Diabetic Control in Patients Treated with Once or Twice-Daily Insulin Injections, Including a Comparison of Conventional Beef and Highly Purified Pork Insulins

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Summary. Twenty patients were changed from a single daily injection of beef insulin (a mixture of soluble and protamine zinc insulin) to two daily injections (mixtures of soluble and isophane insulins). This was associated with a reduction, one month later, in the concentration of glycosylated haemoglobin (HbA₁) and in the degree of late evening glycosuria. A reduction was shown 6 months later in antibody binding of beef and pork insulin by serum. Subsequent conversion to a twice daily regime of highly purified pork insulin was not associated with further improvement in diabetic control, but was associated after 1 month with a reduction in daily insulin dose, and after 5 months with a further reduction in antibody binding of beef and pork insulin by serum. Patients failing to show a C-peptide response to intravenous glucagon had a fall in HbA₁ after conversion from a once to a twice daily insulin regime, which correlated inversely with insulin antibody binding estimated at the beginning of the study.

Key words: HbA₁, glycosuria, insulin antibody, C-peptide, conventional beef insulin, highly purified pork insulin

Assay of the total glycosylated haemoglobin (HbA₁) concentration in blood provides an index of the integrated blood glucose concentration of the previous few weeks [1–3]. We have studied HbA₁ concentrations in blood in a group of young Type 1 (insulin-dependent) diabetics who were treated sequentially as outpatients with once daily conventional beef insulin, then with twice daily conventional beef insulin and finally with twice daily highly purified pork insulin. Our main aim was to establish whether diabetic

control can be improved by substitution of a twice daily for a once daily insulin regime or by substitution of highly purified pork for conventional beef insulin.

Patients and Methods

Patients

Twenty patients (seven males and 13 females) were studied with a mean age of 27 years (range 16–38 years), a mean duration of diabetes of 8 years (range 2–23 years) and mean weight of 103% (range 87–120%) ideal body weight [4] at the start of the study. Their mean age at diagnosis of diabetes was 19 years (range 7–32 years). Each patient had received a single morning (before breakfast) injection of soluble insulin and protamine zinc insulin, both beef insulins (Wellcome), since diagnosis of diabetes. The mean post-prandial plasma glucose concentration at six consecutive outpatient clinic attendances before this study, was less than 15 mmol/l in all subjects.

Each patient had a normal full blood count, normal concentrations of serum creatinine, plasma urea and electrolytes, normal liver function tests and plasma proteins. None had proteinuria and only three patients had minimal background diabetic retinopathy.

Study Protocol

All patients attended the outpatient clinic, 2–3 h after breakfast and morning insulin injection, at monthly intervals for 13 months (14 attendances) during which no attempt was made to alter their dietary or exercise regimens. For the first two months (three attendances) they continued to receive a single daily injection (before breakfast, 0800–0830 h) of soluble and protamine zinc insulin and were then all converted to twice daily (before breakfast and before tea, 1730–1800 h) soluble and isophane insulin (Wellcome) – the initial dose of each insulin being recommended by a doctor, subsequent changes being made by the patient as indicated by urine tests for glucose and the occurrence of hypoglycaemia. Throughout the study, patients were told to adjust insulin doses to reduce glycosuria to a minimum without inducing symptomatic hypoglycaemia.

At the ninth clinic attendance, the 20 patients were divided into two groups with a similar duration of diabetes and C-peptide response to IV glucagon (vide infra). There were no significant differences between the two groups in respect of age, insulin anti-

Fig. 1. Change in antibody binding of beef and pork insulin by serum (mean $\pm$ SEM) and in daily insulin dose following conversion from a once daily conventional beef insulin regime to a twice daily conventional beef insulin regime, and then to a twice daily highly purified pork insulin regime. SI = soluble insulin, PZI = protamine zinc insulin, NPH = isophane insulin, AR = Actrapid insulin, MT = Monotard insulin

body binding, percentage ideal body weight or daily insulin dose. Ten patients were converted to twice daily Actrapid (Novo) insulin and Monotard (Novo) insulin having been advised to reduce their daily dose by approximately 10% according to the manufacturer's recommendations, while the remaining ten patients continued to take twice daily soluble and isophane insulin.

