A Double-Blind Crossover Trial Comparing Human Insulin (recombinant DNA) with Animal Insulins in the Treatment of Previously Insulin-treated Diabetic Patients

ADRIAN J. CLARK, PHILIP G. WILES, JAMES M. LEIPER, GRAHAM KNIGHT, RODNEY O. ADENIYI-JONES, PETER J. WATKINS, JOHN D. WARD, ANGUS C. MACCUISH, HARRY KEEN, AND RICHARD H. JONES

Ninety-four diabetic patients established on treatment with pork (N = 47) or beef insulin (N = 47) took part in a double-blind crossover trial in which 6-wk treatment periods of their animal insulin were compared with similar periods on human insulin (recombinant DNA). Six patients withdrew during the trial—in three cases for hypoglycemia while taking human insulin. In patients initially treated with beef insulin there was no significant change in the mean blood glucose, the 'M' index, the total daily insulin dose, or the frequency of hypoglycemic attacks after the change to human insulin. Home blood glucose sample values were greater before the morning and evening insulin injection on human insulin (morning: 12.8 mmol/L [beef] versus 14.2 mmol/L [human insulin] [$P < 0.05$]; evening: 10.0 mmol/L versus 11.6 mmol/L [$P = 0.05$]). In pork insulin–treated patients greater values while on human insulin were found for mean glucose (9.0 mmol/L [pork] versus 9.7 mmol/L [human insulin], $P = 0.05$), 'M' index (65.0 [pork] versus 79.6 [human insulin], $P < 0.025$), and total daily insulin dose (50.9 U/day [pork] versus 52.5 U/day [human insulin], $P < 0.001$). The early morning glucose sample was also greater on human insulin (9.6 mmol/L [pork] versus 12.1 mmol/L [human insulin], $P < 0.001$). No significant differences in either insulin antibody levels or *E. coli* protein antibody levels were found between either of the animal-insulin treatment periods and human insulin treatment periods. While human insulin appears to be a safe alternative to beef and pork insulins, it seems likely that pharmacokinetic differences may account for the apparent differences in glycemia and insulin requirement. It may be necessary to adjust the dosage and proportions of short-acting and long-acting insulin formulations in order to obtain maximum benefit from human insulin. DIABETES CARE 5 (SUPPL. 2): 129–134, 1982.

Human insulin (recombinant DNA) was first synthesized using *E. coli* in 1978.[1] As well as providing a new source of supply for the treatment of diabetes, human insulin might differ from animal insulins in man in its action and immunogenicity.

In laboratory, animal, and human studies the properties of human insulin (recombinant DNA) differ little from those of the natural hormone and closely resemble those of purified pork insulin (PPI).[2,3] In nondiabetic volunteers human insulin may be absorbed more rapidly than PPI from the subcutaneous site after injection.[4,5,6]

Treatment of diabetes with human insulin began in 1981, and we report here the results of the first British multicenter clinical trial.

PATIENTS, MATERIALS AND METHODS

Ninety-four insulin-treated diabetic patients from five centers participated in a randomized double-blind crossover trial. All patients had been treated with soluble (regular) or NPH, or both insulins of beef (N = 47) or pork (N = 47) origin for 1 yr before entry, and were judged clinically to have been under stable control for the preceding 6 mo. All but one patient received two injections/day (the exception taking

TABLE 1
Clinical details of 88 patients completing the trial. HbA_1 measured by Corning Agar gel electrophoresis (upper limit normal 9.0%)

	Beef → beef → human insulin	Beef → human insulin → beef	Pork → pork → human insulin	Pork → human insulin → pork
Number of patients	20	22	23	23
Age (± SD) yr	39.0 ± 14.8	43.7 ± 12.1	33.2 ± 11.2	34.3 ± 11.4
Daily insulin dose (U/day ± SD)	61.4 ± 17.7	57.2 ± 25.3	49.5 ± 17.2	51.5 ± 16.1
Number on regular insulin alone	3	6	3	2
Number on isophane alone	1	1	7	3
HbA_1 at end of 'run-in' period (%)	9.4	10.5	10.2	9.1

only a single daily injection), and patients requiring more than 120 U/day or with additional illnesses were not included. Further clinical details are shown in Table 1.

The trial was of 18-wk duration in total, and comprised three 6-wk treatment periods. Patients were randomized at the start to receive human insulin in period 2 or 3. When not receiving human insulin they were treated with the animal insulin they used before entry, although all insulins were contained in special study vials that did not allow the patient or doctor to determine which type of insulin was being used at any time. Period 1 served as a run-in period, and made allowance for the nonspecific effect of increased surveillance on glycemic control. Figure 1 illustrates the trial design and the number of patients completing each section.

