

# Fructosamine or glycated haemoglobin as a measure of diabetic control?

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**SUMMARY** Glycated haemoglobin A<sub>1</sub> (HbA<sub>1c</sub>), fructosamine, and total serum proteins were measured in 30 normal and 61 diabetic children. The normal range for HbA<sub>1c</sub> was 4.7-8.8% and for fructosamine was 0.98-1.88 mmol/l. These were similar to adult normal ranges and there were no significant age differences during childhood. There was a highly significant correlation between HbA<sub>1c</sub> and fructosamine in the diabetic children but this was lost when only concentrations within the established normal ranges were considered. Adjustment of concentrations of fructosamine for total serum proteins made no difference to the results. Changes in HbA<sub>1c</sub> and fructosamine were followed in three newly diagnosed patients and in one whose diabetes was getting worse. HbA<sub>1c</sub> decayed with a half life of 28.7 days and fructosamine decayed with a half life of 16.5 days. Fructosamine concentrations were lower than expected in the patients who were improving and higher than expected in the patient who was deteriorating.

It is suggested that while fructosamine is not a direct substitute for HbA<sub>1c</sub> it may be a useful adjunct in determining whether a patient is worsening or improving in the short term. A change from HbA<sub>1c</sub> to fructosamine for routine assessment of diabetes while retaining HbA<sub>1c</sub> on selected occasions would result in some cost savings while retaining the advantages of having both assays available.

In recent years measurement of glycated haemoglobin A<sub>1</sub> (HbA<sub>1c</sub>) has been widely introduced as a measure of medium term control of diabetes and is used by many diabetologists as the yardstick by which diabetic control is judged. However it is subject to a number of disadvantages. It requires incubation for several hours to remove the unstable intermediate Schiff base, it is quite a time consuming assay to perform and, by comparison with the measurement of other ketoamines, relatively expensive. More recently, a method has been described for the measurement of glycated serum proteins, known as fructosamine, which is based on a colorimetric determination utilising the reducing properties of fructosamine at high pH.<sup>1</sup> It has the advantage that it is rapid, inexpensive, and can be automated, thus reducing the amount of laboratory time required for the assay. We have estimated that each HbA<sub>1c</sub> assay costs roughly 50p to perform while estimations of fructosamine cost only a few pence if the reagents are made up in the laboratory or about 25p if the reagents are obtained in kit form.

We have used both of these assays to establish a normal range for both HbA<sub>1c</sub> and fructosamine in

children and applied them to the study of diabetic children to determine whether fructosamine might be a suitable alternative or a useful adjunct to HbA<sub>1c</sub> in their routine management.

## Methods

HbA<sub>1c</sub> was measured using an immobilised boronic acid microcolumn technique after overnight incubation to remove the unstable fraction.<sup>2</sup> In our laboratory this assay has a coefficient of variation of 4% when HbA<sub>1c</sub> equals 12% and 8% when it equals 5%.

Fructosamine was measured as previously described.<sup>1</sup> Venous plasma was added to carbonate buffer at pH 10.8 containing nitroblue tetrazolium 0.25 mmol/l. The absorbance at 530 nm was measured 10 and 15 minutes after mixing and compared with a secondary serum standard which itself had been calibrated against l-deoxy, l-morpholino fructose in albumin solution (40 g/l) that had been treated in identical fashion. The whole assay was carried out at 37°C. Analysis was made using a Centrifichem centrifugal analyser. This is able to handle 24 samples simultaneously and each batch takes about 20 minutes to perform.

## Patients

A normal range of HbA<sub>1c</sub> and fructosamine concentrations in children was established for our laboratory by measuring both variables on samples taken at the time of routine venepuncture in 30 children who were admitted to hospital either for routine surgery or for medical reasons unrelated to abnormalities of blood glucose control. All of these patients had a blood glucose within the normal range at the time of sampling. Verbal consent was obtained from the parents at the time and the study received ethical committee approval. The children were initially divided into three age groups: 0–5 years, 5–10 years, and 10–15 years to assess any age related changes in either variable. Differences were sought using non-parametric statistical methods (Mann-Witney U test).

HbA<sub>1c</sub>, fructosamine and total protein were measured on the same samples taken from patients attending the children's diabetic clinic between January 1985 and June 1986. The samples were obtained either by venepuncture or by finger prick and stored in fluoride oxalate tubes until sent to the laboratory. A fructosamine:protein ratio was derived by dividing the fructosamine concentration (in  $\mu\text{mol/l}$ ) by the total protein concentration (in  $\text{g/l}$ ). HbA<sub>1c</sub> was then plotted against both fructosamine and fructosamine:protein ratio and the appropriate linear regressions and 95% confidence limits fitted by the method of least squares.

