Fructosamine
Clinical Usefulness and Determination
of Reference Ranges

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Introduction

A new method for determining glycated serum proteins, the fructosamine assay, was first described by Johnson, et al.[1], in 1982. Recently, our company has been investigating the use of fructosamine measurement as an additional diabetes assessment tool.

While serum glucose measurements can indicate an individual's glycemic control at a given point in time, glucose measurements can be adversely affected by pre-analytical handling prior to the specimen arriving at the laboratory. Hemolysis, lipemia, prolonged contact of the serum on the cells, transport temperature and fasting status can all affect glucose measurement.

Fructosamine measurement, on the other hand, exploits the ability of serum proteins to undergo glycation (glycosylation) in conditions of increased ambient glycemia. Glycated proteins are an accepted measure of intermediate glycemia[2,3] as demonstrated by the well established clinical usage of hemoglobin A determinations. Serum proteins are glycated as well, and determination$_{1c}$ of their values reflect time-integrated glucose concentrations over the two- to four- week half-life of these serum proteins.

Clinical Implications

The rationale for measuring glycated hemoglobins or glycated plasma proteins is twofold. First, levels of these glycated proteins have been demonstrated to reflect the time-averaged glucose concentrations over the half-life of the respective protein. Secondly, it is established that glycation of proteins often changes the function of the protein in question.

As examples, glycohemoglobin is known to have an increased affinity for oxygen with the effects apparent in tissue. The resultant tissue hypoxia has been incriminated as a cause of diabetic microangiopathy.[4] Glycation of bovine lens crystalline has been reported and may be important to the genesis of cataracts in human diabetes.[5] The glycation of other plasma proteins may likewise result in alterations of their various physiological functions, such as binding, transport, etc. .

Another demonstration of the clinical utility of fructosamine was described by Baker, et al.[6] in which the fructosamine assay was used as a screening test for occult diabetes in groups of patients referred for oral glucose tolerance tests. Comparison of fructosamine concentrations with the results of oral glucose tolerance tests in selected individuals in whom diabetes mellitus was suspected, yielded a significant difference between subjects with and without diabetes. In this group, the specificity and sensitivity of fructosamine as a true diabetes predictor was 88 percent and 91 percent, respectively.
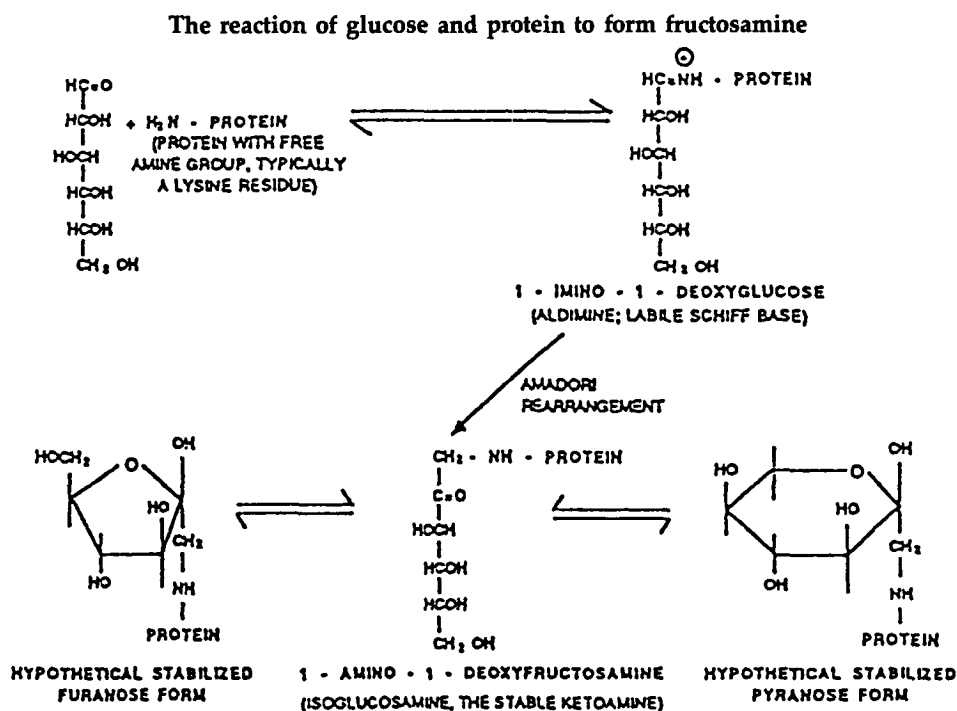
The fructosamine assay's ability to distinguish between subjects with impaired glucose tolerance and those with true diabetes is a decided advantage to the test. Baker, et al.[6] demonstrated very little overlap and good discrimination between these two groups. A positive result on fructosamine screening is therefore consistent with a conservative diagnosis of diabetes.

