Diabetologia (1981) 21: 579–580

Diabetologia

Letters to the Editor

Measurement of Glycosylated Haemoglobins Using Affinity Chromatography

Dear Sir,

Measurement of the glycosylated fractions of haemoglobin has, in recent years, aroused much attention as a means of providing a reliable index of diabetic control [1–3]. Several methods have been proposed for the routine measurement of glycosylated haemoglobin: ion exchange chromatography is the commonest method used, but is known to have certain disadvantages. By this technique the measured amount of glycosylated haemoglobin is particularly sensitive to variations in temperature and buffer pH. The level of haemoglobin F (which elutes with the fast haemoglobin) if raised, may also introduce significant errors [4].

We describe a new method of measuring glycosylated haemoglobin using affinity chromatography with an immobilised aminophenylboronic acid (PBA) [5] which binds diols selectively. We compared this method with ion-exchange chromatography in 32 non-diabetic subjects and 36 randomly chosen known diabetics attending the clinic.

Samples were collected in EDTA tubes and were assayed by both ion-exchange (adapted from [6]) and affinity chromatography. The haemolysate was prepared as described in [6] using a final concentration of haemolysate twice that described. Affinity chromatography was carried out with immobilised PBA columns (PBA-Matrex gel, PBA-30 from Amicon Corporation, Lexington, MA, USA) which were equilibrated at 4 °C in buffer A (pH 8.5, 50 mmol/l morpholine-HCl containing 10 mmol KCN/l). Haemolysate (0.2 ml) was applied and the column was washed with buffer. The void volume and washings (25 ml) were collected and pooled ('unbound' fraction). Adsorbed haemoglobin ('bound' fraction) was eluted with 0.1 mol/l sorbitol in buffer A (10 ml). The absorbance of the 'unbound' and the 'bound' fractions was measured at 413 nm and the percentage glycosylated haemoglobin was calculated.

The results are shown in Figure 1. By ion-exchange chromatography the mean value for glycosylated haemoglobin (± SD) in non-diabetic patients was 7.9 ± 1.5% (observed range 5.3–10.8%), diabetic patients showed a mean value of 10.9 ± 2.7% (observed range 7.0–19.0%).

By affinity chromatography, the mean value in non-diabetics for glycosylated haemoglobin was 5.4 ± 1.2% (observed range 3.3–7.7%, correlation coefficient $r = 0.35$) and for diabetic patients 11.0 ± 2.3% (observed range 7.2–15.8%, correlation coefficient $r = 0.62$). Both methods showed a highly significant difference between the two groups of patients ($p = 0.001$). Figure 1 shows that there is a reasonable correlation between the two methods. This suggests that affinity chromatography is able to detect similar glycosylated species to the ion-exchange technique. In addition, there is an improved separation of the two groups of samples determined by affinity chromatography when compared with the ion-exchange technique. The affinity chromatography technique could therefore prove to be superior in monitoring blood glucose control in patients.

However, further studies would be necessary to confirm this. Preliminary experiments indicate that the affinity technique has good reproducibility and is less sensitive to pH than the ion-exchange method (a change in affinity measured glycosylated haemoglobin of 3% was observed between pH 8.5 and 8.7). Another important aspect of the affinity assay is the speed with which a large number of samples can be processed. Furthermore, because the latter assay presumably depends upon diol-boronate interactions [5], we can expect no interference from haemoglobin F. We believe that this method may be an improvement in terms of accuracy, reliability and laboratory workload in the management of diabetes. However, further studies are necessary to confirm not only which species are bound to the column, but also the correlation between glycosylated haemoglobin measured by affinity chromatography and established methods of assessing diabetic control.