Throughout the study, each patient recorded symptomatic hypoglycaemic episodes, daily insulin dose and second sample urine tests (Clinitest) carried out four times a day (before breakfast, 0800–0830 h, before lunch, 1200–1230 h, before tea 1730–1800 h and late evening, 2200–2230 h) on two days each week. A scoring system was devised to enable each subject's degree of glycosuria to be expressed as a mean for a given time of day, for a given month: 2% = 5, 1% = 4, $\frac{3}{4}$ % = 3, $\frac{1}{2}$ % = 2, $\frac{1}{4}$ % = 1 and aglycosuria = 0.

Estimations

At each clinic attendance, venous blood was assayed for plasma glucose, HbA_{1c}, haemoglobin and reticulocytes. At the third and ninth to fourteenth (inclusive) attendances, venous blood was

obtained for estimation of insulin antibody binding. At the third and ninth attendances an IV 1 mg glucagon stimulation test was performed when post-prandial venous blood samples were obtained at 0, 2, 5 and 10 min for plasma glucose and C-peptide estimation.

The binding of ¹²⁵I-labelled insulin by serum samples was determined by a method using polyethylene glycol separation [5]. Non-specific binding did not exceed 1.5% and all samples were analyzed in one assay, with precision of 2.5. (CV) across the range. Results are expressed as percentage of labelled insulin bound (using either labelled beef or labelled pork insulin).

C-peptide was measured by radioimmunoassay using a modification of the method of Heding [6]. Antibody M1230 raised against human C-peptide was used at an initial dilution of 1/25,000. The detection limit of the assay was 0.04 nmol/l (calculated as 2 SD from 0). Cross reaction with human proinsulin was 11% on a molar basis. The interassay coefficient of variation was 6%. Antibody M 1230, human C-peptide standard and iodinated tyrosylated C-peptide were gifts of the Novo Research Institute, Copenhagen, Denmark. The C-peptide response to the glucagon stimulation test was expressed as the difference between the basal and peak concentration (Δ C-P). Plasma glucose exceeded 2.0 mmol/l throughout the test in each patient.

Plasma glucose was estimated by a standard glucose oxidase method [7].

HbA_{1c} was measured by the column chromatographic technique of Kynoch and Lehmann [8]. Reversible and stable HbA_{1c} were not separated by dialysis of red cells [9]. Between batch precision, estimated by repeat analysis of specimens from patients showed a coefficient of variation of 3.5%. The normal range in non-diabetics in our laboratory is 5.2–8.4%.

Statistical analyses were carried out using the Wilcoxon rank sum test or the Spearman rank correlation test, as appropriate. Results are expressed as mean $\pm$ SEM unless otherwise stated.

Results

Antibody Binding of Insulin and Daily Insulin Dose

Beef and pork insulin antibody binding estimated for serum samples from clinic attendance 9 (23.0 ± 3.7 and $16.2 \pm 3.1\%$) were significantly less ($p < 0.01$) than for serum samples from attendance 3 (32.4 ± 7.2 and $24.2 \pm 3.5\%$) i. e. 6 months after conversion from once daily soluble and protamine zinc insulin to twice daily soluble and isophane insulin (Fig. 1). This was not associated with a significant reduction in the daily insulin dose. Beef and pork insulin antibody binding also fell significantly ($p < 0.05$) between attendances 9 and 14, in patients transferred from soluble and isophane to Actrapid and Monotard insulin. This reduction was not found in patients who remained on soluble and isophane insulin.

One month after conversion from soluble and isophane to Actrapid and Monotard insulin, daily insulin dose had been reduced ($p < 0.05$) from 66 ± 7 to 56 ± 6 units, i. e. by 15%. No further significant reduction in insulin dose was observed thereafter despite the subsequent fall in insulin binding by antibodies.

Fig. 2. Monthly, random, post-prandial glucose and HbA₁ concentrations (mean $\pm$ SEM) following conversion from a once daily conventional beef insulin regime to a twice daily conventional beef insulin regime, and then to a twice daily highly purified pork insulin regime. See Fig. 1 for abbreviations

C-Peptide

Of the 20 patients tested at clinic attendance 3, only nine showed a measurable response with a median Δ C-P of 0.05 nmol/l. When the 20 patients were re-tested at clinic attendance 9, the same nine C-peptide secretors were the only patients to exhibit a measurable response (median Δ C-P 0.04 nmol/l), there being a highly significant correlation between Δ C-P at attendances 3 and 9 ($r = 0.93$, $p < 0.01$). Basal C-peptide concentrations in non-secretors were all less than 0.04 nmol/l.

