

Serum fructosamine and glycated haemoglobin measurements in diabetic control

E J HINDLE, G M ROSTRON, S A CLARK, AND J A GATT

Department of Biochemistry, Infirmary and Department of Paediatrics, Royal Preston Hospital, Preston

SUMMARY Serum fructosamine and glycated haemoglobin (HbA1) were measured in capillary samples from diabetic children and compared with samples from non-diabetic children. Glycaemic control was assessed clinically and by average daily glucose values recorded by home monitoring. Fructosamine correlated with HbA1 and with average glucose values measured over 30 days. HbA1 also correlated with average glucose values measured over 60 days. Changes in fructosamine with time tended to parallel those of HbA1, and advance indication of deteriorating or improving glycaemic control was possible by observing changes in these. Fructosamine has many advantages over HbA1 measurement such as speed, technical ease, and low cost, and is a reliable alternative to HbA1 estimation as an indication of glycaemic control.

Measurement of glycated haemoglobin (HbA1) is well recognised as a method of assessing glycaemic control in the routine management of patients with diabetes mellitus.¹⁻³ It is of particular value in the management of insulin dependent diabetic children.^{2,4} Frequent estimations may be necessary in these patients since their glucose profiles are subject to large fluctuations, causing changes in HbA1c concentrations (in one report, of approximately 1% in a week).⁵ These changes may occur through the formation of an unstable intermediate, and several methods have been described for removal of this before analysis,^{6,7} including a simple incubation with physiological saline for a minimum of five hours.⁸

The fructosamine test is a method of assessing serum glycosyl-protein concentration.⁹ Its measurement is an index of intermediate glucose control (one to three weeks) and is of value as a screening test for diabetes mellitus,⁹ gestational diabetes,¹⁰ type I (insulin dependent) diabetes,¹¹ and type II (non-insulin dependent) diabetes.¹² Measurement of fructosamine may be affected by the concentration of albumin in the specimen, although there is no appreciable change within the albumin reference interval.¹³

The present study was designed to measure fructosamine and HbA1 in capillary specimens from diabetic children and to establish reference ranges. Comparisons of the measurements with clinical observations and home glucose monitoring records were also made.

Subjects and methods

Subjects. The populations studied comprised 57 children and adolescents with diabetes (29 boys and 28 girls, age range four to 17 years) attending the paediatric diabetic clinic of the Royal Preston and Chorley and District Hospitals, and 64 other children with no history of diabetes (26 boys and 38 girls, age range 1 to 17 years) attending a general paediatric clinic. Adult non-diabetic subjects (five men and five women, age range 20 to 40 years) were also used in the study. Specimens were collected from the diabetic patients at each clinic visit (average four times, range one to seven times in a period of one year).

Diabetes management

A diagnosis of diabetes mellitus had been reached using diagnostic criteria such as hyperglycaemia, glycosuria, and ketonuria. The children studied were on a twice daily insulin regimen consisting of a combination of short and medium acting insulins in varying proportions. The insulins used were purified porcine preparations or in a few cases human insulin. Assessment of control at home was by a combination of capillary blood glucose sampling using BM20-800 sticks read visually and by urine testing with Clinitest tablets, and more recently Diabur 5000 Stix. A policy of frequent capillary blood sampling was encouraged but had to be adapted to each child's tolerance. Blood samples were collected by the children four times daily (five),

two times daily (34), three or four times over two to four days (13), less than 12 times per month (four), and on no occasion (one). In addition, urine tests were performed three or four times daily (seven), between three and 20 times per week (17), occasionally (10), and on no occasion (19).

The results were recorded in a diary and the patients or parents, or both, were encouraged to adjust insulin doses according to these. They had telephone access to a specialist diabetic health visitor who could advise further on this. All children were on a controlled carbohydrate diet.

The patients were seen in the clinic at a maximum interval of three months; some requiring to attend more frequently for supervision, encouragement, and advice. At each clinic visit height and weight were measured, urine tested, random blood glucose measured, and capillary blood samples taken for determination of HbA1 and fructosamine concentrations. All patients were seen at every visit by the doctor, specialist health visitor, and dietician.

