Correlation of HbA₁C, Glycated Serum Proteins and Albumin, and Fructosamine with the 24-h Glucose Profile of Insulin-Dependent Pregnant Diabetics

Lauri Suhonen,¹,² Ulf-Håkan Stenman,¹ Velkko Kolviisto,² and Karl Teramo¹

To assess the value of various methods for long-term follow-up of diabetic patients, we compared the concentrations of fructosamine in serum with those of various glycated proteins: hemoglobin (HbA₁C), total serum proteins (G-prot), and albumin (G-alb), assayed in 30 pregnant insulin-dependent diabetics every two weeks after initial determination of a 24-h blood glucose profile. HbA₁C correlated best with the 24-h glucose profile during the succeeding 10–35 days (r = 0.65–0.68, P <0.001). G-prot and G-alb correlated nearly as well as HbA₁C 10–20 days after the glucose profile (r = 0.54–0.64, P <0.01–0.001), but only weakly after 25–35 days.

Monitoring of diabetic control is especially important in pregnancy because of the risk of fetal malformations, intrauterine death, macrosomia, and other complications (1). Such monitoring has been greatly improved by the introduction of methods the results of which reflect the mean blood glucose concentration during a longer interval. Glucose reacts with amino groups of proteins, causing non-enzymatic glycation, the degree of which is proportional to the mean glucose concentration. Depending on the biological half-life of the protein, the proportion of glycated forms reflects the integrated glucose concentration over the previous weeks or months.

Assay of glycated hemoglobin was the first method to be used for monitoring diabetic control (2). Various chromatographic and electrophoretic techniques have been used for this purpose. Either they are manual and laborious or they are automated methods that require expensive equipment. Glycated hemoglobin reflects blood glucose concentrations over a fairly long time, four to eight weeks (2); this limits the use of this assay for the detection of rapid changes in blood glucose.

Glycated serum proteins (G-prot) and albumin (G-alb) reflect the blood glucose concentration during the preceding one to two weeks (3–5). Assay of these is based on the separation of the glycated and nonglycated proteins by affinity chromatography on boric acid–agarose columns, and quantitative determination of each fraction. These methods also are time consuming. Therefore various approaches have been used to simplify them by directly measuring the glycated components, i.e., without prior separation. One such method is based on the reducing properties of glycated proteins. This method, called the "fructosamine assay," is a readily automated colorimetric method, which promises to be a useful alternative to the earlier-mentioned methods (6).

To evaluate the usefulness of various assays in pregnant insulin-dependent diabetic (IDD) patients, we studied prospectively the correlation between the 24-h glucose profile of pregnant IDD patients with the fructosamine concentration determined in samples taken 10–35 days after the 24-h glucose profile was measured. Results of the fructosamine assay were compared with those of HbA₁C, G-prot, and G-alb.

Materials and Methods

Patients

We studied 30 pregnant IDD women. The mean duration of diabetes was 16.5 (SD 5.0) y, and the mean daily insulin dose was 46.4 (SD 11.9, range 25–72) units. Their mean age was 26.8 y (range 19–34 y). Eighteen of the 30 were primiparous.

The group of nonpregnant IDD patients consisted of 26 subjects. The mean duration of diabetes was 11.8 (SD 7.9) y and the mean daily insulin dose was 35.3 (SD 14.3, range 18–70) units. Their mean age was 33.9 y (range 16–66 y). Thirteen were women.

A control group consisted of 18 nonglycated pregnant women with a mean age of 26.2 y (range 21–31 y). They all had normal results for the 75-g oral glucose tolerance test (preprandial blood glucose in capillary blood <5.0 mmol/L, at 1 h <11.0, and at 2 h <8.0) at 28–32 weeks of gestation.

The pregnant IDD patients were admitted to the hospital for a 24-h glucose profile before the end of the 16th week of gestation. Blood glucose was measured at 4-h intervals, beginning at 8 a.m. On the day of admission and every two weeks thereafter, blood was sampled for determination of glycated proteins. Blood was sampled without prior fasting, for assay of glycated and glycated proteins, from the nonpregnant IDD patients during their routine visit to the outpatient clinic. Blood was also sampled from the nonfasting nonglycated control subjects before the end of the 17th week of gestation.