Chemistry

Glycation is a nonenzymatic mechanism resulting in the coupling of a sugar to a protein. In this process, glucose molecules are joined to protein molecules to form stable ketoamines, or fructosamines. In the case of fructosamines, the glycation involves a labile Schiff-base intermediate and Amadori rearrangement.[7] The major component of the fructosamine moiety is glycated albumin, with albumin constituting approximately 52 to 68 percent of total serum proteins. Fructosamine is the generally accepted name for 1-amino-1-deoxyfructosamine, and is sometimes referred to as isoglucosamine. Fructosamine was first synthesized by Emil Fischer in 1886.[8]

As mentioned, fructosamine is a ketoamine, a derivative of the nonenzymatic reaction product of a sugar (usually glucose) to a protein (usually albumin). A glycoprotein, as opposed to a glycated protein, is a protein molecule, typically a globulin, that incorporates a carbohydrate moiety enzymatically during synthesis of the molecule. In contrast, fructosamines arise from a post-translational modification involving a nonenzymatic mechanism and should not be confused with glycopro-

Figure 1

The reaction of glucose and protein to form fructosamine

HC=O
HCOH + H₂N - PROTEIN
HOCH   (PROTEIN WITH FREE
HCOH   AMINE GROUP, TYPICALLY
HCOH   A LYSINE RESIDUE)
CH₂OH

HC=NH - PROTEIN
HCOH
HOCH
HCOH
HCOH
CH₂OH

1 - IMINO - 1 - DEOXYGLUCOSE
(ALDIMINE; LABILE SCHIFF BASE)

AMADORI REARRANGEMENT

CH₂ - NH - PROTEIN
C=O
HOCH
HCOH
HCOH
CH₂OH

1 - AMINO - 1 - DEOXYFRUCTOSAMINE
(ISOGLUCOSAMINE, THE STABLE KETOAMINE)

HYPOTHETICAL STABILIZED
FURANOSE FORM

HYPOTHETICAL STABILIZED
PYRANOSE FORM

Taken from Armbruster, D.A. *Clin. Chem.*, Vol. 33, No. 12, 1987

is presented below in schematic form (Figure 1).

Analytical Approaches

Five different methodologies are described in the literature to measure fructosamine:

1. Phenylhydrazine procedure.
2. Furosine procedure.
3. Affinity chromatography.
4. 2-thiobarbituric acid colorimetric (TBA) procedure.
5. Nitroblue tetrazolium colorimetric (NBT) procedure.

These various procedures are reviewed by Armbruster.[7] The most widely accepted fructosamine assay, the NBT procedure, is used at this company.

The NBT assay has been adapted to a wide variety of automated chemistry analyzers. Baker, et al.[9] found from a six-month study of 33 laboratories utilizing the NBT protocol with both manual procedures and five different analyzers, that within-run, day-to-day and interlaboratory coefficients of variation (CV) were typically in the range of two to seven percent.

Suboptimal analytical sample conditions demonstrate negligible effects on assay results and add to the attractiveness of the fructosamine assay. Hyperlipemia also does not seem to interfere significantly with the assay.[10,11] Very few interfering substances have been found to affect fructosamine determination, although elevated bilirubin may occasionally cause significant alteration in the test values.[12]

It has been shown that serum albumin concentration affects fructosamine values. It was found that hypoalbuminemia influences the assay, but that fructosamine concentrations are generally independent of albumin concentrations when the level of albumin exceeds 30 to 35 grams per liter.[13] McDonald, et al.[14] developed an algorithm to adjust fructosamine levels by accounting for variations in total serum protein and albumin. The formula is:

Corrected Fructosamine = Fructosamine + 0.006 (70 - Total Protein) + (0.013) (40 - albumin).