Methods.

Samples

All samples were collected into polyethylene capillary tubes containing heparin and fluoride. Capillary samples (100 μ l) were collected by finger prick from the diabetic subjects and venous samples were collected from the non-diabetic subjects. Venous and capillary samples were collected simultaneously from the adult volunteers.

After centrifugation (within two hours of collection), plasma was transferred to the centrifugal analyser cups and either analysed immediately for glucose, albumin, and fructosamine or stored at -4°C before analysis. The residual cells were incubated with physiological saline to remove labile component and then analysed for HbA1.

Fructosamine

Fructosamine was determined by the method of

Johnson *et al*⁹ using the Cobas Bio Centrifugal Analyser¹³ (Roche Diagnostics). (Within batch and between batch precision CV= $<1.0\%$, 3.0% respectively.) Variations in assay procedures described in the published reports⁹⁻¹¹ make it essential that each laboratory defines its own reference intervals.

Glucose and albumin

Glucose and albumin were estimated on the Cobas Bio Analyser using hexokinase and bromocresol green respectively.

Glycated haemoglobin

HbA1 was estimated by affinity electrophoresis on cellulose acetate membranes¹⁴ (Glyco-Phore Kit, Gelman Sciences). (Within batch and between batch precision CV= 6.7% , 7.3% respectively.)

Statistical analysis

Linear correlations were estimated using the method of least squares and Student's *t* test for statistical comparison.

Results

Reference ranges for fructosamine and HbA1 are shown in the Table. There was no significant difference between fructosamine and HbA1 estimated in capillary or venous samples from adult subjects ($P>0.05$). It was assumed, therefore, that the same would apply in children.

There was a significant difference between glucose, fructosamine, and HbA1 in the diabetic and non-diabetic groups ($P<0.001$). All subjects had serum albumin concentrations within the laboratory adult reference range (mean (SD), 45 (10) g/l), and the values in the non-diabetic and diabetic groups did not differ significantly ($P>0.05$).

Correlation of fructosamine and HbA1. Fructosamine correlated with HbA1 ($r=0.86$), Fig. 1(a).

Table Reference ranges for fructosamine and glycated haemoglobin HbA1. Values are mean (2 SD)

| Subjects | Age range (yrs) | Albumin (g/l) | Glucose (mmol/l) | Fructosamine (mmol/l) | HbA1 (%) |
|---------------------------------|-----------------|---------------|------------------|-----------------------|-------------|
| <i>Children</i> | | | | | |
| Diabetic (n=56) (capillary) | 4-17 | 41.4 (7.2) | 12.5 (10.4) | 1.36 (0.88) | 10.2 (6.1) |
| Non-diabetic (n=64) (venous) | 1-17 | 40.5 (7.14) | 4.76 (1.84) | 0.59 (0.26) | 5.29 (1.97) |
| <i>Adults</i> | | | | | |
| Non-diabetic (n=10) (capillary) | 20-40 | — | 4.78 (2.1) | 0.68 (0.12) | 6.0 (2.3) |
| Non-diabetic (n=10) (venous) | 20-40 | — | — | 0.69 (0.10) | 5.8 (2.2) |

