Specific Glycation of Albumin Depends on Its Half-Life

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Some suggest that measurements of plasma fructosamine concentration should be corrected for the plasma protein (or albumin) concentration because the extent of glycation per volume depends on both protein and glucose concentrations. Several reports, however, demonstrate a poor correlation between plasma fructosamine and albumin concentrations in diabetic patients. In vitro kinetic and in vivo studies have shown that glycation is also dependent on the half-lives of plasma proteins. Because a decrease in plasma albumin diminishes its catabolism, we speculated that low albumin concentrations are associated with a greater extent of glycation on a molar basis (specific glycation) and vice versa. To test this hypothesis, we studied plasma albumin, total protein, and fructosamine in 63 nondiabetic subjects with normal plasma fasting glucose concentrations and hemoglobin A₁c between 5.1% and 5.9%. Plasma fructosamine was poorly correlated with albumin concentration \( r = 0.348 \) but a logarithmic plot of the specific glycation of albumin vs albumin concentration showed a better correlation \( r = -0.842 \), suggesting that the kinetic considerations were operating in vivo. Therefore, because lower specific glycation of plasma albumin "compensates" for higher concentration, correction of fructosamine for albumin content in patients will overestimate mean blood glucose when albumin is low and underestimate it when albumin is high.

Indexing Terms: glycohemoglobin • fructosamine • diabetes • total protein • metabolism kinetics

In view of the importance of strict blood glucose control for the delay of onset of late complications of diabetes, the assay of glycohemoglobin to monitor blood glucose is now well established. More recently, various assays of glycated plasma proteins have been introduced, with claims of improvement in simplicity, automatization, and specificity (1–7). The fructosamine assay was first described as a simple concentration measurement of glycated plasma protein without reference to total protein (3). Because the original assay lacked specificity (8), it has been improved and a novel calibration method was applied (9). This improved fructosamine assay also was not corrected for serum protein or albumin content. However, the extent of glycation of albumin accounts for more than half of total plasma protein glycation (10, 11).

Measurement of glycohemoglobin has traditionally been expressed as the percentage of total hemoglobin that is glycated. Likewise, glycated plasma protein measured by affinity chromatography, by hydroxymethylfurfural, or by the fructose method has been related to protein or albumin content (4–6). This calculation is based on the assumption that the extent of glycation per volume depends on both protein and glucose concentrations. Glycation/protein and glycation/albumin, i.e., specific glycation on a molar basis, should therefore reflect diabetic control most accurately.

Numerous workers have reported statistically significant correlations between plasma albumin and plasma fructosamine within the reference range (12, 13), during treatment (14), in subjects with different plasma albumin values (15), and in pregnant women (16). Other studies report less or no significant correlations between fructosamine and plasma albumin. For example, Baker et al. (17, 18) suggested that fructosamine values need not be corrected for albumin concentrations when albumin values are normal. From results obtained in 61 diabetic children, Allgrove and Cockrill (19) concluded that correction for albumin did not offer any advantage. The problems associated with the fructosamine assay, including protein correction, have recently been reviewed (20).

Two studies with large cohorts of normal subjects (768 and 492, respectively) found no narrowing (21) and even an increase (22) of the range of the reference values when corrected for albumin. These studies did not, however, take into account that the half-life of albumin is dependent on its plasma concentration (23).

Our aim in this study is to demonstrate that the fructosamine concentration is influenced by both the concentration and the half-life of albumin. Because these two variables are inversely related (2), they exert compensating effects on plasma fructosamine concentration.

Materials and Methods

We measured glycohemoglobin (HbA₁c) by ion-exchange chromatography, using the Diamat HPLC system (Bio-Rad, Munich, Germany). Between-assay imprecision was 1.3% at an HbA₁c concentration of 8.5%. Plasma fructosamine content was determined with commercial reagents (Boehringer Mannheim, Mannheim, Germany) and a Hitachi 717 analyzer, according to the method of Johnson et al. (3) as modified by Siedel et al. (24). The between-assay coefficients of variation were 1.5% at a fructosamine concentration of 194 \( \mu \text{mol/L} \) and 1.8% at 485 \( \mu \text{mol/L} \). The central 95% reference interval was 203–289 \( \mu \text{mol/L} \) (mean 248 \( \mu \text{mol/L} \)). An in vitro glycated human-based plasma was used as calibrant (9).

