FRUCTOSAMINE CONCENTRATIONS IN THE SERUM OF DIABETICS AND HEALTHY INDIVIDUALS IN SINGAPORE

L L Tho, E S C Koay, A C Thai, J K Candlish

SYNOPSIS

A commercial kit for the assay of fructosamine was subjected to partial assessment with a sample of 68 diabetics attending outpatient clinics. It correlated well with glycated haemoglobin (using two commercially available kit methods) and with serum lipids. With a healthy population a reference interval of 1.9-2.9 mmol/L (as 1-deoxy-1-morpholinofructose equivalents) was generated. The components of analytical imprecision were acceptable; the Harris ratio as calculated from intra-and interindividual variances was calculated for six subjects and ranged from 0.23 to 1.14. It is thus more prudent to use the patient as his/her own referent in respect of this analyte.

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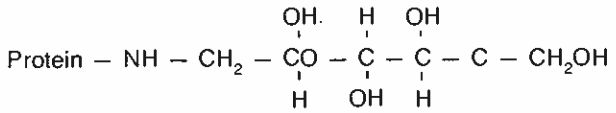
INTRODUCTION

It appears to be Peterson and Jones (1) who first suggested that proteins with a shorter circulating lifetime than haemoglobin could be examined for glycation in an effort to assess diabetic control over a period of a few weeks rather than months. The suggestion was taken up and several papers have appeared in an attempt to assess the efficacy of the assay of serum protein glycation (2-4). In the course of these investigations it was established that albumin was the main protein undergoing glycation, as might be expected, and so measurements were related to its half-life, and offer a view of hyperglycaemia over the previous 2-4 weeks. A kit, "Fructosamine Test <Roche>", which depends on the ability of a ketoamine to reduce nitroblue tetrazolium under alkaline conditions, also came on the market.

Since the sugar adduct can be represented as



that is, a fructose-protein anhydride, the estimation is that of "fructosamine" although fructosamine itself is not present and it is rather the ketoamine function which is being assayed. What is measured by this method cannot be entirely coextensive with glycated serum proteins, since Lim and Staley (4) found a correlation coefficient of only 0.817 between fructosamine and glycated protein as measured by an affinity chromatography method.

In approaching an evaluation in modern clinical chemistry one must be guided by the various protocols, some of them provided by authoritative bodies such as the International Federation of Clinical Chemistry (5). It is however obvious that the full protocols cannot apply to every analyte. For example, recovery experiments are usually prescribed, in order to test systematic error, and perhaps accuracy. However such manoevres depend upon the existence of an ultrapure analytical standard, obviously unavailable in the case of glycated serum proteins. The protocols (6) also usually state the desirability of assessing variances at the medical decision levels, but again such a concept has little relevance to the analyte presently under scrutiny. Detectability (often referred to as "the sensitivity of the assay") is equally lacking in applicability to serum fructosamine.

A truncated evaluation schedule is therefore more suitable for this comparatively novel analyte. It seems appropriate to: 1) assess the various components of imprecision; 2) determine interindividual and intraindividual variances to assess the reference interval and its utility; 3) determine whether suitable correlations with other determinants of diabetes can be obtained.

MATERIALS AND METHODS

A population of 104 apparently "healthy" subjects (medical students and their relatives) agreed to cooperate for six apparently "healthy" normal subjects agreed to give blood samples at 0800, 1000, 1200, 1400, and 1600 h on 4 separate days spaced at weekly intervals (with only the 0800 h specimen being a "fasting" one). 67 nonselected diabetic patients attending clinics at the National University Hospital, Singapore, from whom blood was taken in the course of normal clinical assessment, formed the group for the comparison of fructosamine to other markers of the condition. (It was considered that trying to obtain fasting samples in the absence of some policing operating to verify total abstinence from food might produce seriously misleading results.) Heparinised syringes were used in all cases.

Fructosamine was assayed by the Fructosamine Test <Roche>, This uses deoxymorpholinofructose as standard, and constitutes a manual procedure, though it could be automated with suitable apparatus.

Glycated haemoglobin was measured by both the

Departments of Biochemistry[1] and Medicine[2], Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore 0511

L L Tho, BSc, Research Student
E S C Koay, PhD, Lecturer
J K Candlish, PhD, Assoc. Professor
A C Thai, M Med, Senior Lecturer

**Department of Medicine,
Faculty of Medicine,
National University of Singapore.**

Correspondence to: Prof Candlish

Perce Gylcotest 4200 kit (for "glycosylated haemoglobin") and the Boehringer Mannheim Test-combination Haemoglobin A1 (for "HbA1"). The manufacturers of both of these kits claim that they elimate the "labile" or "pre" HbA_1 fraction; Glucose, triglycerides and cholesterol were analysed on the Beckman ASTRA analyser.