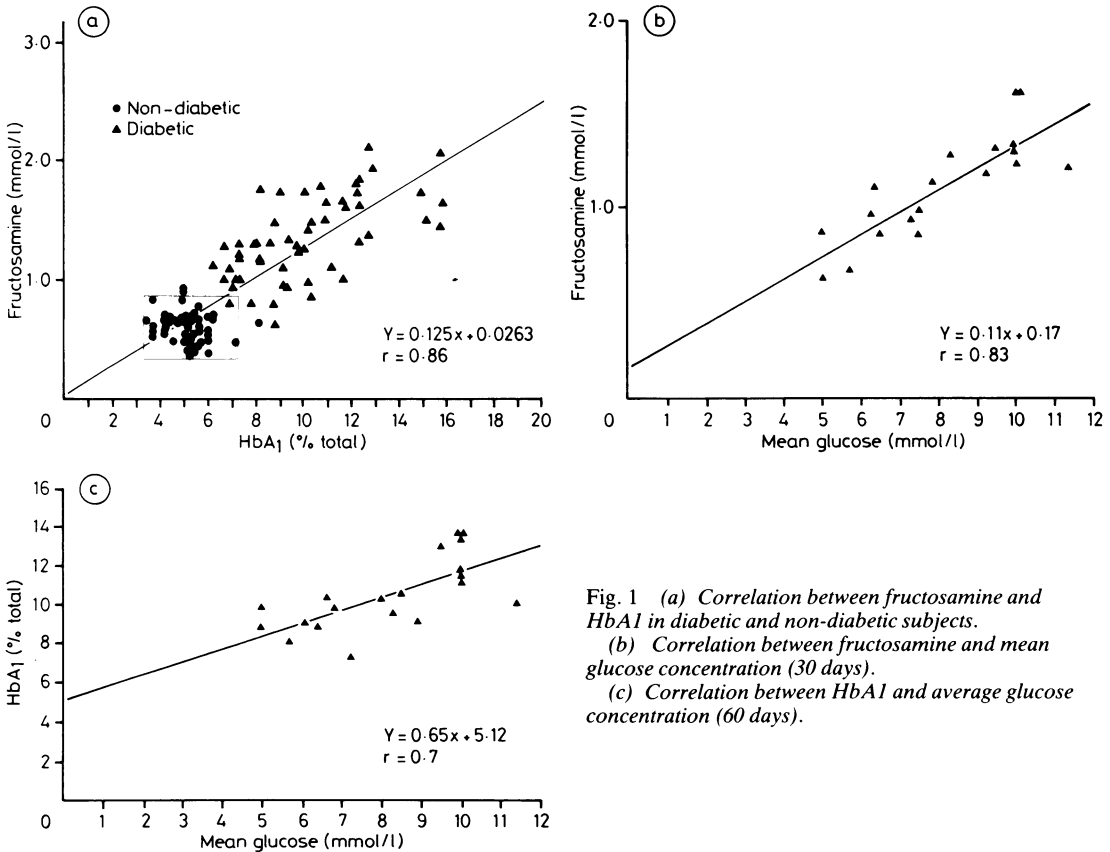


Fig. 1 (a) Correlation between fructosamine and HbA1 in diabetic and non-diabetic subjects. (b) Correlation between fructosamine and mean glucose concentration (30 days). (c) Correlation between HbA1 and average glucose concentration (60 days).

Correlation of fructosamine and HbA1 with glucose. Diabetic diaries were examined from 20 children who made an adequate number of regularly recorded home blood glucose measurements. Fructosamine correlated with the mean blood glucose value (representing, on average, 50 individual estimations) measured over 30 days before a clinic visit ($r=0.83$) (Fig. 1(b)). HbA1 also correlated with the mean blood glucose value (representing, on average, 110 individual estimations) measured over the preceding 60 days ($r=0.7$) (Fig. 1(c)).

Changes in fructosamine and HbA1. Fructosamine and HbA1 values were compared on the first and second visits in all patients, and in many instances biochemical evidence of improvement in diabetic control was seen. In general, those patients who did not perform any form of home monitoring regularly showed poor biochemical control compared with

those who performed frequent daily blood tests and adjusted their insulin requirements accordingly.

Examples of serial estimations of fructosamine and HbA1 in two patients are shown in Fig. 2. Patient 1 shows fructosamine and HbA1 values above the non-diabetic ranges. These decreased during the first three months studied, approaching the non-diabetic range. Subsequent stabilisation at slightly higher values was effected. Clinical evidence of improving diabetic control was also evident, and was supported by data from the diabetic diaries. Subject 2 showed high fructosamine and HbA1 concentrations which initially tended to show improvement during the first month. Evidence of suboptimal control was subsequently shown biochemically, and in consequence intensive counselling of the patient prevented further deterioration and a return to reasonable control. The weight of this patient, measured at each clinic visit, shows good