Total protein content and glucose concentration were determined with commercial reagents (Boehringer Mannheim) with a Hitachi 717 by the biuret and glucose dehydrogenase reaction, respectively. Plasma album. 
min concentrations were determined by the bromcresol purple method. The central 95% reference intervals for albumin and total protein were 32–55 (mean 44 g/L) and 61–82 g/L (mean 72 g/L), respectively. Between-assay imprecisions of albumin and total plasma protein were 2.8% and 2.7% for concentrations of 36 and 61 g/L, respectively.

Regression analyses were performed by the method of least squares, and the correlation coefficient was determined by using a curve-fit program on an IBM AT computer.

Blood was sampled after overnight fasting from 63 subjects who were selected on the basis of HbA1c values between 5.1% and 5.9%. Anyone with glucose concentrations >5.9 mmol/L was excluded.

Results

Mean plasma values in these nondiabetic subjects were albumin 36 g/L (range 17–48), total protein 73.1 g/L (49–93), and fructosamine 228 μmol/L (165–303). We found a poor correlation between plasma fructosamine concentrations and albumin \( (r = 0.348) \) (Figure 1, top). Furthermore, the minimal slope of the linear-regression line means, for example, that doubling the albumin concentration from 25 to 50 g/L would yield only a minor increase in the fructosamine value, i.e., from 214 to 240 μmol/L. The same relationship holds also for total protein correction. Specific glycation of albumin (and protein) was calculated as fructosamine concentration/albumin (protein) concentration. Plotting the logarithm of the specific glycation of albumin against the albumin concentration yielded an inverse linear relationship \( (r = -0.842) \) (Figure 2, top).

Applying the same considerations to total plasma protein showed a poor correlation with either fructosamine concentration \( (r = 0.397) \) (Figure 1, bottom) or specific glycation of total protein \( (r = 0.55) \) (Figure 2, bottom). Comparison of HbA1c and fructosamine, and specific glycation of albumin, respectively, yielded no statistically significant correlation (data not shown).

To prove that our results are not influenced by unknown factors due to nonspecific reducing activity, we isolated by preparative electrophoresis (11) plasma albumin from five nondiabetic subjects with low albumin concentrations and five subjects with high albumin. Subsequent determination of specific glycation with the frurosine method yielded values of 137%, 142%, 131%, 120%, and 145% of normal albumin glycation \( (n = 20) \) for albumin concentrations of 32.6, 35.0, 37.0, 20.0, and 21.0 g/L, respectively. Accordingly, in subjects with albumin concentrations of 47, 49, 48, 50, and 47 g/L we measured specific glycation values of 79%, 63%, 87%, 99%, and 77%, respectively.

![Fig. 1. Comparison of plasma fructosamine measured in 63 nondiabetic subjects with known concentrations of albumin (top) and total protein (bottom) 
(Top) \( y = 1.21x + 183; r = 0.348 \) (Bottom) \( y = 1.20x + 196; r = 0.397 \)](image1)

![Fig. 2. Semilogarithmic plot of specific glycation of albumin with the corresponding albumin concentration (top) and total protein (bottom) 
Same values as shown in Fig. 1. (Top) \( y = -0.0105x + 1.18; r = 0.842 \) (Bottom) \( y = -0.0035x + 0.747; r = 0.55 \)](image2)
**Discussion**

It has been widely recognized that glycation of hemoglobin is dependent not only on blood glucose concentration but also on the lifetime of the erythrocyte. For example, when erythrocytes were separated according to their age, higher glycations of hemoglobin and erythrocyte membrane protein were measured in the older cells (25).

Baynes et al. (10) and our group (26) have analyzed the kinetics of the glycation reaction of albumin in detail. When albumin was glycated in vitro under steady-state conditions, we found glycation comparable with that found in normoglycemic subjects, suggesting that in vitro glycation corresponds to physiological glycation. If it is assumed that (a) steady-state equilibrium is reached, (b) glycated and native albumin are eliminated at the same rate, and (c) the glycated albumin is stable under physiological conditions, the following relations are valid:

\[ [G-A] = k[G][A]T_{1/2} \]
\[ [G-A]/[A] = k[G]T_{1/2} \]

That is, the concentration of glycated albumin [G-A] at steady state is proportional to the mean glucose concentration [G], to the albumin concentration [A], and to the half-life of albumin (T_{1/2}) (equation 1). Therefore, specific glycation of albumin (G-A)/[A] is proportional to T_{1/2} and the glucose concentration (equation 2).