Use of this formula will negate the effects of total serum protein and albumin until extreme levels are reached. However, with multipliers of 0.006 for protein and 0.013 for albumin, the corrections are typically not significant.

Method

We have adapted to automation a method for the determination of fructosamine described by Johnson, et al.[1] In this method, glycated serum proteins (fructosamines) reduce nitroblue tetrazolium (NBT) to form a purple-colored formazin.

A sample volume of 15 mmol/L of serum is added to 250 mmol/L of NBT reagent (Sigma, St. Louis, MO) containing 0.25 mmol/L of NBT in sodium carbonate/sodium bicarbonate buffer at pH 10.4. Calibration is with Sigma Accuset Calibrator. The set point of this liquid-stabilized calibrator is established with a glycated human albumin product. The set point corresponds to 1-deoxy-1-morpholino-D-fructose

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The analysis is conducted on an Olympus AU5000 Analyzer, set to record a bichromatic end point reaction. The reaction is run at 37. The wavelength pair is programmed at 540/750 nanometers; the reaction is read initially at 5.3 minutes, and again at 8.3 minutes.

It should be noted that the reference ranges for fructosamine are highly dependent on methodology as well as values and the types of calibrators utilized. There have been a number of problems with the stability of DMF calibrators, and it is generally agreed that secondary calibrators, such as the ones described above, are preferential.[12] Although absolute comparisons between studies are difficult because reference ranges and absolute numerical values of fructosamine are not comparable, the usefulness of correlation studies are apparent.

Support Studies

Correlation

It is well known that random nonfasting glucose measurements are a relatively poor measure of glycemic control. Therefore, correlation is also poor for fructosamine or hemoglobin A_{1c} to random nonfasting blood sugars or even mean blood sugars over short intervals,[15] (despite the good correlation between fructosamine and hemoglobin A_{1c}). Comparison of glycated proteins with similar half-lives (two to four weeks for fructosamine and 60+ days for hemoglobin A_{1c}) would most likely find statistically significant correlations. Indeed, statistically significant correlations between serum fructosamine concentrations and glycated hemoglobin concentrations have been observed by many researchers.[16-25]

Daubresse, et al.,[19] reported an interesting correlation of fasting blood glucose relative to glycemic control, using A1 determinations, and fructosamine utilizing a similar test

methodology. Their correlations (Figure 2) match well with empirically-derived percentile cut-offs as determined in our studies (Figure 3) in the subjective categories of glycemic control: "Excellent," "Good," "Fair," and "Poor."

In addition, Baker, et al.[6] published a normal range of 1.23 - 2.15 mmol/L for fructosamine, using the NBT assay, which corresponds well to our established ranges. As fur-

Figure 3
HORL Glycemic Control Ranges
(based on percentile ranges for > 5000 specimens)

Glycemic Control	Hemoglobin A1c %	Fructosamine (mmol/L)
Excellent	< 6.0	1.2 - 2.1
Good	6.1 - 8.0	2.2 - 2.5
Fair	8.1 - 10.0	2.6 - 3.0
Poor	> 10	≥ 3.1

ther evidence of the diabetes predictive value of fructosamine determination, Baker found that fructosamine values ranged from 1.74 to 3.10 mmol/L in patients who subsequently were diagnosed as diabetic.

Correlation of hemoglobin A_{1c} values with fructosamine measurements in the accurate determination of glycemic control also provides confirmation of the utility of fructosamine measurement. This correlation was performed in our studies by comparing the two tests for proposed insureds whose blood glucose value was greater than 115 mg/dl. The fructosamine versus hemoglobin A_{1c} correlation study graph (Figure 4) shows a high degree of correlation between the two tests ($r = 0.8909$, $n = 269$). As mentioned above, minor discrepancies in the correlations between these two tests may be attributable to the different time windows of glycemic control that are being measured for each moiety.