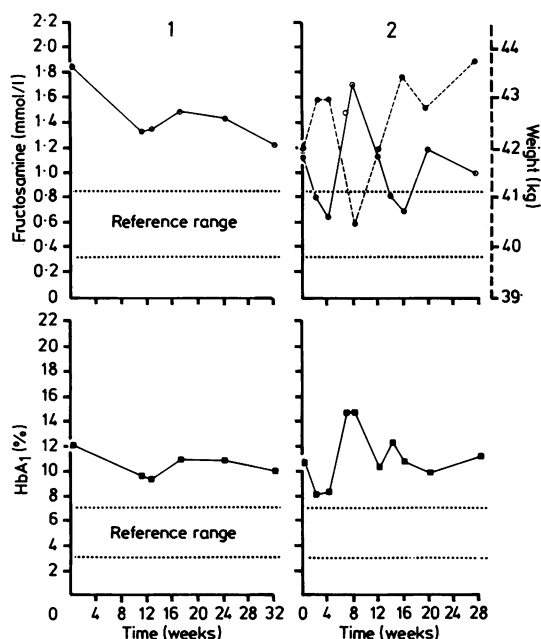


Fig. 2 Changes over time in fructosamine (○), HbA1 (■) (for patients 1 and 2), and weight (●) (for patient 2).

clinical evidence of varying diabetic control supporting the biochemical findings.

Discussion

Chronic hyperglycaemia may well be the cause of the vascular complications of diabetes mellitus,¹⁵ and methods of accurate assessment of glycaemic control must surely be encouraged. This is of particular importance in children, where early and continuing stabilisation may establish a pattern of diabetic control for life.³

The purpose of this study was to measure fructosamine and HbA1 concentrations in capillary blood specimens from diabetic children as a measure of glycaemic control, and to compare these with clinical observations and home glucose monitoring records. Each is subject to analytical and physiological factors which may affect the estimation. For instance, the labile intermediate formed by haemoglobin and glucose in vivo and vitro^{6,7} may cause overestimation of HbA1, and the presence of low serum albumin concentrations may cause underestimation of fructosamine.¹³

Fructosamine and glycated haemoglobin can be measured in the same sample of capillary blood. The reference range for plasma fructosamine in the

non-diabetic group (mean (SD), 0.59 (0.26) mmol/l) is lower than that found in adults¹³ (1.04 (0.4) mmol/l), which was derived from analysis of serum samples.

Fructosamine and HbA1 correlated with average blood glucose values measured by home monitoring over 30 days ($r=0.83$) and 60 days ($r=0.7$) before attending the clinic. While this correlation depends on an estimate of glucose concentration, which is relatively subjective, it serves to give a numerical expression to a wholly subjective assessment performed in clinic when the diabetic diaries are reviewed, and is therefore a relevant exercise. In our opinion, estimations of fructosamine and HbA1 at a clinic visit are valuable aids in the interpretation of the diabetic diary records.

Changes in fructosamine and HbA1 with time (for example, Fig. 2) provide a profile which, together with clinical data, gives advance warning of deteriorating or improving glycaemic control. Fructosamine is thought to reflect average glucose values over two to three weeks, while glycated haemoglobin reflects glucose values over a longer period (about six weeks). In our experience changes in fructosamine and HbA1 generally occur in parallel in stable subjects.

We believe that fructosamine and HbA1 estimations are of considerable value in the management of diabetic children. Most methods of estimation of HbA1 are, however, technically demanding, time-consuming, labour intensive, and relatively costly. The method in use in our laboratory is no exception. The fructosamine assay, however, is rapid, technically simple, inexpensive, and requires small volumes of sample (20 μ l). While the analytical equipment we have used is sophisticated, most district general laboratories will possess a similar or equivalent piece of apparatus, making the analysis potentially available to paediatric departments. Further studies may indicate which is the more suitable analytically and in clinical application.

We thank the staff of the paediatric clinics for their help in this study and Mrs S Jackson for preparation of the manuscript.

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Correspondence to Dr E J Hindle, Department of Biochemistry, District Laboratory, The Infirmary, Preston, Lancashire.

Received 26 September 1985