If mean plasma glucose concentration in nondiabetics is assumed to be constant, then the specific glycation should depend only on albumin’s half-life:

\[ [G-A]/[A] = k'T_{1/2} \]

where \( k' = k[G] \).

To prove or falsify these relationships, we selected nondiabetic subjects on the basis of their HbA_1c values. To ensure that the subjects had comparable mean blood glucose concentrations within a narrow range, we studied only subjects with HbA_1c values between 5.1% and 5.9%. Therefore, [G] should be relatively constant in these subjects.

Because the half-lives of the different plasma proteins vary considerably and because albumin is the most prominently glycated protein in plasma, and assuming that T_{1/2} of albumin is constant, glycation should be proportional to albumin concentration (equation 1), and the specific glycation of albumin should be independent of other variables, including albumin concentration (equation 2). However, we found that correlation of fructosamine with albumin was very poor and specific glycation clearly depended on albumin concentration. This obvious discrepancy can be explained only by the third variable, the half-life of albumin. Plasma albumin influences its own catabolism in such a way that lower plasma concentrations of albumin are catabolized substantially more slowly; i.e., they remain longer in the circulation (23). Therefore, on a molar basis, albumin in low concentrations is glycated to a higher extent than when present in high concentrations. For quantitative considerations one must realize that albumin concentration and its catabolic rate \( k_{cat} \) are semilogarithmically related (23):

\[ \lg k_{cat} = \lg (0.693/T_{1/2}) = k^*[A] \]
\[ \lg T_{1/2} = \lg 0.693 - k^*[A] \]

Substituting equation 4 in equation 3, and recognizing that \( \lg 0.693 = 0.16 \), yields:

\[ \lg [G-A]/[A] = \lg k' + 0.16 - k^*[A] \]

\[ \lg [G-A]/[A] = \text{const.} - k^*[A] \]

**Equation 5** shows that the logarithm of the specific glycation of albumin is indirectly proportional to the albumin concentration itself. Plotting the variables and calculating the linear-regression line showed a good correlation \( r = -0.842 \) for glycation and concentration of plasma albumin (Figure 2, top).

We also isolated albumin from five subjects with low albumin and from five subjects with high albumin concentrations and measured specific glycation by a different, more specific method. This was performed to verify our results by another method, in case our original results might have been influenced by an unknown reducing activity interfering with the fructosamine assay (8). Again, higher specific glycation was found for low plasma albumin concentration and vice versa. These results verify that the half-life of albumin indeed determines specific glycation. To get an idea of the quantitative relationship between albumin concentration and its half-life, consider that a decrease in albumin by about one third causes a 50% decrease in the rate of its catabolism. These considerations explain why specific fructosamine values approximately double when albumin concentrations in plasma are halved.

We also related our fructosamine values to the concentration of total protein, given the suggestion of Hill et al. (20) to correct fructosamine values for total protein content. We obtained essentially the same results but the relations were less pronounced than were observed for albumin. This is as expected because total plasma protein includes proteins such as immunoglobulins or fibrinogen, which show little or no influence of their plasma concentration on their own catabolism (23).

Recently, this laboratory demonstrated (26) that glycation of other plasma proteins is also influenced by half-life. Isolation of low-density lipoprotein from nondiabetic subjects revealed that low-density lipoprotein...
with a longer half-life, e.g., because of hypothyroidism, was glycated to a higher degree than in subjects whose lipoprotein had a shorter half-life (26).

To our knowledge, this is the first observation in clinical chemistry that albumin should not be used for correction because its concentration influences its own turnover, which in turn influences the amount of glycation. It might be wise to reflect on whether our results also apply for other systems. We conclude that the amount of glycated plasma albumin may not be related to albumin content for the reasons discussed above. Therefore, correcting plasma fructosamine values for albumin content may provide neither a more accurate interpretation of metabolic control nor a smaller reference range.

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References