Both variables were measured at diagnosis and at frequent intervals for up to 19 weeks in three patients who were newly diagnosed. In all three the diabetes was rapidly brought under control and the blood glucose returned to near control values. The concentration of fructosamine at equilibrium (C) is determined by the 'black box' relationship<sup>3</sup> and is dependent upon the rate of glycation ( $\Delta G$ ) and the half life ( $t_{1/2}$ ) of the proteins which are glycated according to the formula:

$$C = \Delta G \times t_{1/2} / \ln 2$$

Preliminary data in our laboratory suggest that the rate of synthesis of fructosamine in vitro is directly proportional to the concentration of glucose in serum over a wide range. If the same is true in vivo,<sup>4</sup> the concentration of fructosamine at any one time is directly proportional to the integrated blood glucose concentration during a period determined by the half life of the glycated proteins, assuming that the concentration of those proteins remains constant, which was shown in the case of our patients.

From the 'black box' relationship it can be seen that it is only possible to estimate the half lives of

HbA<sub>1c</sub> and fructosamine if their rates of synthesis are known. These can only be calculated precisely if the mean blood glucose concentration over the period studied is recorded. As this was not possible, for the purposes of these calculations, the mean values of the normal range of HbA<sub>1c</sub> and fructosamine were then subtracted from the appropriate observed value to eliminate the effect of de novo synthesis and the half life of each estimated from these derived values.

The observed values of HbA<sub>1c</sub> and fructosamine in these three patients, together with those of a fourth patient whose condition had been improving but then progressively worsened over several weeks (HbA<sub>1c</sub> concentration rising from 13.3% to 20%), were plotted against one another. Comparison was made with the regression line previously derived from the combined data of all the patients in the clinic in order to determine any differences between improving and worsening diabetes in the observed as against expected concentrations of fructosamine for a given concentration of HbA<sub>1c</sub>.

## Results

Table 1 shows the means and standard deviations of HbA<sub>1c</sub> and fructosamine concentrations in the 30 normal children divided into the three age groups. There were no significant differences between the values in the groups so they were all combined. Mean (SD) HbA<sub>1c</sub> concentration was 6.30 (1.08)% and fructosamine concentration was 1.33 (0.23) mmol/l with observed normal ranges (in the 30 children studied) of 4.7–8.8% and 0.98–1.88 mmol/l. The upper limits of normal values were therefore taken as 9% and 1.9 mmol/l, respectively. There was no significant relation between HbA<sub>1c</sub> and fructosamine in these subjects.

Individual concentrations of HbA<sub>1c</sub> and fructosamine in 129 samples from 61 diabetic children are shown in fig 1. A highly significant relation ( $p < 0.001$ ,  $r = 0.69$ ) was indicated and the linear regression and 95% confidence limits are shown. When data points corresponding to values of HbA<sub>1c</sub>

Table 1 Mean (SD) glycated haemoglobin A<sub>1</sub> and fructosamine concentrations in 30 normal children

Age	Glycated haemoglobin (%)	Fructosamine (mmol/l)
0–5	6.0 (1.0)	1.3 (0.2)
5–10	7.0 (0.8)	1.3 (0.2)
10–15	6.2 (1.1)	1.5 (0.3)
Mean (SD)	6.3 (1.1)	1.3 (0.2)

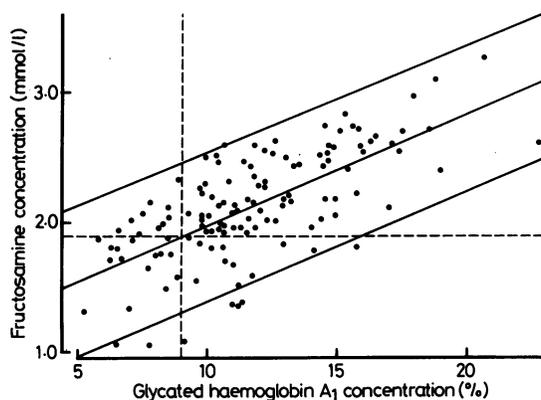


Fig 1 Relation between glycated haemoglobin A<sub>1</sub> and fructosamine in 129 samples from 61 diabetic children. The linear regression and 95% confidence limits are shown. The interrupted lines represent the upper limits of normal of the two assays. For glycated haemoglobin A<sub>1</sub>  $\leq 9.0\%$   $r=0.34$ ,  $p=NS$ .