Figure 2
HbA1 Versus Fructosamine and the Clinical Appreciation of Diabetes Control

Diabetes control	n	Fasting blood glucose (mg/d)	HbA1 (%)	Fructosamine (mmol/l)
Very good	11	109 ± 10	6.8 ± 0.2	2.20 ± 0.09
Good	34	167 ± 8	8.8 ± 0.2***	2.67 ± 0.09*
Poor	31	198 ± 15**	9.5 ± 0.3***	2.96 ± 0.12***
Bad	37	274 ± 19***	10.4 ± 0.2***	3.46 ± 0.10***
		F=15.2 p <0.001	F=18.7 p <0.001	F=18.5 p <0.001

Different from very good control (*p <.05; **p <.01; ***p <.0001)
*According to the clinical judgement of the diabetologist in charge (see material and methods).

Taken from Daubresse, J.C. et al, *Diabete & Metabolisme*, 13, 217-221, 1987

Frequency

The purpose of this study was to establish a normal range for fructosamine. The fructosamine frequency graph (Figure 5) was compiled over a six-day period using specimens received (n = 5000). For that population, the 95th percentile, arbitrarily defined as the upper limit of normal, was 2.1 mmol/L. Subsequent testing on additional specimens confirmed the 95th percentile at 2.1 mmol/L.

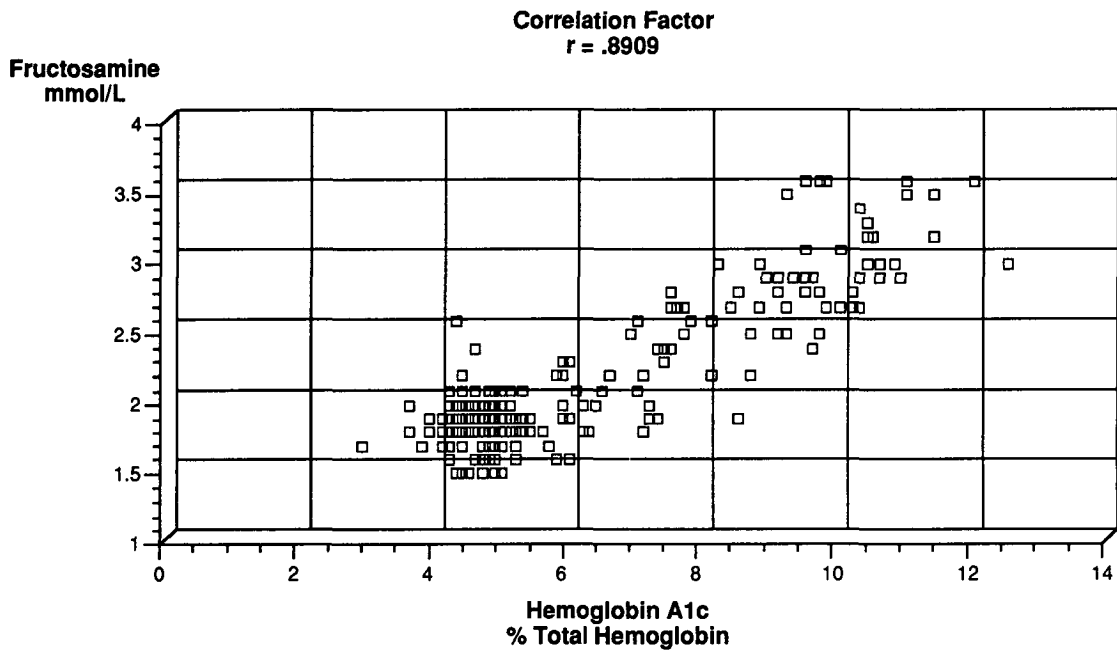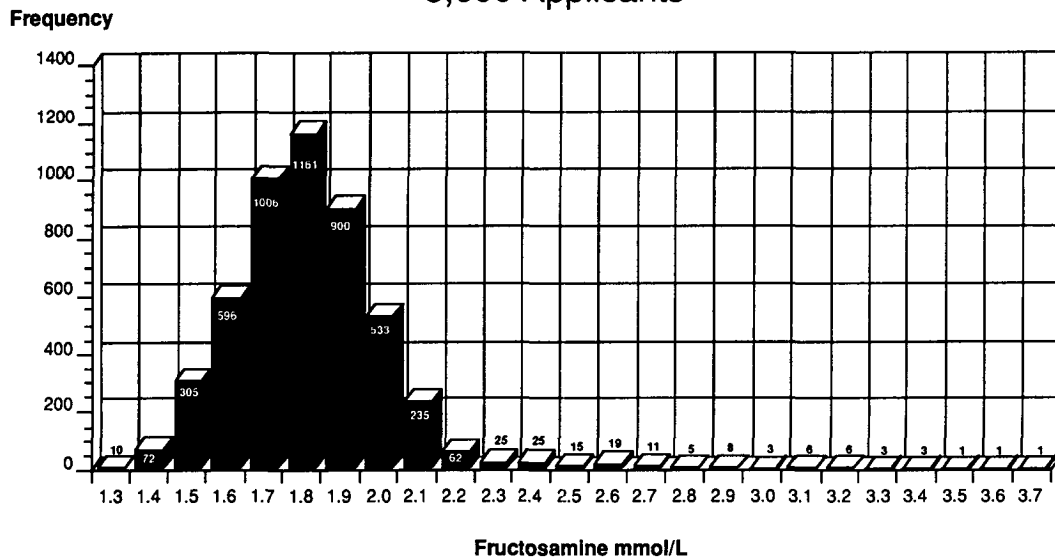#### Figure 4.

