmol/L, and bilirubin interfered substantially at concentrations >68.4 μmol/L. A normal reference range of up to 282 μmol/L (mean ± 2SD = 242 ± 40) was derived from results obtained from 228 nondiabetic subjects. In 222 diabetic subjects, serum fructosamine concentration correlated well with fasting glucose concentration (r = 0.74) and with Hb A1c concentration (r = 0.80). This automated fructosamine assay has the advantages of technical simplicity, low cost, and reduced analytical time compared with the Hb A1c method.

INDEXING TERMS: glycohemoglobin • diabetes mellitus

Measurement of glycohemoglobin has been in common use as a screening tool in monitoring long-term glycemic control since the 1970s. Analytical methods for determining glycohemoglobin concentration include ion-exchange chromatography, HPLC, affinity chromatography, colorimetry, electrophoresis, and isolectric focusing [1]. However, these methods are labor intensive and expensive.

Because of the relatively long half-life of the erythrocyte, the hemoglobin A1c (Hb A1c) assay does not reflect recent changes in blood glucose concentration. Measuring serum glycated proteins (fructosamine) with shorter half-lives ranging from 17 to 20 days has been suggested as more sensitive than Hb A1c for assessing short-term glycemic control [2, 3].

This study investigated analytical performance of the fructosamine assay with the BM/Hitachi 747-200 Automatic Analyzer. We used a second-generation fructosamine assay [4], which is a modification of the method described by Johnson et al. [5], and evaluated possible interference with the fructosamine assay by lipemia, hyperbilirubinemia, and hemolysis. We investigated the correlation between fructosamine and other measures of diabetic control, particularly Hb A1c and fasting glucose.

Materials and Methods

REAGENTS AND INSTRUMENTATION

Glucose, triglyceride, albumin, total protein, and fructosamine assays were done with the BM/Hitachi 747-200 Automatic Analyzer (Boehringer Mannheim, Indianapolis, IN). Glucose was measured by the hexokinase method, triglyceride by the glycerol phosphate oxidase method, albumin by the bromocresol green method, total protein by the biuret method, and fructosamine by the nitroblue tetrazolium method (all standard BM/Hitachi methods). All reagents, as well as control and calibrator materials, were obtained from Boehringer Mannheim. The fructosamine assay was calibrated by using a glycated polypeptide (high-molecular-mass compound), a synthetic protein that reacts similarly to physiological fructosamine [6, 7]. Fructosamine assay reagents contained 200 mmol/L carbonate buffer (pH 10.3), 2.88 μmol/L nitroblue tetrazolium, >15 U/L uricase, and detergent.

The BM/Hitachi 747-200 Automatic Analyzer was set to record the absorbance increase in the interval 9.0 to 10.0 min after the start of the reaction. The absorbance was measured bichromatically at 546 and 700 nm. The incubation temperature was 37 °C. Sample volume was 14 μL and reagent volume was 250 μL. Hb A1c assays were done with the Bio-Rad/Diamat™ analyzer (Bio-Rad Labs., Hercules, CA) by using ion-exchange HPLC. d-Glucose (Sigma Chemical Co., St Louis, MO) was used for linearity testing. Precinorm™ fructosamine control and Precirol™N (Boehringer Mannheim) were used for precision tests. Dimethyl sulfoxide, sodium carbonate, hydrochloric acid, and sodium chloride (all obtained from Sigma), Intralipid™ (Kabi Pharmacia, Clayton, NC), and bilirubin

1 Department of Chemistry, The Permanente Medical Group, Inc., Regional Laboratory, 1725 Eastshore Highway, Berkeley, CA 94710-1798.
2 Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA.
* Author for correspondence. Fax 510-559-5204.
Presented at the 47th Annual Meeting, American Association for Clinical Chemistry, July 16-20, 1995, Anaheim, CA.
Received July 18, 1995; accepted November 1, 1995.
(Pfanstiehl Labs., Waukegan, IL) were used to evaluate interference by the procedure of Glick et al. [8].