Methods

Blood glucose was measured by a glucose dehydrogenase method (Gluc-DH-Method, Merck) in a Multistat centrifugal analyzer (Instrumentation Laboratory).

HbA₁C was determined by a rapid, fully automated HPLC method. The intra- and interassay coefficients of variation (CV) of the method were both 2–3%. The reference interval for nonpregnant diabetic women is 4.2–6.6% of total Hb (7).

We separated G-prot and G-alb from nonglycated proteins by affinity chromatography (Glycotest; Pierce Chemical Co.). Protein was measured spectrophotometrically by
the Coomassie Brilliant Blue dye-binding method (8). The intra- and interassay CVs for the method were both 4–5%. G-alb was measured by immunoturbidimetry with reagents from Orion Diagnostica. The intra- and interassay CVs for this method were both 3–4%.

Fructosamine was measured colorimetrically (Fructosamine test; P. Hoffmann-La Roche) in the Multistat III centrifugal analyzer. The pH of the reaction mixture was 10.35, and the change in absorbance at 530 nm was measured between 10 and 15 min after the reaction was initiated. Intra- and interassay CVs for this method were both 3–5.5%.

Three to five blood specimens were obtained from each pregnant diabetic patient after the 24-h glucose profile. Correlation coefficients were calculated between the mean blood glucose concentration of the 24-h glucose profile and the glycated proteins at five-day intervals from 10 to 35 days after the 24-h glucose profile.

Results
The mean blood glucose concentration in the 24-h glucose profile of pregnant IDD patients was 7.1 (SD 2.3) mmol/L, and the range was 4.4–10.9 mmol/L. The mean postprandial blood glucose was 8.5 (SD 3.7, range 2.5–16 mmol/L). In the nonpregnant IDD patients the mean postprandial blood glucose was 11.4 (SD 7.5, range 2.1–37 mmol/L).

Table 1 shows the mean concentrations of HbA1C, G-prot, G-alb, and fructosamine. For all assays the nonpregnant IDD patients had higher values than the pregnant IDD patients. The capacity of various assays to differentiate between diabetic and healthy subjects is reflected by the difference in mean values between patients and control subjects. G-alb showed the largest difference between IDD groups and controls: 3.3- and 2.2-fold for nonpregnant and pregnant IDD patients, respectively. For G-prot these differences were 2.7- and 2-fold, for HbA1C 1.9- and 1.5-fold, and for fructosamine 1.5- and 1.4-fold, respectively.

The ability of the different assays to reflect the integrated blood glucose concentration was assessed by correlating the observed values with the 24-h glucose profile 10–35 days before the sample was taken. HbA1C correlated well with the 24-h glucose profile observed 10–35 days earlier (Figure 1, r = 0.65–0.68, P < 0.001). G-prot and G-alb correlated nearly as well 10–20 days after the glucose profile (Figure 1; r = 0.54–0.64, P < 0.01–0.001), but after 25–35 days the correlation started to be poorer. Fructosamine was not correlated significantly with the 24-h glucose profile at any time (Figure 1; r = 0.23–0.36).

Table 1. Mean (and SD) Concentrations of HbA1C, Glycated Serum Proteins (G-prot), Glycated Albumin (G-alb), and Fructosamine (Fra) in 26 Nonpregnant and 18 Pregnant Diabetics and in 18 Pregnant Controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonpregnant</td>
<td>Pregnant</td>
<td>Pregnant controls</td>
</tr>
<tr>
<td></td>
<td>(n = 26)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>9.9 ± 2.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.8 ± 1.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>G-prot, %</td>
<td>8.4 ± 2.7&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>6.4 ± 1.8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>G-alb, %</td>
<td>7.5 ± 3.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.1 ± 1.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Fra, mmol/L</td>
<td>3.7 ± 0.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.3 ± 0.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

* P < 0.001 (higher than controls). <sup>a</sup> P < 0.01 (higher than pregnant diabetics). <sup>b</sup> P < 0.05 (higher than pregnant diabetics).

Fig. 1. The correlation of HbA1C (O), glycated serum proteins (●), glycated albumin (□), and fructosamine (■) with 24-h glucose profile in 30 pregnant insulin-dependent diabetics during 10–35 days after the glucose profile.

