Serum Fructosamine in Diabetic Pregnancy
Erik K. Frandsen, Tahir Sabagh, and Robby A. Becchus

Serum fructosamine was measured in 275 blood donors, in 559 subjects with a normal pregnancy, in 32 gestational diabetics being treated with insulin and 69 being treated by diet only, and in 53 pregnant subjects with established diabetes. In none of the pregnant subgroups did the mean fructosamine concentration exceed that of the donor group. The concentration in normal pregnant subjects showed a modest but significant decrease with gestational age and an increase with maternal age. Hyperglycemic non-pregnant subjects (n = 24) had significantly increased serum fructosamine concentrations, and 96% of these subjects exceeded the upper 95% confidence limit for fructosamine in the donor group. A highly significant correlation was demonstrated between serum fructosamine and preprandial plasma glucose in the hyperglycemic subjects. A weak, but significant, correlation was shown for the entire population sample of antenatal patients, while this was non-significant within each of the sub-groups comprising established diabetics and gestational diabetics, respectively.

Additional Keyphrases: age-related effect \ influence of albumin \ hyperglycemia \ glycated proteins

Glycated hemoglobin is widely measured in the assessment of glycemic control in diabetes mellitus. Recently, however, a method for the measurement of glycated serum proteins has been attracting increasing attention. This method, the fructosamine method, is an alternative to the more cumbersome, time-consuming, and expensive procedures for measuring glycated hemoglobin. The fructosamine method (1) is well suited for automation (2–8). The automated assay combines the advantages of accuracy of estimation with a high analytical capacity and low cost. The assay is based upon measurement of the reducing activity of fructosamines at alkaline pH, with nitroblue tetrazolium as the redox indicator. Serum fructosamine concentration correlated well with preprandial blood glucose (1–3, 7, 9, 13) and with the concentration of glycated hemoglobin (3, 4, 7–9). Recently the fructosamine assay was reported to be of potential value for detection of gestational diabetes (2, 13). In this study, we measured serum fructosamine in normal and diabetic pregnancy, to investigate the influence of maternal and gestational age on concentrations of glycated protein in serum and to evaluate the efficacy of patients' treatment.

Methods and Materials

Methods

The measurement of serum fructosamine was automated by using a Cobas Bio centrifugal analyzer (Roche Diagnostic Systems) and a commercial kit from Roche Diagnostica in which a secondary protein standard is used. The analyzer settings were as recommended by the kit manufacturers except that the sample dilution was increased (10 \( \mu L \) sample, 27-fold final dilution) and the time interval decreased. Our use of a 10-\( \mu L \) sample volume ensured a linear relation between absorbance and dilution of serum and standards with saline (NaCl, 9 g/L). The fructosamine-induced reduction of nitroblue tetrazolium was followed by absorbance measurements 4 to 7 min after initiation of the reaction. This choice of assay timing doubled the assay capacity as compared with the more frequently used interval of 10–15 min (2, 5, 7, 8). However, the main reason for reducing the assay timing was not to increase the assay capacity, but to minimize use of the AutoAnalyzer, which in a busy routine laboratory is heavily scheduled for many other analyses. Other groups have recently reported similar short assay timing (4, 6, 14, 15).

The fructosamine assay is based on simple reduction chemistry, so the assay has a potential for interference from other reducing substances in serum. Such interference would result in overestimation of the fructosamine concentration. As yet, we have not rigorously tested the specificity of the assay under our assay conditions. The results on using these conditions tended, however, to be slightly lower by an average factor of 0.92 than those derived by use of incubation intervals of 10 and 15 min. Thus no systematic overestimation due to interference from serum constituents was evident.

Subjects

Blood was sampled by venepuncture from healthy male blood donors and from subjects attending the antenatal clinic. Our use of the donor blood for the establishment of the normal reference range was based on the lack of any sex-related difference in serum fructosamine (4, 8, 9). The samples were separated at room temperature, and the sera were stored at \(-20 ^\circ C\) before analysis.

The normal pregnant group comprised 559 subjects with a mean maternal age of 26 years (SD, 6.5 y) and a mean gestational age of 31 weeks (median, 33 weeks). The diabetic pregnant group comprised 69 gestational diabetics who were controlled by diet only, 32 gestational diabetics who received insulin, and 53 established diabetics on insulin therapy. All pregnant subjects attending the antenatal clinic had a 1-h screening test for blood sugar after orally ingesting 50 g of glucose (16). Subjects with plasma glucose values \( \geq 7.8 \text{ mmol/L} \) (1.40 g/L) were referred for further evaluation. The criteria for the diagnosis of gestational diabetes were those of O'Sullivan and Mahan (17), except that the glucose load was decreased to 75 g. The remaining subjects included in this study consisted of men and women who were selected by one of the criteria of a fasting blood glucose value \( \geq 7.8 \text{ mmol/L} \) (1.40 g/L) or a random value \( \geq 11.1 \text{ mmol/L} \) (2.00 g/L).

Statistical Analysis

Correlations were determined by linear regression analysis with the least-squares method. Student's \( t \)-test was used for statistical comparisons.