SAMPLES
All blood samples were originally submitted for other assays; no samples were obtained solely for this study. Blood samples from nondiabetic and diabetic patients seen in the 23 Northern California Region Kaiser Permanente Medical Centers were used. Serum specimens were stored at 2–8 °C and analyzed within 1 week. Whole-blood specimens (preserved with EDTA) were stored at 2–8 °C and analyzed for Hb A1c within 3 days.

ANALYTIC PROCEDURE
The manufacturer's published values for precision, accuracy, and linearity for the fructosamine assay were evaluated by using the NCCLS EP10-T protocol [9]. Because a suitably high-concentration fructosamine assay control was not available, Precinorm fructosamine control (diluted to half the original concentration) was used as the low-concentration control and Precitrol-N was used as the high-concentration control. The fructosamine assay value of the Precinorm fructosamine control was 278 μmol/L, which was established by the manufacturer. The Precitrol-N fructosamine assay value was 634 μmol/L, as determined at our request by Boehringer Mannheim. According to NCCLS EP10-T protocol, the mid-concentration samples must be exactly halfway between the high and low concentrations; this was accomplished by mixing equal volumes of the high- and low-concentration controls. The following specific sequence was analyzed in a run, without change, interruption, or intervening samples: mid, mid, high, low, mid, mid, low, low, high, high, mid. The first two samples functioned as primers; only the nine subsequent sample results were used in computation. One run a day was performed for 5 consecutive days; this is the minimum required for the experiment according to the NCCLS EP10-T protocol [9]. To assess the linearity of the assay, we prepared a human serum pool (initial fructosamine value 800 μmol/L) supplemented with D-glucose to a final concentration of 66.6 mmol/L glucose and incubated at 37 °C for 20 h; the resulting fructosamine value was >1000 μmol/L. This supplemented pool was then diluted with 9 g/L NaCl to produce concentrations corresponding to 20%, 40%, 60%, and 80% of the high-concentration pool. The effects of hemolysis, lipemia, and icterus were determined by supplementing separate serum pools with graded concentrations of fresh hemolysate (Hb up to 155 μmol/L), Intralipid (Intralipid 10 g/L = triglyceride 28.25 mmol/L), and bilirubin (up to 1026 μmol/L) according to the procedure of Glick et al. [8]. Comparison of the triglyceride effect on serum fructosamine concentration was assessed in specimens from diabetic patients and from nondiabetic patients. In addition to measuring fructosamine in sera with triglyceride concentrations <1.70 mmol/L (obtained from 100 nondiabetic and 100 diabetic subjects), we also measured fructosamine in sera with triglyceride concentrations between 2.26 mmol/L and 24.86 mmol/L (obtained from 100 nondiabetic and 100 diabetic subjects).

CORRECTION FOR SERUM PROTEIN
Different concentrations of protein may influence fructosamine concentration. To eliminate this factor, some have recommended correcting the fructosamine value for the total protein value or the albumin value [10–12]. The fructosamine value in this study was corrected for the protein or albumin concentration by using the following formulas:

\[
\text{[Fructosamine]corrected for total protein} = \frac{\text{[fructosamine/total protein]}}{70 \text{g/L}}
\]

\[
\text{[Fructosamine]corrected for albumin} = \frac{\text{[fructosamine/albumin]}}{41 \text{g/L}}
\]

The concentrations of 70 g/L protein and of 41 g/L albumin correspond to the mean values found in the diabetic patients in this study.

STATISTICAL ANALYSES
The significance of differences in the two categories (normal and increased triglyceride samples) was determined by Student's t-test. Correlations were analyzed by linear regression.

Results
Precision (within-run CVs and total imprecision CVs) and bias are shown in Table 1. Linearity was established up to 1000 μmol/L (r = 1.00; slope = 0.99) (Fig. 1).