Fructosamine versus Hemoglobin A1c Correlation Study

Correlation Factor
r = .8909

Figure 5.

Fructosamine Frequency

Stability

The stability study analyzed the effect of pre-analytical specimen conditions on fructosamine values.

One red-top tube (no preservative) was centrifuged after clotting and analyzed the same day to represent the ideal fresh specimen collected in a clinical setting. A portion of these serum samples were allowed to stand at room temperature for three days and again analyzed. The correlation coefficient for fresh serum versus serum at room temperature for 72 hours was 0.90.

The other fresh serum samples were incubated at 40C for 72 hours to approximate the serum samples in transport during the summer months. These heat-treated specimens were then analyzed and compared to results obtained for the fresh specimens. The correlation coefficients for fresh serum samples versus serum stored at 40C for 72 hours was 0.93.

Some specimens received for insurance testing are not centrifuged immediately after clotting, and occasionally specimens are received which are uncentrifuged. To simulate these conditions, one red-top tube from each specimen set was allowed to remain uncentrifuged for 72 hours. These specimens were then centrifuged, analyzed and compared to results obtained from the fresh serum specimens. The correlation coefficients for fresh serum versus serum in contact with red blood cells at room temperature for 72 hours was 0.76.

To mimic the effects of heat on uncentrifuged specimens transported during the summer months, one tube from each specimen set was allowed to remain uncentrifuged and incubated at 40C for 72 hours. The incubated specimens were then centrifuged, analyzed and compared to the results obtained from the fresh serum specimens. The correlation coefficients for fresh serum versus serum in contact with red blood cells at 40C for 72 hours was 0.67.

It can be concluded that centrifuged and separated serum is relatively stable regardless of storage conditions. Ideally, all specimens should be centrifuged and separated prior to testing.

Discussion

The fructosamine assay is more sensitive than random glucose measurement for glycemic control determinations and contributes a different view of glycemia than that of hemoglobin A1c. Because the half-life of albumin and other serum proteins is considerably shorter than that of hemoglobin, the concentration of fructosamine levels can change more rapidly than those of hemoglobin A1c.

Although fructosamine can be affected by severe protein pathologies (particularly IgA gammopathies), these protein abnormalities are uncommon in the general population. Therefore, they do not detract from the usefulness of fructosamine analysis as a screening procedure, especially for the relatively healthy insurance-buying population.

The fructosamine test is technically reliable and can be adapted to many high volume, automated, chemistry-profiling instruments. Fructosamine is measured bichromatically as an end-point reaction based on the ability of glycated proteins to reduce NBT in an alkaline solution. The test requires a small sample volume, and results are resistant to the effects of storage and heat on the specimens.

Fructosamine's intermediate picture of glycemic control coupled with confirmation by hemoglobin A1c gives a dynamic advantage to both tests and increases the predictive value of the blood profile for glucose intolerance. Automation adaptability, sensitivity and relative lack of pre-analytical interference offer strong evidence that fructosamine analysis is a useful screen for diabetes and a benefit to the underwriting process.

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