Clinical Importance of the Reversible Fraction of Haemoglobin A_{1c} in Type 2 (Non-Insulin-Dependent) Diabetes

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Summary. Two studies demonstrating the clinical relevance of the labile fraction of haemoglobin A_{1c} are presented. Haemolysates, dialysed at 37 °C against 0.9% NaCl, showed an average decrease of haemoglobin A_{1c} value of 7.0% of total glycosylated haemoglobin, post-dialysis. There was a close correlation between the pre- and post-dialysis haemoglobin A_{1c} values (r = 0.9644). In four Type 2 (non-insulin-dependent) diabetic subjects, blood glucose and pre- and post-dialysis haemoglobin A_{1c} values were determined at weekly intervals over an 18-week period, including 6 weeks when oral hypoglycaemic therapy was withheld. During this time, pre- and post-dialysis haemoglobin A_{1c} values responded synchronously to changes in blood glucose concentration. The mean coefficient of variation of the pre- and post-dialysis haemoglobin A_{1c} values did not differ significantly over the 18-week period. It is concluded that the routine elimination of the labile fraction of haemoglobin A_{1c} is not necessary to assess control in Type 2 diabetes.

Key words: Haemoglobin A_{1c}, Type 2 diabetes, reversible fraction, pre-dialysis, post-dialysis, glucose.

During the life span of the erythrocyte, haemoglobin A_{1c} (HbA$_{1c}$) is formed by the post-translational association of the haemoglobin and glucose molecules in a reversible aldimine linkage [1]. Subsequent Amadori rearrangement results in the formation of a stable ketoamine. As blood glucose concentration is the major factor affecting the extent of glycosylation, HbA$_{1c}$ levels are increased in diabetic patients, and to the extent that the glycosylated moiety is in the ketoamine form, provide a representative measure of the blood glucose concentrations in antecedent weeks [2]. However, the aldimine fraction may change rapidly in response to changes in blood glucose concentration [3, 4], and it has been suggested that this labile fraction should be removed by dialysis before analysis [5]. This is difficult and time consuming, so we have explored the clinical relevance of the labile HbA$_{1c}$ fraction to assess the need for the inclusion of a dialysis step in the routine assay of glycosylated haemoglobin.

Patients and Methods

Patients

Samples for the in vitro study were obtained from patients attending the Waikato Hospital Diabetic Clinic for assessment of diabetic con-

trol. Informed written consent was obtained from the four patients participating in the in vivo study.

Methods

A high performance liquid chromatography system described previously, but using Waters Associates equipment (Waters Associates, Milford, Massachusetts, USA) was used for the determination of HbA$_{1c}$ [6, 7]. The system gave a linear response to increasing quantities of HbA$_{1c}$ in standard solutions. Between-day and within-day coefficients of variation were between 3 and 5.5%. The reference range for HbA$_{1c}$ was 4.0–5.5% (mean ± 2 SD).

In vitro dialysis studies: Haemolysates were prepared from samples of EDTA blood as described previously [6] and dialysed for 12 h at 4 °C or 37 °C in viscose cellulose membranes (12,000–14,000 daltons, Union Carbide Company, USA) against 0.14 mmol/l NaCl. Aliquots were analysed for HbA$_{1c}$ pre- and post-dialysis.

In vivo study: Four Type 2 diabetic subjects with stable diabetic control on diet and oral hypoglycaemic therapy were studied over three consecutive 6-week periods. Fasting blood glucose concentrations and HbA$_{1c}$ values were determined at weekly intervals during each period. In the second 6-week period, the oral agents were stopped, and then reinstated in the final 6 weeks.

Results

At 4 °C, the dialysis produced no significant decrement in HbA$_{1c}$ values in samples from 26 non-diabetic, 27

Type 1 and 19 Type 2 diabetic subjects. In contrast, at 37 °C (21 non-diabetic, 15 Type 1 and 20 Type 2 diabetic samples), an average decrement of 7% of total HbA_{1c} was observed in both the diabetic and the non-diabetic samples. There was a close correlation between the pre-

and post-dialysis values ($r = 0.9644$; $y = 0.88x + 0.335$; $p < 0.001$; Fig. 1).

Mean ± SD values for blood glucose, pre-dialysis HbA_{1c} and post-dialysis HbA_{1c} in the four Type 2 diabetic subjects are shown in Figure 2. Mean blood glucose values fluctuated during the first 6 weeks of the study while the subjects were on treatment, and then increased substantially during the second 6 weeks after cessation of tablet treatment. Reinstatement of tablet therapy in the final 6 weeks of the study produced a considerable reduction in blood glucose concentrations during the first 2 weeks, but the mean values then stabilised at a higher level than in the first 6 weeks (Fig. 2). With one exception, mean pre-dialysis HbA_{1c} values exceeded the mean post-dialysis values; this also applied for the individual values of the four subjects. Synchronous changes were noted in the pre- and post-dialysis HbA_{1c} values (Fig. 2) in response to the change in blood glucose concentrations produced during the second and third study periods. The predialysis HbA_{1c} values possibly increased more abruptly in the first 2 weeks of the second study period but, for the most part, the absolute difference between the two means was small and remained relatively constant throughout, i.e. 0.4% at 6 weeks, 0.9% at 9 weeks, 0.5% at 12 weeks, 0.3% at 15 weeks and 0.5% at 18 weeks at the termination of the study. Coefficients of variation of blood glucose concentrations, pre- and post-dialysis HbA_{1c} values were determined for each of the four subjects for the entire 18-week study period. The mean coefficient of variation
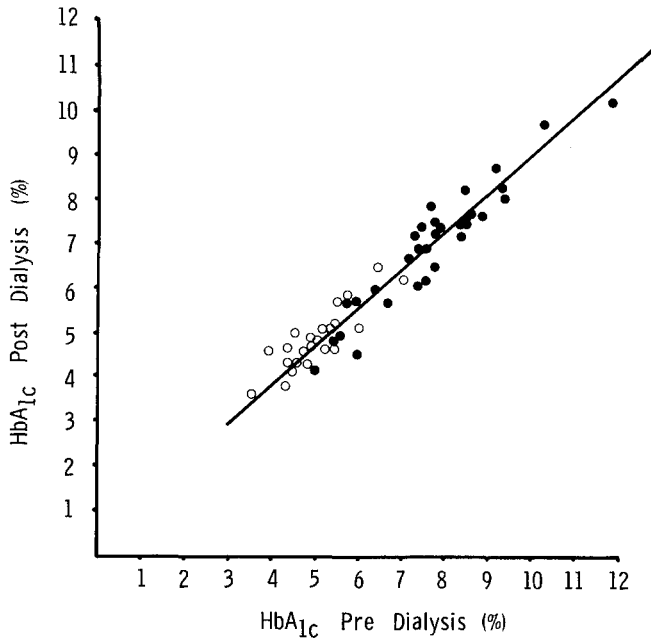