The fructosamine assay showed no discernible change from lipemia (because a triglyceride concentration of up to 28.25 mmol/L in the specimens caused the final results to be within ±3% of the original value) (Fig. 2A). No change in results occurred at Hb up to 15.5 μmol/L/Hb, but positive interference (an increase of ≥10% from the original value) occurred between 15.5 and 77.5 μmol/L Hb (Fig. 2B); little to no interference was observed between 77.5 and 139.5 μmol/L Hb. Negative interference (a decrease of ≥10% from the original value) occurred at Hb concentrations >139.5 μmol/L. Bilirubin caused positive interference (an increase of ≥10% from the original value) at concentrations >68.4 μmol/L (Fig. 2C). Fructosamine concentration increased ~0.9% for each 10 μmol/L increase in bilirubin concentration.

Mean fructosamine concentration in serum from 100 nondiabetic subjects and 100 diabetic subjects is shown in Table 2. No statistically significant difference was found in serum fructosamine concentration between those with normal vs those

| Table 1. Precision and accuracy of fructosamine assay. |
|----------------------------------|------------------|------------------|------------------|
|                                  | Control          |                  |                  |
|                                  | Low              | Mid              | High             |
| Stated value, μmol/L             | 139              | 386              | 634              |
| Within-run CV, % (n = 15)        | 1.52             | 0.93             | 0.89             |
| Total imprecision CVs, %         | 1.56             | 1.25             | .99              |
| Difference from stated value, μmol/L | +5.93           | +17.70           | +15.46           |
* Over 5 days.
with increased triglyceride concentration in either nondiabetic or diabetic subjects (P > 0.05).

The reference intervals were established by using fasting samples from 228 nondiabetic physical examination outpatients with fasting glucose concentration < 5.0 mmol/L. These data were normally distributed. The reference interval (mean value ± 2SD, 242 μmol/L ± 40) for fructosamine was 202–282 μmol/L (n = 228); the reference interval for fructosamine corrected for albumin was 191–265 μmol/L (mean value 228 μmol/L) (Fig. 3). Albumin values in diabetic patients were 33–49 g/L (mean ± 2SD = 41 ± 8, n = 440); in nondiabetic patients, albumin values were 35–52 g/L (43 ± 3, n = 228). Hemolyzed and icteric sera were excluded.

Correlations between fructosamine concentration, Hb A1c concentration, and fasting glucose concentration are shown in Table 3. Uncorrected, total-protein-corrected, and albumin-corrected fructosamine showed statistically significantly higher correlation with fasting glucose concentrations than Hb A1c did (P < 0.001).

**Discussion**

Results in this study were similar to the manufacturer’s published values [within-run CVs: 1.5% (low concentration), 0.9% (mid concentration), 0.7% (high concentration); total imprecision CVs: 1.8% (low concentration), 1.7% (mid concentration), 1.5% (high concentration)]. This study verified that the fructosamine assay done with the BM/Hitachi 747-200 Automatic Analyzer had acceptable imprecision (<2%) for use in the clinical laboratory. Its linearity of up to 1000 μmol/L, which was roughly 3.5 times the upper reference limit, was sufficient to cover the pathological range of diabetic patients in poor metabolic control (in this study, the highest fructosamine concentration in patients with poorly controlled diabetes was 938 μmol/L).

Regarding the effects of hypertriglyceridemia on fructosamine concentration, Woo et al. [13] and Baker et al. [14] reported that triglyceride caused some minor interference in the fructosamine assay. Our study found that measured serum fructosamine concentration was free from interference from

**Table 2. Mean serum fructosamine concentration (μmol/L) in diabetic and nondiabetic subjects.**

<table>
<thead>
<tr>
<th></th>
<th>Diabetic (n = 100)</th>
<th>Nondiabetic (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal triglyceride</td>
<td>309.1 (SD 85.4)</td>
<td>242.6 (SD 20.1)</td>
</tr>
<tr>
<td>(≤1.70 mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased triglyceride</td>
<td>317.2 (SD 72.9)</td>
<td>242.7 (SD 20.3)</td>
</tr>
<tr>
<td>(2.26–24.86 mmol/L)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean triglyceride value = 0.98 mmol/L (nondiabetic), 1.22 mmol/L (diabetic).