outside the normal range were excluded, however, the relation significance could not be shown. When HbA<sub>1c</sub> was plotted against the fructosamine:protein ratio rather than fructosamine almost identical results were obtained. There was very little variation in total protein between patients (mean (SD) 65.25 (4.36) g/l) and the similar relation ( $r=0.68$ ,  $p<0.001$ ) suggested that the fructosamine:protein ratio calculation confirmed no advantages over fructosamine in these patients and has therefore not been considered further.

The changes in HbA<sub>1c</sub> and fructosamine with time from diagnosis in the three patients who were newly diagnosed and being brought under control as shown in figs 2 and 3. In all three patients fructosamine concentration had returned to within the normal range by 40 days. In contrast the HbA<sub>1c</sub> concentration was still raised at this time in all three and in one case was still raised at 80 days. Half life values of HbA<sub>1c</sub> and fructosamine were calculated as described above for all three patients; both showed an apparent single half life of decay and the results are shown in table 2. In all cases the half life of fructosamine was shorter than that of HbA<sub>1c</sub> (mean 16.5 v 28.7 days). Also shown in figs 2 and 3 are the concentrations of HbA<sub>1c</sub> and fructosamine plotted against time in the one patient whose condition worsened.

Fig 4 shows the HbA<sub>1c</sub> and fructosamine concentrations in all four patients plotted against each other and compared with the observed regression line. In all three newly diagnosed patients the fructosamine concentrations were lower than ex-

Table 2 Half life of glycated haemoglobin A<sub>1</sub> and fructosamine in three newly diagnosed patients

Patient No	Sex	Days from diagnosis	Half life (days)	
			Glycated haemoglobin A <sub>1</sub>	Fructosamine
1	F	82	34.5	18.2
2	F	99	22.3	13.6
3	F	133	29.3	17.6
Mean			28.7	16.5

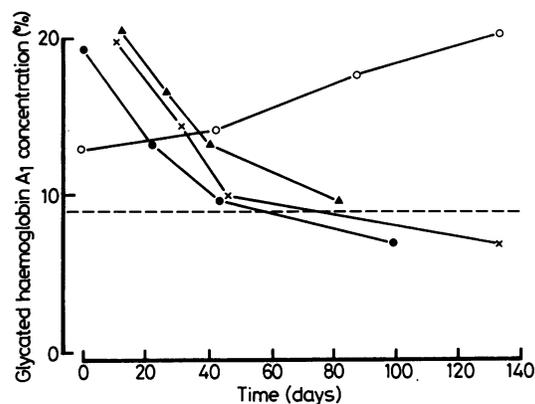


Fig 2 Changes in glycated haemoglobin A<sub>1</sub> in three newly diagnosed diabetics (●—●; x—x; ▲—▲) who were rapidly brought under control and one other patient (○—○) whose diabetes worsened. The interrupted line represents the upper limit of normal.

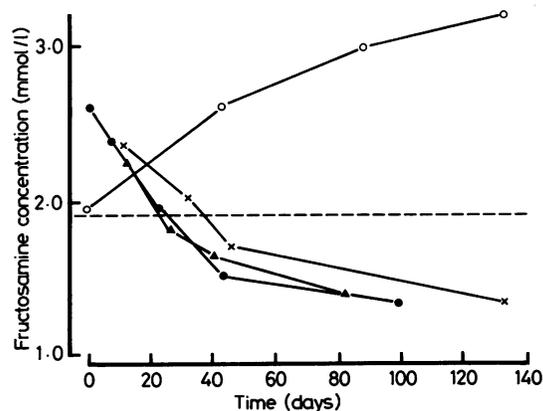


Fig 3 Changes in fructosamine in three newly diagnosed diabetics (●—●; x—x; ▲—▲) who were rapidly brought under control and one other patient (○—○) whose diabetes worsened. The interrupted line represents the upper limit of normal.