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Results

Assay Variables

Precision. The intra-assay imprecision, within-day inter-assay imprecision, and between-day interassay imprecision (i.e., the CVs) were 1.04%, 1.3%, and 3.1%, respectively.

Fructosamine was measured in 180 subjects with normal pregnancies, absorbance readings being made at 4-7 min and at 10-15 min. The mean ratio of the latter to the first measurement was 1.09 (SD 0.07). Thus the assay timing used in this study caused a slight but significant (P <0.001) lowering of the values as compared with those obtained by using measurements at 10-15 min.

Albumin. Figure 1 depicts the dose–response relationship of fructosamine reactivity vs the concentration of glycated human albumin. The albumin (40 g/L) was glycated in vitro and subsequently dialyzed against saline to remove excess glucose. The fructosamine reactivity was determined at various albumin concentrations (sample dilution in saline) and also at a fixed albumin concentration (sample dilution in saline containing 40 g of albumin per liter). We assayed 30-μL aliquots of these dilutions for fructosamine reactivity, using a total reaction volume of 270 μL. At fixed total albumin concentrations the reactivity was a linear function of the nominal fructosamine concentration. In contrast, for total albumin concentrations changed by dilutions of the glycated sample in saline, the dose–response plots were curvilinear.

Similar effects of albumin were observed on substituting quality-control sera, patients' sera, or a synthetic fructosamine, 1-deoxy-1-morpholinofructose, for the glycated albumin sample. The inhibitory effect of albumin was minimal at assay concentrations <1.5 g/L, equivalent to use of a serum volume of 10 μL. At total albumin concentrations >1.5 g/L a pronounced and dose-dependent inhibitory effect of albumin was evident. The broken line in Figure 1 is derived from the experimental graph by simple blank correction, i.e., by subtracting the reactivity due to the intrinsic glycation of the albumin. The slope of the curvilinear graph increased as the total albumin concentration decreased. At concentrations <1.5 g/L the graph was essentially linear, indicating that at these concentrations the inhibitory effect of albumin is negligible. The albumin-induced inhibition can be calculated from the difference in absorbance change between the curvilinear graph and the extrapolation of its linear segment.

Reference Values

The mean fructosamine concentration in serum of the 275 blood donors was 1.90 mmol/L (Figure 2). The reference interval in this population sample was 1.51–2.29 mmol/L (mean ±1.96 SD). Serum fructosamine in 559 subjects with a normal pregnancy did not differ significantly from that for the donor group (P >0.1; Figure 3A, Table 1).

The influence of the maternal and gestational age on the value for fructosamine in normal pregnant subjects is depicted in Figure 4. The mean fructosamine concentration in third-trimester subjects was 3% lower than that in second-trimester subjects (Table 2). The concentration remained constant after 20 weeks of gestation (Figure 4B; Table 2). It tended to increase with the age of the subjects (Figure 4A), but this increase was only significant when the youngest was compared with the oldest age-group (Table 3).

The correlation between serum fructosamine and total protein was poor (r = 0.28; n = 96; P <0.01) and we did not attempt to correct the fructosamine values for serum protein content.

Fructosamine in Diabetes

The mean fructosamine concentration in the pregnant diabetics receiving insulin and that in the donor group did not differ significantly (Table 1). Conversely, the concentration was lower in the gestational diabetics controlled by diet only (P <0.001) and higher in the non-treated hyperglycemic subjects (P <0.001) than that in the donor group. Most of the hyperglycemic subjects (96%) had fructosamine concentrations exceeding the upper 95% confidence limit for the donor group.

The best estimate of a linear relationship between serum fructosamine and fasting plasma glucose in the non-treated
Table 1. Serum Fructosamine in Various Patient Groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>275</td>
<td>1.90</td>
<td>0.20</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>559</td>
<td>1.89</td>
<td>0.15</td>
</tr>
<tr>
<td>Gestational diabetics, diet only</td>
<td>69</td>
<td>1.70</td>
<td>0.15</td>
</tr>
<tr>
<td>Gestational diabetics, insulin treated</td>
<td>32</td>
<td>1.86</td>
<td>0.10</td>
</tr>
<tr>
<td>Pregnant diabetics, insulin treated</td>
<td>53</td>
<td>1.88</td>
<td>0.21</td>
</tr>
<tr>
<td>Non-pregnant hyperglycemic subjects</td>
<td>24</td>
<td>3.41</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Table 2. Influence of Gestational Age on Serum Fructosamine in Normal Pregnancy

<table>
<thead>
<tr>
<th>Gestation, weeks</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–26*</td>
<td>105</td>
<td>1.94</td>
<td>0.18</td>
</tr>
<tr>
<td>26–40*</td>
<td>317</td>
<td>1.88</td>
<td>0.14</td>
</tr>
<tr>
<td>20–30*</td>
<td>113</td>
<td>1.90</td>
<td>0.16</td>
</tr>
<tr>
<td>30–40*</td>
<td>253</td>
<td>1.88</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 3. Influence of Maternal Age on Serum Fructosamine in Normal Pregnancy

<table>
<thead>
<tr>
<th>Maternal age, y</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–26*</td>
<td>79</td>
<td>1.87</td>
<td>0.17</td>
</tr>
<tr>
<td>20–40*</td>
<td>360</td>
<td>1.90</td>
<td>0.15</td>
</tr>
<tr>
<td>40–60*</td>
<td>12</td>
<td>1.98</td>
<td>0.14</td>
</tr>
</tbody>
</table>

hyperglycemic subjects was calculated by the least-squares method. The regression equation had the form:

Fructosamine (mmol/L) = 0.15 × glucose (mmol/L) + 1.16

(r = 0.88; P < 0.001).