Each patient visited an assessment clinic at 2-wk intervals, having collected a 7-point series of capillary blood samples in plastic capillary tubes[7] (four centers), or in fluoride/oxalate bottles (one center) within the previous 2 days. The seven points chosen were three pre-meal samples (one after overnight fast), three samples 90 min after each meal, and one bedtime sample; these were returned to the laboratory at each center for glucose analysis by glucose oxidase methods.

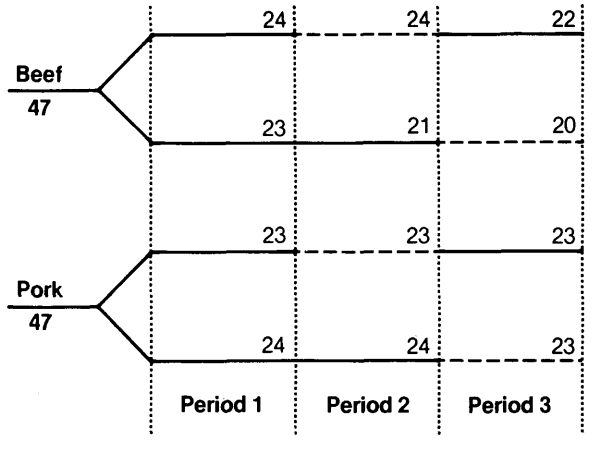

FIG. 1. Design of the trial: 47 BIT and 47 PIT patients were randomized to receive human insulin in either period 2 or 3—period 1 serving as a run-in period. The figures at the end of each period denote the number of patients completing that section.

Both patients and their attendant doctors were instructed to adjust insulin dose in accordance with their usual practice. No deliberate dose adjustment was made on passing from one treatment period to the next.

Serum samples for *E. coli* antibodies and insulin antibodies were taken at the start of the study and at the end of each treatment period. Insulin antibodies were measured by a polyethylene glycol precipitation technique on acid/charcoal–treated sera using a beef insulin ligand for all samples from beef-treated patients and a pork ligand for all samples from pork-treated patients.[8]

E. coli antibodies were measured using specially devised techniques described previously.[9]

With the exception of antibody values, results were analyzed by standard crossover trial methods,[10] which involve comparing pooled results for each insulin for period 2 and 3 using analysis of variance. Assessment of glycemic control included calculation of the modified 'M' index,[11] results of which were compared after log transformation.

Longitudinal changes in insulin antibody results were compared using paired *t* tests. *E. coli* antibody results obtained at the first visit were used to define a "normal" range for insulin-treated diabetics (mean + 2 SD), and values exceeding this range are reported.

This study had the approval of participating hospitals' ethical committees, and written consent was obtained from each patient.

RESULTS

Six patients withdrew from the trial over the 18-wk study period, in three cases for failure to comply with the 2-wk visit requirement for reasons unrelated to insulin treatment. The remaining three cases, who withdrew because of hypoglycemia, had changed to human insulin from pork insulin (one case) or beef insulin (two cases) in the preceding 3 wk. Details of

TABLE 2
Clinical details of three patients withdrawing from the trial because of hypoglycemia

Patient		1	2	3
Age/sex		50/F	31/F	20/M
Insulin species		Pork	Beef	Beef
Total insulin dose (U/day)	0 wk	40	38	116
	6 wk	28	44	128
	12 wk	40	—	—
HbA_1 (%)	0 wk	13.1	12.6	10.2
	6 wk	12.6	8.9	11.0
	12 wk	11.7	—	—
Reported hypoglycemia	period 1	11	0	2
	period 2	6	—	—
Days on human insulin before withdrawal		20	6	7
Reason for withdrawal		Severe hypoglycemia. Hospital admission. Treated with pork insulin.	Hypoglycemia. Revived with oral glucose. Patient requested withdrawal.	Irritability and frequent hypoglycemia. Patient requested withdrawal.

these patients are shown in Table 2. The following analysis relates to the 88 subjects who completed the study.

Home blood glucose samples. There was excellent compliance with this technique—86% of patients returning 90% or more of samples, and 35% returning all 70 samples requested of them over the 18-wk period.

Figure 2 shows the mean daytime glucose profiles of patients during animal insulin and human insulin treatment in beef insulin–treated (BIT) patients. Mean glucose concentration was significantly higher before the morning ($P < 0.05$) and evening ($P < 0.05$) injections while taking human insulin. For pork insulin–treated (PIT) patients, samples 1 and 2 (pre- and postbreakfast) were both significantly higher on human insulin ($P < 0.001$ and $P < 0.002$); however the small difference between the means of the pre-evening injection sample (No. 5) did not achieve statistical significance ($P > 0.05$). This trend was present when the profiles preceding the visits 2, 4, and 6 wk after change to human insulin were examined individually.

The mean of all glucose concentrations and the mean 'M' index for the 2-, 4-, and 6-wk visits in each period were significantly greater on human insulin in the PIT group, but not in the BIT group (see Table 3).

