Sensitivity of Serum Fructosamine in Short Term Glycemic Control

THOMAS W. PRIOR, Ph.D.,
JOHN F. CHAPMAN, Dr.P.H.
and DANIEL D. BANKSON, Ph.D.

Division of Laboratory Medicine,
North Carolina Memorial Hospital,
1071 Patient Support Tower,
Chapel Hill, NC 27514

ABSTRACT

The serum fructosamine concentrations measured in 64 diabetic patients correlated \( r = 0.73 \) with glycated hemoglobins (HbA1c). However, 23 percent of the diabetic patients had normal fructosamine and abnormal HbA1c levels. In order to determine whether or not the discrepant values were the result of recent glycemic regulation by the diabetic patient, fructosamine levels of patients suffering from diabetic ketoacidosis (along with \( \beta \)-hydroxybutyrate levels) were closely monitored. It was shown that short term alterations (one to three days) in serum glucose did not significantly affect fructosamine levels. Therefore, disagreements between fructosamine and HbA1c are most likely due to longer term improvement in glucose control by the diabetic or the result of the higher imprecision of the HbA1c assay.

Introduction

The measurement of the glycated serum protein fraction, termed fructosamine, has been proposed as a means of monitoring glucose control in diabetic patients.\(^2\),\(^9\) Fructosamine levels provide a measure of short term glycemic control (one to three weeks) in diabetic patients, unlike the glycated hemoglobin (HbA1c) which reflects glucose control over a longer period (two to three months).\(^1\),\(^3\),\(^11\) However, the actual interval of blood glucose control and the clinical value of fructosamine has not been well established. In the literature, there are conflicting reports regarding its usefulness in monitoring diabetics. Some authors have found a high degree of correlation existing between fructosamine and HbA1c and have, therefore, suggested that it be used as a reliable alternative to the more technically demanding, time consuming, and costly HbA1c measurements.\(^4\),\(^6\),\(^14\) Others have found poor correlations between the two tests and recommend that it not be used as an alternative.\(^7\),\(^13\) Finally, Jentorp et al recently stated\(^8\) "fructosamine should be considered a unspecific tool until its value in clinical practice becomes clearer."

The purpose of this study was to determine the sensitivity of fructosamine to rapid changes in glucose concentration and thus better estimate the interval
of glucose control that is reflected by a fructosamine measurement. An initial evaluation of the Roche fructosamine kit was also undertaken in order to assess the kit’s analytical reliability.

Materials and Methods

Fructosamine

Fructosamine was measured spectrophotometrically using the Roche fructosamine kit and the Roche Cobas BIO Centrifugal Analyzer.* The method is based on the ability of fructosamines to reduce nitroblue tetrazolium (NBT) at an alkaline pH. The kit manufacturer’s protocols were used and the assay was calibrated using the 1-deoxy-1-morpholino-D-fructose standard (3.2 mmol/L) supplied by Roche.

Glycated Hemoglobin

The concentration of glycated hemoglobin was determined by a cation exchange chromatography method.†

β-Hydroxybutyrate

β-hydroxybutyrate (BHBA) was quantitated enzymatically with β-hydroxybutyrate dehydrogenase using the Cobas BIO Centrifugal Analyzer. The reaction involves measuring the increasing absorbance at 340 nm associated with the reduction of nicotinamide adenine dinucleotide.⁵,¹²

Glucose

Glucose was measured by an oxygen electrode using glucose oxidase on an Astra 8 analyzer.‡

Statistical Methods

Linear correlations were estimated using the method of least squares. The unpaired students t test was used to determine statistical significance between groups.

Results and Discussion

Method Evaluation

Reference Range. Fructosamine values for 40 non-diabetic individuals are shown in figure 1. The observed range was 2.14 to 2.75 mmol per L, with a mean of 2.40 mmol per L and a standard deviation of 0.15. Although the distribution of fructosamine values is not gaussian (demonstrating slight positive skew), nonparametric testing yielded a similar range of 2.17 to 2.71 mmol per L.

Precision. Two pools of human serum were used to assess within-run precision. The CVs were 1.0 percent (n = 22, mean fructosamine = 2.44 mmol per L) and 0.89 percent (n = 22, mean fructosamine = 4.19 mmol per L). The between-run precision was measured using two commercially available lyophilized quality control sera. The CVs were 2.3 percent (n = 12, mean fructosamine = 2.64 mmol per L), and 1.1 percent (n = 12, mean fructosamine = 4.15 mmol per L).

Linearity. The linearity was determined by serially diluting a commercially available serum with isotonic saline. The upper limit of linearity was 5.5 mmol per L (r = 0.999).