Linear-regression analysis of the correlation between fructosamine and glucose in plasma of the fasting pregnant subjects showed a weak correlation for the entire population sample (0.02 < P < 0.05), but no significant correlation within each of the sub-groups comprising established diabetics and gestational diabetics, respectively. The mean value for plasma glucose concentration, measured preprandially, was 5.42 (SD 0.95) mmol/L for the established diabetics and 4.93 (SD 0.59) mmol/L for the gestational diabetics.

Discussion

The fructosamine concentration in serum is measured in terms of deoxymorpholinofructose equivalents, either by direct comparison with the reactivity of 1-deoxy-1-morpholinofructose or, indirectly, by comparison with a protein standard calibrated with the synthetic fructosamine. Ordinarily, this standard is reconstituted in media containing 40 g of albumin per liter, and it is well known that the albumin

Fig. 3. Frequency distribution of values for serum fructosamine in pregnant females
(A) normal pregnancy; (B) established diabetics on insulin therapy; (C) gestational diabetics on insulin therapy; (D) gestational diabetics controlled by diet only

Fig. 4. Serum fructosamine in normal pregnancies vs maternal age (A) and gestational age (B).
The hatched areas represent mean values ± 1.96 SD. The open areas represent mean values ± 1.96 SEM.
contributes to the fructosamine-induced reduction of nitroblue tetrazolium (1, 4, 7, 14, 18). This albumin effect is composite in nature, consisting of a positive element, which is ascribable to the inherent glycation of the albumin, and a negative element, which causes decreased reduction of the redox indicator. The interference by the positive element can be overcome by blank correction, and such correction is now commonly applied (4, 7, 18). The fructosamine concentration in serum was underestimated in the early reports (1–3) and in some more recent works (8, 9), owing to lack of blank correction. The inhibitory effect of albumin is less well recognized as a source of potential interference in the fructosamine assay. We found that this interference is minimized when concentrations of albumin in the assay mixture are <1.5 g/L, as we easily achieved by simply decreasing the sample volume to 10 μL. The inhibitory effect may be a general protein effect caused by partial binding of the oxidized form of the redox indicator to the protein matrix (1).

The present study confirms the findings of others that serum fructosamine concentrations in pregnant subjects are influenced by gestational age (13, 19) as well as maternal age (13). However, the age dependency demonstrated in these studies was more pronounced than that reported in the present study. The mean fructosamine concentration in third-trimester subjects was 3% less than that in second-trimester subjects, about half the change reported in two other studies (13, 19).

The mean value for serum fructosamine in insulin-treated pregnant subjects did not differ significantly from that found in normal pregnancy or blood donors, thus indicating that, on the average, the glycemia in the diabetic patients was likewise normalized. In contrast, significantly higher concentrations of fructosamine in serum were measured in non-treated hyperglycemic patients, and in this patient group the fructosamine concentration correlated highly significantly with the preprandial glucose concentration in plasma. Such correlation has also been well documented by others (1–3, 7, 9, 13). The correlation between preprandial plasma glucose and fructosamine in antenatal patients was much less pronounced. Within each of the antenatal subgroups the correlation was non-significant, and only for the entire population sample was this correlation significant. Such poor correlation was not unexpected when one considers the narrow ranges of preprandial glucose values. Furthermore, the degree of protein glycation is a function of time and of the time-averaged protein and glucose concentration. Thus a correlation with glucose concentration should only be expected insofar as this variable reflects the average glycemic situation.

Our results regarding the normalization of fructosamine concentration in insulin-treated pregnant subjects are at variance with those reported by Roberts et al. (2), who found significantly higher concentrations than in non-diabetics. However, the values reported by these investigators for the insulin-treated patients were the maximum values recorded throughout the pregnancy, and thus any direct comparison with our results may be flawed.

Our observation that the serum fructosamine concentration in normal pregnancy did not differ from that in male blood donors accords with recent reports that non-diabetic antenatal patients do not differ in this respect from non-diabetic hospitalized and ambulatory patients (8), and that no sex-related difference was seen (4, 8, 9).

An improvement in glycemic control in antenatal patients improves the perinatal outcome. Owing to the longer biological half-life of hemoglobin as compared with serum proteins, the concentration of glycated hemoglobin is a less-sensitive index of the glycemic control than is serum fructosamine. The simplicity and good performance of the fructosamine assay, combined with its low cost and suitability for automation, recommend this assay for use in assessment of glycemic control in antenatal patients.

References