* Mean triglyceride value = 3.84 mmol/L (nondiabetic), 4.57 mmol/L (diabetic).
lipemia produced by addition of Intralipid (triglyceride up to 28.25 mmol/L) and also free from interference from hypertriglyceridemia in vivo in both diabetic and nondiabetic subjects. This discrepancy may result from using different commercial reagents and analyzer, although all belong to the second-generation fructosamine assay.

The pattern of interference observed with addition of Hb was unusual: Both negative and positive interference were observed. Hb, which absorbs light at 540 nm, can cause optical interference at lower concentration, and chemical interference combining with optical interference can occur at higher concentration [15]. Bilirubin at concentrations >68.4 μmol/L caused artfactually increased fructosamine values. Therefore, moderately to grossly hemolyzed or icteric samples are not acceptable for fructosamine analysis.

The reference value (up to 282 μmol/L, n = 228) for nondiabetic patients was similar to the reagent manufacturer’s published reference value (up to 285 μmol/L, n = 997), the reference value (up to 289 μmol/L, n = 230) reported by Cefalu et al. [16], and the reference value (up to 296 μmol/L, n = 2211) reported by Baker et al. [14].

Correction of fructosamine value for albumin improved correlation between fructosamine and well-accepted biochemical measures of diabetic control. Fasting glucose correlated better with fructosamine corrected for albumin than with fructosamine, with fructosamine corrected for total protein, or with Hb A₁c. Hb A₁c value correlated best with fructosamine corrected for albumin. Our findings regarding correlation between fructosamine and fasting glucose or fructosamine and Hb A₁c agree well with values supported by Cefalu et al. [16].

The fructosamine assay has several advantages over the Hb A₁c assay. Samples for the fructosamine assay require no pretreatment, and because serum is used, the assay can be done with other routine chemistry tests with the same instrument simultaneously (no separate whole-blood sample is necessary). In this study, 350 fructosamine assays could be done in 2 h on one BM/Hitachi 747-200 analyzer, but 50 h would be needed for the same number of Hb A₁c assays done with one Bio-Rad/Diamat analyzer. The fructosamine assay is considerably less expensive (in terms of labor and reagent costs) than the Hb A₁c assay. Serum fructosamine concentration is not affected by Hb variants or decreasing Hb half-life [17, 18].

Preliminary clinical data tend to support the concept that the fructosamine assay can provide a good index of glycemic control [16], especially in patients involved in intensive intervention programs, e.g., diabetic pregnant patients [3]. Because of the reliability, technical simplicity, low cost, and reduced analytical time of the fructosamine assay, we conclude that this assay can be considered an alternative choice for first-line monitoring of diabetic patients. However, additional studies are needed to prove its clinical usefulness.

The Medical Editing Department, Kaiser Foundation Research Institute, provided editorial assistance.

**References**

10. Thomas L. Correction of the fructosamine value to protein:

---

**Table 3. Correlation coefficients between fructosamine, Hb A₁c, and fasting glucose in 222 diabetic subjects.**

<table>
<thead>
<tr>
<th>Fructosamine</th>
<th>Fructosamine corrected for albumin</th>
<th>Fructosamine corrected for total protein</th>
<th>Hb A₁c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A₁c</td>
<td>0.80</td>
<td>0.87</td>
<td>0.85</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.74</td>
<td>0.79</td>
<td>0.75</td>
</tr>
</tbody>
</table>

All values significant at the P < 0.001 level.

*Not applicable.*