Yours sincerely

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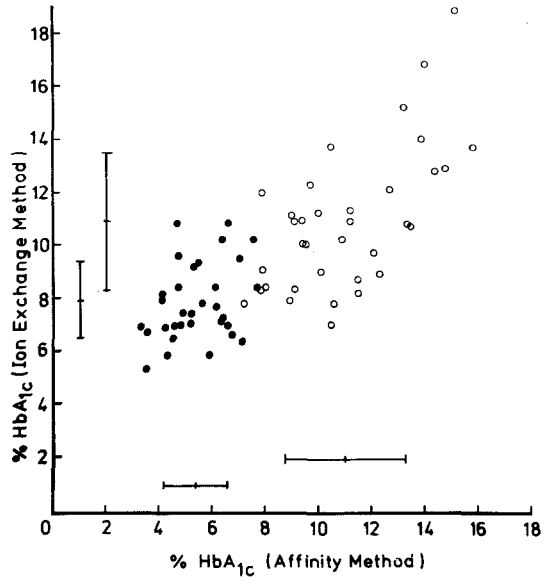

Fig. 1. Haemolysates from each patient were assayed both by the ion-exchange method and the affinity method. ● non-diabetics; ○ diabetics. Mean values ± SD are shown for non-diabetics and diabetics

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Diabetologia (1981) 21: 580

Glucose or Glucose Monohydrate for Glucose Tolerance Tests?

Dear Sir,

Recently a pharmaceutical company sought our advice on the amount and chemical form of glucose which should be incorporated in a drink suitable for oral glucose tolerance testing. We had some difficulty in answering the question.

In the second report of the WHO Expert Committee on Diabetes Mellitus, a standard oral glucose load of 75 g was recommended for adults [1]. However, unlike the first report of the Expert Committee, published in 1965, the chemical form of glucose to be used in the load was not stated. The earlier recommendations specified the use of 'glucose (dextrose monohydrate)' [2]. A load of 75 g of anhydrous glucose (mol. wt. 180.2) is equivalent to 416 mmol glucose, whereas 75 g glucose monohydrate (mol. wt. 198.2) represents only 378 mmol glucose, a 38 mmol or 9.1% decrease.

The terminology used in pharmacopoeias is confusing. The 1980 British Pharmacopoeia defines 'anhydrous dextrose' as 'D-gluco-pyranose, anhydrous glucose'. However, 'dextrose monohydrate' is defined as 'glucose' [3].

Other recommendations concerning oral glucose tolerance testing do not clarify the nature of the glucose dose. The suggested criteria presented to the Committee of the European Association for the Study of Diabetes state: 'Load 75 g glucose in 200–500 ml or 1.75 g/kg for children' [4]. The National Diabetes Data Group of the National Institutes of Health in America require a 75-g glucose dose for non-pregnant adults, or 'a commercially prepared carbohydrate load equivalent to this glucose dose' [5]. Fajans and Conn's early criteria for the diagnosis of diabetes do not specify the use of anhydrous glucose or glucose monohydrate in glucose tolerance tests [6]. A recent report of a Working Party representing the Australian Diabetes Society, the Royal College of Pathologists of Australasia and the Australian Association of Clinical Biochemists stated that '75 g (416 mmol) of glucose should be dissolved in 250–350 ml water and drunk within 5 min'. Thus this report implies the use of anhydrous glucose.

The size of the glucose load used in glucose tolerance tests has been shown to have no influence on results in normal subjects [7]. However, the same study reported that, in subjects with mildly impaired glucose tolerance, there was an increase of 3.0 mmol/l in the 2-h post-glucose level of plasma glucose when 100 g glucose was ingested, compared with 50 g. Thus, assuming that glucose monohydrate was used, changing the load from 50 g (252 mmol) to 100 g (504 mmol) resulted in an increase in plasma glucose concentration of 3.0 mmol/l. If the relationship of increasing glucose load to increasing 2-h plasma glucose level is linear, then the 38 mmol difference between 75 g glucose and 75 g glucose monohydrate would result in a 0.45 mmol/l increase in 2-h plasma glucose concentration. This difference may affect the classification of a subject.

A definitive statement is required concerning the amount of glucose to be used in 'the 75 g dose' in oral glucose tolerance tests for the diagnosis of diabetes mellitus. Glucose monohydrate is cheaper than anhydrous glucose and is the stable crystalline form of glucose at temperatures less than 50 °C. We suggest that the recommended glucose load should be 75 g glucose monohydrate.

Yours faithfully
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