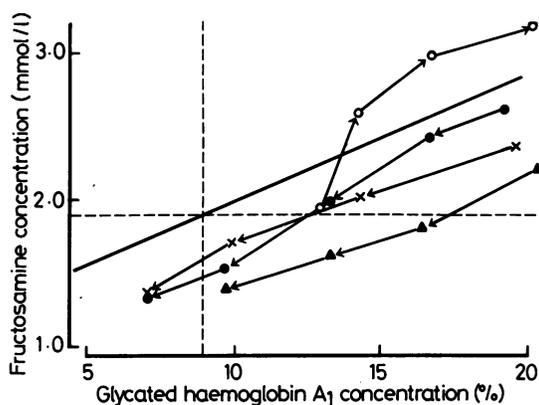


Fig 4 Relation between glycated haemoglobin A<sub>1</sub> and fructosamine in four patients compared with the regression statistic. The three newly diagnosed patients (●—●; ×—×; ▲—▲) were rapidly brought under control; in the other patient (○—○) the diabetes worsened. Arrows indicate direction of progress.

pected since the points fell below the regression line. Conversely all the points derived from the period of deterioration in the fourth patient lay above the regression line indicating a concentration of fructosamine that was higher than expected.

## Discussion

Poor diabetic control leads to an increased incidence of long term complications, particularly retinopathy and nephropathy,<sup>5-7</sup> and maintenance of good control can help to delay their onset. Objective methods of assessing diabetic control have been increasingly used in the past decade to help achieve the aim of near normoglycaemia. HbA<sub>1c</sub> has been the mainstay of these methods but recently fructosamine has been introduced and is now measured routinely in some clinics including our own. The results of these studies show that the ranges of HbA<sub>1c</sub> and fructosamine in normal children are very similar to those of normal adults and no age related differences were shown.

Fructosamine concentration measured as described here has been shown to reflect mainly the concentration of high molecular mass ketoamines or glycosyl proteins<sup>2</sup> with little or no interference by other physiologically important reducing substances under the conditions used. It is therefore a useful indicator of glycaemic state in the period before its measurement.

The method of estimating the half lives of HbA<sub>1c</sub> and fructosamine assumed that the blood glucose in the diabetic children promptly returned to a mean

normal value at the start of treatment and was maintained so thereafter. It also assumed that a mean normal blood glucose would yield mean normal HbA<sub>1c</sub> and fructosamine was unaffected by the diabetic state. While the latter two assumptions are reasonable, clearly the first is not and the effect of this is to give values of the half lives that are slightly longer than the true one. Nevertheless, it is valid to compare the two half lives as, during the period studied, both were subject to the same influences of blood glucose fluctuations.

We have shown that the half life of HbA<sub>1c</sub> is about four weeks and confirmed the finding of others that the half life of fructosamine is substantially shorter at two and half weeks.<sup>8,9</sup> It is, therefore not surprising that the correlation between HbA<sub>1c</sub> and fructosamine was only 0.69 and that this disappeared when only values within the normal range were included, although it has been claimed that the different half lives of the substances is not the only contribution to this finding.<sup>10</sup> Fructosamine is therefore not a direct substitute for HbA<sub>1c</sub> and the advantages and disadvantages of each assay must be considered when deciding whether it might be a suitable alternative, as has been previously suggested.<sup>11,12</sup> The introduction of fructosamine in addition to HbA<sub>1c</sub> would inevitably increase the overall costs of assessing diabetic control (by about 10–15% if the reagents are made up in the laboratory) and cannot be reasonably justified as a routine measure. Conversely, a complete change to fructosamine as an alternative to HbA<sub>1c</sub> would only lead to small annual savings (in a paediatric clinic) and is difficult to justify on clinical grounds at least until more widespread experience has been gained with the assay. The relatively short half life of fructosamine leads to a rapid improvement in its concentrations after a short period of good control and may give a false sense of security regarding longer term control that measurements of HbA<sub>1c</sub> might identify unless more frequent fructosamine estimations are made. In addition, conditions associated with reduced plasma proteins or increased rates of protein turnover such as pregnancy or nephrotic syndrome would be expected to give falsely low fructosamine concentrations.

Perhaps the best policy is to use fructosamine instead of HbA<sub>1c</sub> for most routine purposes while retaining occasional HbA<sub>1c</sub> estimations (for example, once a year as part of an 'annual check') in addition. Furthermore, as we have shown that as expected, fructosamine concentrations are lower than predicted by HbA<sub>1c</sub> when patients are improving and vice versa, it may be possible to use the two parameters together, under certain circumstances, to determine whether a patient is improving

or worsening in the short term. Fructosamine may be a useful adjunct to HbA<sub>1c</sub> in this respect. Such a policy would still reduce the overall costs of assessing diabetic control while retaining the flexibility of having both assays available. Further studies are needed to test this hypothesis.

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