Statistical assessment was carried out using the Abstat package (Anderson Bell Co). There was no rejection of outliers in this study.

RESULTS AND DISCUSSION

Imprecision studies

Imprecision figures are given in Table 1. Imprecision is generally given in the literature as "within-run", "within-day" or "between-run" and "day-to-day" or "total", with the implication, of course, that these should be of increasing magnitude. In a manual assay such as this the concept of within-day, or between-run imprecision is hardly applicable, since few laboratories would run such a procedure more than once per day. The within-run and day-to-day imprecisions are however highly acceptable. A previous study (4) quotes an "interbatch" variation of 6.5%.

Table 1
IMPRECISION CHARACTERISTICS OF THE FRUCTOSAMINE ASSAY (COEFFICIENTS OF VARIATION)

	Level 1		Level 2	
	mean (mmol/L)	CV(%)	mean (mmol/L)	CV(%)
within-run *	2.3	2.9	3.6	2.8
day-to-day **	2.7	4.3	3.7	3.8

* 5 replicates for each level
** single assay on 5 consecutive days

Legends for figures

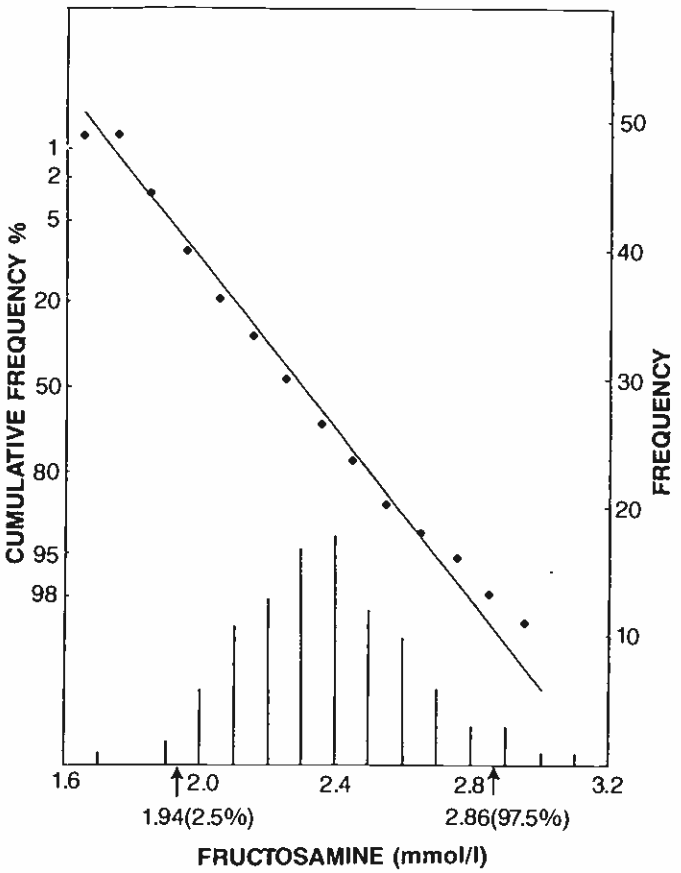


FIG 1
SERUM FRUCTOSAMINE CONCENTRATIONS IN THE POPULATION STUDIED. ARROWS SHOW CUT-OFF POINTS FOR THE MID-0.95TH FRACTILE FOR THE CUMULATIVE FREQUENCY PERCENT PLOT.

Reference intervals and Harris ratio

The reference interval was generated by plotting the results on cumulative frequency paper (Fig 1). Baker and coworkers (3) in their pioneering work found fructosamine to show a Gaussian distribution, and this is borne out by the appearance of the distribution obtained here and the linearity of the cumulative frequency percent plot. The range adopted is a little wider than that stated by the manufacturers of the kit (2.0 -2.8 mmol/L) although presumably it is only the upper cut-off point which is of interest.

As Harris (7) formalised, the square root of the ratio of the intraindividual to the interindividual variances is a measure of the utility of a reference interval, the optimal figure being 1.4. Harris ratios were computed using the data for the six individuals who gave blood at different times of the day as follows. Since these were analysed in duplicate, the total analytical variance was first calculated (8) using the formula

$$\frac{\Sigma \text{ differences between duplicates}^2}{2 \times \text{number of pairs}}$$

and yielded a result of 0.0164.

Interindividual variance was calculated as the variance of the normal population (apparent group variance) minus the analytical variance (0.0164). Intraindividual variance was calculated for each of the six subjects as the variance associated with single measurements of each specimen over the whole time period (apparent individual variance), minus the analytical variance. Thereafter the quantity

$$\sqrt{\frac{\text{intraindividual variance}}{\text{interindividual variance}}}$$

computes as 0.23, 0.30, 0.71, 1.1, 1.1, and 1.2 for the individuals, showing considerable scatter.