Diabetic Control

There were no significant differences between consecutive HbA₁ levels, random plasma glucose concentrations, degrees of glycosuria or frequency of symptomatic hypoglycaemia during the first two months of the study (three attendances) when the patients received soluble and protamine zinc insulin once daily, and these variables were, therefore, each averaged and expressed as a basal value.

One month after conversion to twice daily soluble and isophane insulin, HbA₁ fell from a basal value of 12.9 ± 0.5 to $11.6 \pm 0.4\%$ ($p < 0.01$) (Fig. 2). The mean degree of late evening glycosuria fell ($p < 0.01$) from a basal value of 2.7 ± 0.3 to 1.7 ± 0.3 units one month later, an improvement which was sustained throughout the study. When degree of glycosuria for each time of the day was expressed as a mean value for months 3–13 inclusive, only late evening glycosuria (1.6 ± 0.04 units) showed a significant reduction from the basal value ($p < 0.01$). There were no significant differences between esti-

mates of basal glycosuria before breakfast (2.1 ± 0.3 units), before lunch (1.3 ± 0.2 units) and before tea (2.1 ± 0.2 units) and those recorded for months 3–13 (1.6 ± 0.04 , 1.1 ± 0.02 and 1.7 ± 0.02 units). Figure 2 shows the mean plasma glucose concentration recorded at each clinic attendance. With the above exceptions, no significant differences were observed when comparing consecutive monthly indices of diabetic control. However, the mean HbA₁ concentration at attendance 14 ($10.5 \pm 0.3\%$) was lower ($p < 0.01$) than that at attendance 5 ($11.3 \pm 0.3\%$) when all 20 patients were considered together.

There were no significant differences between patients treated with soluble and isophane insulin and those treated with Actrapid and Monotard insulin in respect of plasma glucose and HbA₁ concentrations (Fig. 2), nor in respect of glycosuria nor symptomatic hypoglycaemia.

There was no significant difference between mean basal HbA₁ values of C-peptide secretors ($12.6 \pm 0.7\%$) and non-secretors ($13.1 \pm 0.6\%$), nor between patients with low ($13.6 \pm 0.8\%$) and high ($12.1 \pm 0.4\%$) insulin antibody binding. For the latter comparison, the 20 patients were ranked according to their insulin antibody binding estimated at the third attendance and divided into two groups of ten, having the lowest and the highest binding respectively.

Each patient's basal HbA₁ concentration was compared with the mean concentration recorded at attendances 5–14 inclusive, whereby individual changes in HbA₁ in response to conversion from a once to twice daily insulin regimen were assessed. Eighteen patients (85%) showed a reduction, while three patients (15%) showed an increase in HbA₁.

When the mean change in HbA₁ following conversion from a once to a twice-daily insulin regimen was compared between C-peptide secretors ($-1.7 \pm 0.7\%$) and non-secretors ($-1.9 \pm 0.3\%$) and between patients with low ($-2.3 \pm 0.5\%$) and high ($-1.2 \pm 0.4\%$) insulin antibody binding, respectively, no significant difference was found. However, when C-peptide non-secretors were considered alone, patients with low insulin antibody binding showed a significantly greater ($p < 0.01$) change in HbA₁ ($-2.8 \pm 0.3\%$) than in patients with high insulin antibody binding ($-1.2 \pm 0.5\%$). Individual reductions in HbA₁ following conversion from a once to a twice daily insulin regime showed a significant negative correlation with beef insulin antibody binding estimated at attendance 3 ($r = 0.62$, $p < 0.05$), in C-peptide non-secretors, but not in C-peptide secretors ($r = 0.38$).

Throughout the study each patient maintained a normal haemoglobin and reticulocyte count.