Daily insulin dose. As shown in Table 3, total daily insulin dose was not changed significantly in BIT patients during the 6-wk period studied, although PIT patients showed a mean increase of 1.6 U/day during the human insulin period ($P < 0.01$). When the visit 6 wk after the change to human insulin was assessed rather than the mean of all three visits, BIT patients had not changed dose significantly (beef = 62.8 U/day; human insulin = 62.2 U/day; NS), whereas PIT patients had increased dose by 2.3 U/day (pork = 50.7 U/day; human insulin = 53.0 U/day; $P < 0.02$).

Hypoglycemia. The number of reported hypoglycemic episodes was not significantly different between beef insulin and human insulin, or between pork insulin and human insulin. Eighteen beef insulin–treated patients reported more hypoglycemia in the human insulin period, while 15 reported more episodes during the beef period (NS; Wilcoxon matched pair signed rank test). For pork insulin–treated patients, only ten reported more hypoglycemia on human insulin, as compared with 21 who reported more hypoglycemia on pork insulin (NS).

Home blood glucose sample profiles were also analyzed for the frequency of glucose values below 2.0 mmol/L, and below 3.0 mmol/L. No differences between beef and human insulin or pork and human insulin groups were apparent.

Insulin antibodies. Changes in insulin-binding antibodies are shown in Figure 3. The only significant differences noted are in the PIT group that received human insulin in period 3. The largest fall in antibody binding in this group appears

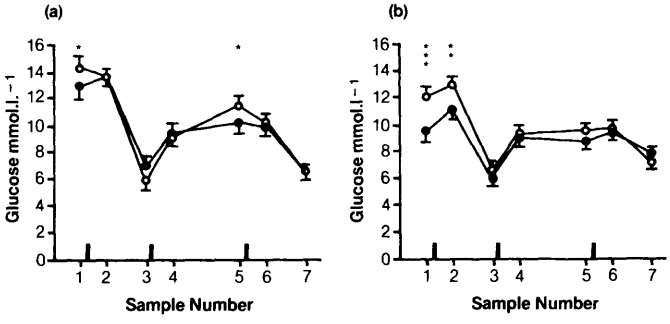

FIG. 2. *Daytime blood glucose profile (a) in BIT patients, and (b) in PIT patients.* ○ = *human insulin treatment;* ● = *animal insulin treatment. Vertical bars denote the 3 main meals.* * = $P < 0.05$, ** = $P < 0.002$, *** = $P < 0.001$.

TABLE 3
Mean values of average glucose levels, 'M' index, and total daily insulin dose for each insulin treatment

	Beef group			Pork group		
	Beef insulin	Human insulin Mean (± SEM)	Significance	Pork insulin	Human insulin Mean (± SEM)	Significance
Average glucose (mmol/L)	9.8 (0.46)	9.7 (0.42)	NS	9.0 (0.40)	9.7 (0.40)	$P < 0.05$
'M' index	80.5 (8.8)	85.0 (7.5)	NS	65.0 (5.9)	79.6 (6.3)	$P < 0.025$
Total insulin dose (U/day)	62.5 (3.4)	62.7 (3.3)	NS	50.9 (2.4)	52.5 (2.4)	$P < 0.01$

to take place in period 2, and thus this change is not likely to be due to human insulin treatment.

E. coli *antibodies.* Against upper limits of normal of 152 counts per minute (c.p.m.) and 194 c.p.m. for the BIT and PIT group respectively, six patients were recorded as having elevated levels of *E. coli* antibodies (three BIT; three PIT), on one or more occasions. (See Table 4.) In all cases these patients' initial values exceeded the mean for the group (beef mean = 100 c.p.m.; pork mean = 94 c.p.m.), and in only one patient (no. 6) did a rise in *E. coli* antibodies follow treatment with human insulin. In three patients (nos. 1, 2, and 5) there was a fall in antibody level to within the normal range during the human insulin treatment period. Patient no. 4 had elevated levels throughout, and the highest level was recorded during a pork treatment period.

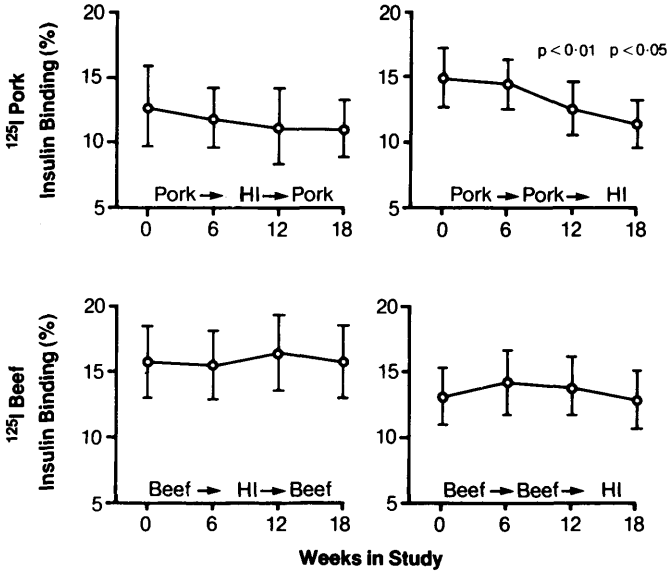

FIG. 3. Insulin antibody changes during the study in PIT (top graphs) and BIT patients (bottom graphs), expressed as the mean insulin binding (%) ± SEM. Statistical analysis compares one set of values with the values obtained 6 wk previously.