The correlation between results obtained by the various assays was also studied by linear regression analysis. There was a highly significant correlation between assays for glycated proteins and fructosamine (Table 2 and Figure 2). However, in samples from the nondiabetic pregnant women, a significant correlation was observed only between G-alb and G-prot and between G-alb and fructosamine, respectively (Table 2). The y-intercept for the regression equation of fructosamine (y) as a function of G-prot (x) was 1.6, suggesting that there was an average nonspecific background of 1.6 mmol/L in the fructosamine assay.

Table 2. Correlations between HbA1C, Glycated Serum Proteins (G-prot), Glycated Albumin (G-alb), and Fructosamine (Fra) in 26 Nonpregnant and 18 Pregnant Diabetics and in 18 Pregnant Controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonpregnant</td>
<td>Pregnant</td>
<td>Pregnant controls</td>
</tr>
<tr>
<td></td>
<td>(n = 26)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>G-prot/HbA1C</td>
<td>1.09 ± 0.86&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.07 ± 0.84&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.37 ± 0.42</td>
</tr>
<tr>
<td>G-alb/HbA1C</td>
<td>1.33 ± 0.85&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.93 ± 0.81&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.12 ± 0.23</td>
</tr>
<tr>
<td>Fra/HbA1C</td>
<td>1.79 ± 0.67&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.23 ± 0.53&lt;sup&gt;#&lt;/sup&gt;</td>
<td>0.03 ± 0.09</td>
</tr>
<tr>
<td>G-alb/G-prot</td>
<td>1.22 ± 0.99&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.86 ± 0.95&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.29 ± 0.50&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fra/G-prot</td>
<td>1.89 ± 0.90&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.23 ± 0.67&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.14 ± 0.41</td>
</tr>
<tr>
<td>Fra/G-alb</td>
<td>1.54 ± 0.91&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.29 ± 0.76&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.37 ± 0.63&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* P < 0.001. <sup>a</sup> P < 0.01. <sup>b</sup> P < 0.05.

Fig. 2. Correlation between glycated serum proteins and fructosamine in the samples taken from 26 nonpregnant (0) and 18 pregnant insulin-dependent diabetics (●) and 18 pregnant controls (□).

The y-intercept, calculated by the standardized principal component method, was 1.6. The correlation equation, calculated by the least-squares method, was y = 1.75 ± 0.23x (r = 0.91).
The concentration of fructosamine is given in mmol/L; others are percent of total.

(Figure 2). Of the various assays, G-alb and G-prot showed the best correlation and HbA1C and fructosamine the worst.

Figure 3 shows the change of the various glycated proteins with time in four individual pregnant IDD patients. In all four patients the change in fructosamine was much smaller than the change in results of the other three assays for glycated proteins, and often the change was opposite to that of the other assays (Figure 3, b, c, and d). The change in HbA1C was smaller (Figure 3b) and occurred later (Figure 3d) than that of G-prot and G-alb. This is compatible with the longer biological half-life of HbA1C. G-alb, G-prot, and HbA1C displayed the same change in most of the cases. We studied the concordance between an increase or decrease of the various assay results in 30 pregnant IDD patients. The changes in G-alb and G-prot between consecutive samples were concordant in 55 of 65 cases (85%), but the change in fructosamine was discordant with G-alb and G-prot in only 38 of 65 (58%) and 37 of 67 samples (55%), respectively.

Discussion

Assays of glycated proteins are used to estimate the mean blood glucose concentration during a longer interval. Our results confirm that, in pregnancy, HbA1C, G-prot, and G-alb do this fairly well, and that for this purpose the fructosamine assay is clearly inferior to the other methods.

The difference in the ways that fructosamine and the other assays reflect changes in glycemic control in individual pregnant IDD patients is demonstrated by Figure 3. We selected four representative patients to illustrate the changes in the four components assayed during the follow-up period. The changes in HbA1C, G-prot, and G-alb generally paralleled one another. In contrast, the changes in fructosamine were very small, and relatively often (44%) were opposite to changes for the other assays. This suggests that the fructosamine assay measures components in serum that are not dependent on the mean glucose concentration.

These results may seem surprising, considering the good correlation between all these components observed in earlier studies (9-12). However, a significant correlation between two methods is easily obtained if samples containing low and very high concentrations are compared. In this study we also observed a good correlation when we used results for samples from nonpregnant IDD patients, but the correlation was barely significant when samples from pregnant diabetics were compared. The weak correlation between fructosamine and the previous mean blood glucose also supports such a conclusion. Although fructosamine was compared with only one diurnal glucose profile, this profile has been shown to be representative of daily control in pregnant diabetic patients (13).