Correlation with glycated haemoglobin

Fig 2 shows the regression plots of fructosamine on glycated haemoglobin; although both correlation coefficients are significant, the Pierce method yielded the higher (0.65 as opposed to 0.42 for the Boehringer-Mannheim kit). Winocour et al (9) using the same fructosamine kit obtained a correlation coefficient of 0.70 against the Boehringer kit, so there is evidently the potential for much variability in different hands. However we computed the standard error of the regression, Syx, as being almost 0.50 for both methods.

Regressions against other serum constituents

The subjects were not fasting, so a plot of glucose against fructosamine would provide but a rough and ready relationship at best; in fact, there was no apparent correlation, an observation also made previously (9).

There was however some relationship to the serum lipids (Fig 3), although the correlation coefficients were low.

CONCLUSION

Assays of protein glycation in serum appear to be on the verge of general introduction into clinical chemistry. There is an especially persuasive case for their use in the management of diabetes in pregnancy (10).

The present work substantiates the impression that fructosamine is a potentially valuable test, and that the commerical kit employed in the present survey fulfills a recognisable role, in view of its relationship to glycated haemoglobin and accepted imprecision characteristics, as long as it is borne in mind that the individual himself is probably the best referent.

ACKNOWLEDGEMENT

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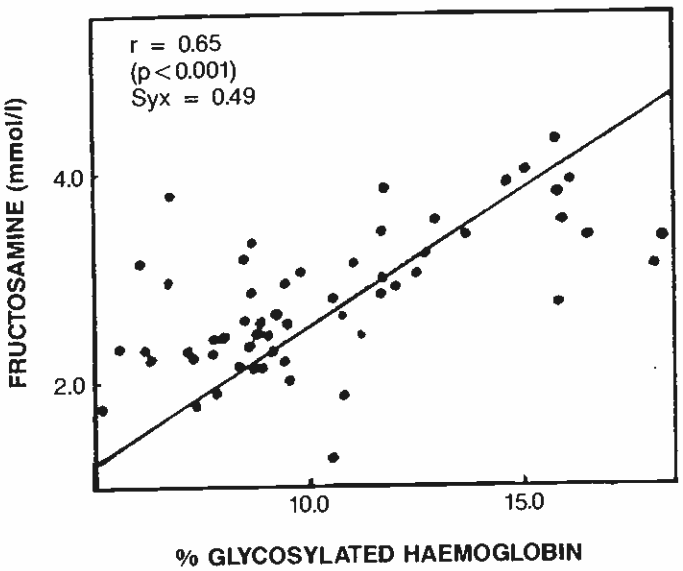

FIG 2
SCATTER PLOTS FOR SERUM FRUCTOSAMINE AGAINST TWO METHODS FOR GLYCATED HAEMOGLOBIN, THE PIERCE KIT ("GLYCOSYLATED HAEMOGLOBIN") ON THE LEFT AND THE BOEHRINGER-MANNHEIM KIT ("HbA1") ON THE RIGHT.

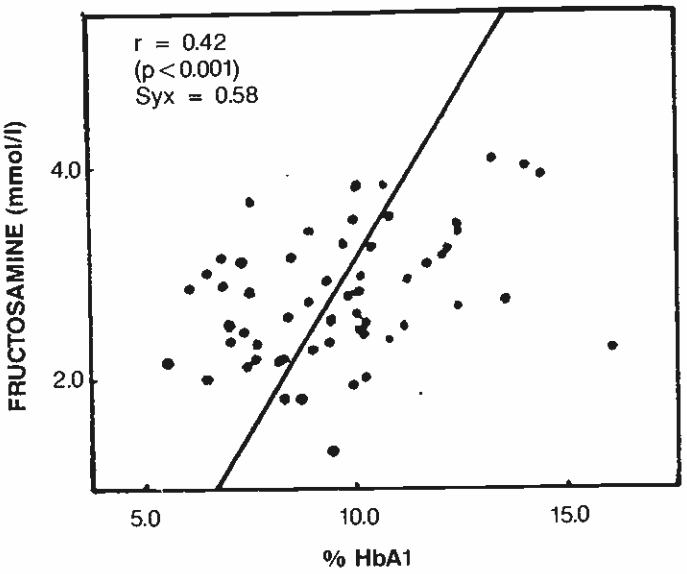

FIG 3
SCATTER PLOTS OF SERUM FRUCTOSAMINE AGAINST SERUM CHOLESTEROL AND TRIGLYCERIDES

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