Fig. 1. Correlation of HbA_{1c} values pre- and post-dialysis, against 0.9% normal saline at 37 °C, in diabetic (•; $n = 35$) and non-diabetic (○; $n = 21$) subjects. ($y = 0.88x + 0.335$; $r = 0.9644$).
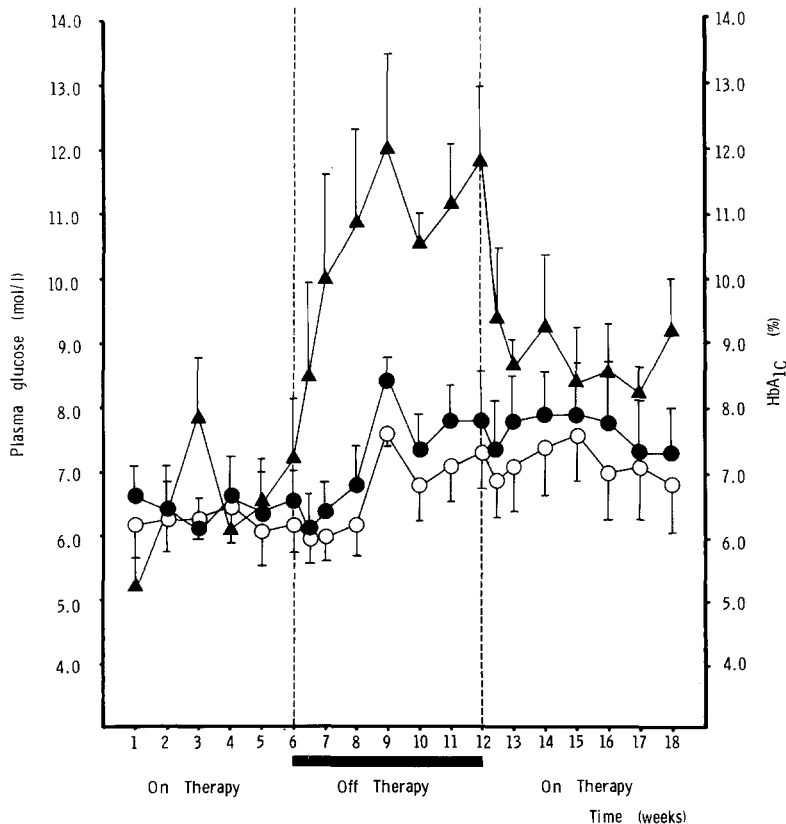

Fig. 2. Mean ± SD values for plasma glucose (▲), HbA_{1c} pre (●) and post-dialysis (○) at 37 °C in four diabetic subjects. On oral therapy, period 1; off therapy, period 2; and on oral therapy, period 3.

was 25.2% for the blood glucose, 10.2% for the pre-dialysis HbA_{1c} values and 9.0% for the post-dialysis HbA_{1c} values. The latter two values did not differ significantly.

Discussion

The present studies support others in demonstrating a reversible component of the HbA_{1c} fraction as measured by ion-exchange chromatography [5, 8]; the temperature dependence of the dialysis effect is comparable to that described by Mortensen et al. [9]. Importantly however, the labile fraction present in both non-diabetic and diabetic subjects is quantitatively minor, even in the diabetic subjects with relatively poor control and high HbA_{1c} values. Indeed, the magnitude of the difference between pre- and post-dialysis values is sufficiently small that it may not be detected by a number of the simple mini-column methods in common use. On that basis alone, prior dialysis of samples at 37 °C would appear to be of little value. In addition, it should be noted that this procedure may introduce a further source of error, as the denaturation of haemoglobin occurs at an accelerated rate of 37 °C and the denaturation products, plus glutathione haemoglobin may produce an artefactual increase in the commonly determined HbA_1 fraction [10].

In an earlier study, we observed that HbA_{1c} values sampled repetitively over a 4-month period did not vary significantly more in diabetic than non-diabetic subjects, yet the blood glucose concentrations were considerably more variable in the diabetic group [10]. It did not appear that a reversible aldimine fraction, if present, was of clinical significance. That observation is strengthened by the present study where substantial but slow changes in blood glucose values induced in four Type 2 diabetic subjects were associated with quantitatively similar and synchronous changes in pre- and post-dialysis HbA_{1c} values. It would therefore appear that a reversible component is present in both diabetic and non-diabetic samples but it is quantitatively minor and of little clinical significance when a measure of HbA_{1c} is used as an index of diabetic control. There seems little point in the routine elimination of this component by dialysis or similar techniques, a conclusion recently published by others [11].

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