Discussion

Twice daily insulin regimes may help in the management of "unsatisfactorily" controlled [10], "severe" [11], or "labile" [12] diabetics, whose metabolic control has been assessed in terms of pre-meal plasma glucose and ketone concentrations, or degree of glycosuria or ketonuria. The present study shows that in our patients receiving once daily insulin the change to a twice daily regime was associated with a fall in HbA₁ concentration. Such a change in insulin regime cannot be effected "blind" and, therefore, the possibility remains that part of the improvement in HbA₁ concentration may be due to greater effort on behalf of patient and doctor to improve diabetic control. On the other hand, it seems likely that the reduction in late evening glycosuria is due to the evening dose of soluble insulin and may largely account for the concomitant fall in HbA₁. Our findings contrast with a previous comparison [13] of 24 h metabolic profiles of inpatients receiving once daily Actrapid and Monotard insulin and twice daily Actrapid and Semitard (Novo) insulin. In the latter study, no significant difference in mean 24 h plasma glucose nor 24 h urinary glucose excretion was observed, possibly because Semitard's duration of action may be shorter than that of isophane. Alternatively, the indices of diabetic control used by Werther et al. [13] may have been insufficiently sensitive, by comparison with HbA₁, to detect an improvement in control. Nevertheless, the once daily regime was accompanied by significantly greater hyperglycaemia in the late evening than the twice daily regime [13], in accordance with our own findings.

Of further interest is the considerable individual variation in HbA₁ response to a change from a once to a twice daily insulin regime. It has been suggested [14, 15] that in patients who have no endogenous insulin secretion, high insulin antibody binding may improve diabetic control by prolonging the half-life of insulin [16, 17]. Thus, C-peptide non-secretors with low insulin antibody binding might be expected to gain particular advantage from an evening dose of insulin. In accordance with such a hypothesis, our findings suggest that in C-peptide non-secretors the improvement in HbA₁ following conversion from a once to a twice daily insulin regime, shows an inverse correlation with initial insulin antibody binding. This observation also encourages us to believe that the reduction in HbA₁ is not solely due to improved patient compliance. It is of interest that the continuing secretion of C-peptide, which has been shown to reduce HbA₁ concentrations in insulin treated diabetics [18], is not associated with a reduction in HbA₁ response to the change in insulin regime.

A number of reports [19–22] claim an improvement in metabolic control following conversion from conventional beef to highly purified pork insulin. However, in these studies, the patients were selected on the basis of metabolic lability, high insulin requirement or insulin allergy and diabetic control was assessed by unsatisfactory means. Moreover, the studies were uncontrolled. We now show that highly purified pork insulin, when delivered as a twice-daily insulin regime, does not carry any advantage over conventional beef insulin. A small but significant reduction in HbA₁ was observed between attendances 5 and 14 when all twenty patients were considered together. It seems likely that this improvement in diabetic control is due to the close supervision of the patients studied leading to greater compliance. Omission of a control group of patients would have led to the erroneous conclusion that the highly purified pork insulins were responsible for the improvement in HbA₁ concentrations.

The method used to characterise insulin antibody binding restricts us from commenting on changes in insulin antibody binding capacity or affinity characteristics. Nevertheless, in accordance with previous studies [5, 23, 24] we have shown that beef and pork insulin antibody binding is reduced after conversion from conventional beef to highly purified pork insulin. We have also shown considerable reduction in insulin antibody binding after conversion from protamine zinc to isophane insulin, suggesting that the high ratio of protamine to insulin in protamine zinc insulin may be increasing insulin's antigenicity [25]. It is of interest that this reduction in insulin antibody binding has little influence on daily insulin dose. The

immediate fall in insulin dose after conversion to highly purified pork insulin may reflect lower binding affinity of insulin antibody for highly purified pork as compared with conventional beef insulin [21]. However, we cannot deny the possibility that the reduction in insulin dose merely reflects the advice given to each patient, according to the recommendations of the insulin manufacturers. Our patients did not encounter frequent, profound hypoglycaemia, as described by Asplin et al. [21], many of whose patients had required a large dose of once daily insulin and had been poorly controlled prior to conversion of insulin regime.

In conclusion, our findings suggest that a twice daily insulin regime provides better glycaemic control than a once daily regime, but that highly purified pork insulin offers no such advantage over conventional beef insulin, when administered twice daily. C-peptide non-secretors with low insulin antibody binding show the greatest improvement of control on changing from once to twice daily insulin.

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