Clinical Correlation

Fructosamine Levels in Diabetics. The mean serum fructosamine concentration measured in 64 diabetics was 3.07 mmol per L (SD = 0.79), which was significantly different from that found in the normal group (p < 0.001). The fructos-
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Fructosamine concentrations correlated significantly with HbA1c values ($r = 0.73$, figure 2). However, when the reference ranges were considered, discrepancy between the two tests was observed. Twenty-three percent of the diabetic population had elevated HbA1c values (>6.6 percent) and normal fructosamine levels. It has been shown that fructosamine is depressed by low concentrations of protein.10,13 This, however, was not a factor because these patients had normal (6.8 to 8.3 g per dl) total protein levels. Some of the disagreements may be analytical in nature, as a result of the greater imprecision of the HbA1c measurements ($CV = 9.6$ percent). The disagreements may also be due to the fact that the tests measure different periods of glucose control. Since the fructosamine levels are indicators of shorter term glucose control, it is possible that the high frequency of normal fructosamine values were due to the efforts of the diabetic patient to gain glycemic control shortly before the clinical visit.8 If this is the case, then the serum fructosamine may be too sensitive to recent changes in glucose levels and thus provide the physician with misleading information regarding overall glycemic control for the previous two to three weeks.
In order to determine the sensitivity of fructosamine to changes in glucose levels, the fructosamine concentrations were studied in three diabetic patients who entered the hospital in diabetic ketoacidosis and whose glucose and \( \beta \)-hydroxybutyrate were monitored. Ketoacidosis was clearly evident by the severely elevated \( \beta \)-hydroxybutyrate levels in all three patients (normal = 40 to 480 \( \mu \)mol per L). Patient A (figure 3) had an initial glucose of 1,295 mg per dl (71.9 mmol per L), a \( \beta \)-hydroxybutyrate of 13,610 \( \mu \)mol per L, and a fructosamine of 5.6 mmol per L. After insulin treatment, his glucose had fallen to 211 mg per dl (11.6 mmol per L) and remained less than 150 mg per dl (8.3

**Figure 3.** Glucose, fructosamine, and \( \beta \)-hydroxybutyrate in patient A.
mmol per L) during the next 40 hours. Although there was an initial 85 percent decrease in glucose over the first 15 hours, the fructosamine decreased by only 25 percent (5.6 to 4.2 mmol per L) and remained above the reference limit throughout the next 40 hours. Patient B (figure 4) had an initial glucose of 995 mg per dl (55.2 mmol per L), β-hydroxybutyrate of 8,306 μmol per L, and a fructosamine of 6.62 mmol per L. The glucose values fluctuated throughout the four day period: 153 mg per dl (8.4 mmol per L) at 13 hours, 450 mg per dl (25.0 mmol per L) at 26 hours, and 82 mg per dl (4.5 mmol per L) at 70 hours. Patient B's fructosamine initially decreased from 6.62 to 5.10 during the first 13 hours and then remained fairly constant during the following four days, despite the fluctuat-
ing glucose levels. Patient C (figure 5) entered the hospital with a glucose of 695 mg per dl (38.6 mmol per L) a β-hydroxybutyrate of 11,618 μmol per L, and a fructosamine of 6.21 mmol per L. After four hours, the glucose had fallen to 185 mg per dl (10.3 mmol per L) and gradually returned to normal by 22 hours. The fructosamine values paralleled, to a much lesser degree, the decreasing glucose levels and remained severely elevated after 22 hours (>3.7 mmol per L).

In conclusion, a proportion of the diabetic population (23 percent) exhibited normal fructosamine and elevated HbA1c levels. Some of the disagreement may be due to the higher imprecision of
the HbA1c test. However, absolute agreement should not be expected, since the tests are expressions of different aspects of metabolic control. It was shown that the differences were most likely not the consequence of abrupt improvement in glucose control before the clinic visit. Large glucose and β-hydroxybutyrate shifts, observed in the three patients, resulted in relatively modest changes in fructosamine. Fructosamine has been shown to be more responsive than HbA1c for detecting early changes in glycemic control and, therefore, can be used to assess the adequacy of glucose control over a shorter period of time. However, short term (one to three days) improvement in glucose control could not appreciably be demonstrated by the fructosamine levels and poor glycemic regulation would not be apparent over the relatively short term. Our experience with the Roche fructosamine kit demonstrated a procedure which was analytically reliable, correlated reasonably well with HbA1c results on the same patients (figure 2), and was economical and convenient to perform.

References