DISCUSSION

Clinical trials of any new insulin should assess the response in new (insulin-naive) patients, and in established insulin-treated diabetic subjects who are switching to the new insulin for the first time. While trials in the former group are still in progress, it is the latter group on whom we report our findings here.

It is particularly important in this group to undertake double-blind trials, in order to limit bias in either the patient or his physician towards either the 'new' or the 'old' insulin. There are both ethical and analytical advantages in using a crossover design, so long as the 'study' periods are preceded by a 'run-in' period in order to make allowance for the non-specific effects of increased surveillance on glycemic control. The choice of 6-wk treatment periods is sufficiently short to aid patient compliance with trial procedures, while allowing assessment of early changes in metabolic control and insulin requirement.

Results presented here show that in BIT patients, no significant changes in mean glucose, 'M' index, or the daily insulin dose take place, suggesting that transfer of patients from beef to human insulin should be accompanied by few problems in practice. PIT patients, however, appear to need a small increment in insulin dose when changed to human insulin, and this is accompanied by slightly less good glycemic

TABLE 4
E. coli antibodies (expressed as counts per minute) in the six patients with values above normal on one or more occasions during the trial. Upper limit of normal (defined as the mean + 2 standard deviations): beef patients = 152 c.p.m., pork patients = 194 c.p.m. Values in italic represent samples taken after the human insulin treatment period

	Beef-treated patients			Pork-treated patients		
Visit no.	1	2	3	4	5	6
1	126	142	128	313*	166	135
2	162*	152	91	330*	202*	129
3	126	187*	98	415*	*137*	*203**
4	*104*	*119*	154*	*334**	134	147

*Elevated values.

control. Very possibly treatment with human insulin for longer than the 6-wk undertaken here may be accompanied by a still slightly greater insulin increment in order to achieve the same degree of control as previously. This pork–human difference may also explain the slight reduction of hypoglycemia that was apparent when pork insulin–treated patients received human insulin.

The blood glucose profile changes shown in Figure 2 may account for some of these small differences. Treatment with human insulin appears to be accompanied by higher glucose levels before morning and evening injections. This effect may be the result of different pharmacokinetic properties of NPH human insulin specifically, or of human insulin in general. There is increasing evidence that human insulin is absorbed more rapidly from the subcutaneous injection site,[4–6] and it may be that its earlier entry to the circulation leads to its earlier removal, and hence a shorter duration of action.

Hypoglycemia is a difficult response to assess. Three patients withdrew from the trial because of symptomatic hypoglycemia which occurred after the change to human insulin, a trend not found in the majority of patients. Glucose profile results with human insulin do not suggest greater overall hypoglycemic potency than with beef or pork insulins, but more rapid absorption may favor early hypoglycemia as well as later hyperglycemia. This probable difference in time course of action should perhaps be borne in mind when changing patients to human insulin. It may be that some diabetics are more susceptible than others, although the three withdrawals did not appear to differ from the rest in any obvious way. Consequently, on transfer to human insulin patients should be carefully supervised. It may be necessary to adjust timing of injections, dosage, and proportions of short-acting and long-acting insulin formulations in order to minimize early hypoglycemia and obtain maximum benefit from human insulin.

We were not able to show a significant fall in insulin antibodies after changeover to human insulin, although we would not have expected to do this in view of the short period of human insulin treatment. Others have shown that significant changes in antibody levels are only seen 3 mo or more after a switch to a less immunogenic variety of insulin.[12–15]

E. coli antibodies do not rise in response to human insulin treatment—a finding in keeping with earlier work.[9] Only one patient (who had high initial values of *E. coli* antibodies)—patient 6 (Table 4)—showed a rise to a value just outside the normal range, following human insulin treatment, as compared with five patients who had values above normal on beef and pork insulin treatment.

In summary, human insulin appears to be a safe and effective alternative to currently available animal insulins, although in this trial we were unable to show any specific advantages in this group of patients of human insulin as compared to their previous treatment.

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Address reprint requests to Dr. A. J. Clark, Unit for Metabolic Medicine, Hunt's House, Guy's Hospital Medical School, London. SE1 9RT, United Kingdom.

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