The fructosamine assay is not specific for protein-bound fructosamine. Other reducing compounds also react with nitro blue tetrazolium (6). By choosing the pH (10.35) and reaction time (10–15 min) carefully, one can diminish the effect of reducing compounds such as blood glucose and creatinine. Nevertheless, interfering compounds still cause a background interference (14). Moreover, a recent study has demonstrated unexpectedly long half-life for fructosamine in type 1 diabetic children, suggesting that the fructosamine assay does not specifically measure protein glycation (15).

The nonspecific factors in the fructosamine assay have not been systematically studied, but a rough estimate is obtained from the regression equation between results for fructosamine assay and glycated serum proteins. This suggests that nonspecific factors contribute as much as 1.6 mmol of "fructosamine" (Figure 2), which represents 67% of the measured fructosamine in healthy pregnant women.

If this background changes from day to day, fructosamine assay may in fact be unsuitable for monitoring long-term control of blood glucose. Owing to the high background, the difference in fructosamine values between IDD patients and control subjects is also much smaller (1.4–1.5-fold) than that of G-prot (2.0–2.7-fold), although these assays should measure the same components.

Earlier studies have also shown that the fructosamine assay does not measure glycated proteins specifically. Johnson et al. (6) found that at least half of the fructosamine activity was ascribable to nonspecific reducing components, and Howey et al. (16) observed a nonspecific reducing activity corresponding to 0.9 mmol/L. Schleicher et al. (17) found that only about half of the reducing activity of the fructosamine was attributable to specific nonenzymatic glycation of proteins in healthy subjects and well-controlled diabetics. Smart et al. (18) correlated fructosamine with HbA1, and found a y-intercept of about 1.7, suggesting that there was an average nonspecific background corresponding to 1.7 mmol/L in the fructosamine assay.

The concentration of proteins in serum, 70 g/L, roughly corresponds to 1 mmol/L. Of these, about 3% are glycated—i.e., 30 mmol/L. The average apparent concentration of "fructosamine" in serum is 2.5 mmol/L. This would imply that each glycated protein molecule should carry 83 glucose molecules, which is unlikely. For example, glycated Hb carries an average of only one glucose. Part of this discrepancy apparently stems from the use of a standard that does not correspond to the analyte, but this is certainly only part of the explanation. This calculation adds further support to the notion that the main problem with the fructosamine assay is its unacceptably high nonspecific background.

The results for G-alb and G-prot were very similar, and therefore the choice between these methods may be based primarily on technical aspects. For assay of albumin, we used an immunological method, because the conventional
dye-binding methods are too sensitive to differences in buffer composition between the two albumin fractions obtained by affinity chromatography. Thus the assay of G-alb requires more complicated methodology than does the assay for G-prot.

The G-prot value depends not only on the integrated blood glucose value but also on the relative proportion of various proteins in the serum (19). Under conditions associated with rapid changes in serum protein concentration, such as acute infections or protein loss, the time available for non-enzymatic glycation of proteins will vary, and this will affect the concentration of G-prot. These factors would affect fructosamine and G-prot to the same extent. Therefore, changes in serum protein composition do not explain the weak correlation between mean glucose and fructosamine concentrations.

In a recent cross-sectional study, fructosamine was found to be a potential alternative to HbA1 for long-term control of nonpregnant IDD patients (18). However, these authors pointed out that a longitudinal study is necessary for a comprehensive assessment. This is clearly demonstrated by the present study. If only a cross-sectional correlation had been performed, the fructosamine assay would have given quite acceptable results. Only the longitudinal study revealed its limitations. It remains to be determined whether our results also hold true for nonpregnant diabetics.

Our results also may not be applicable to all methods for assay of fructosamine. However, the method we used was designed to minimize the known nonspecific effects (recently reviewed by Armbruster, 14). All methods for fructosamine are essentially similar, but whether our results are valid for other, similar methods remains to be determined.

We conclude that use of the fructosamine assay for long-term blood glucose control in pregnant IDD patients does not appear to be justified. Despite its technical advantages, the fructosamine determination provides less reliable information on blood glucose control than does either HbA1C or the assays for G-alb and (or) G-prot.

References