Receptor Binding Studies and Clinical Effects of Human Insulin (recombinant DNA): Studies in Patients with Newly Diagnosed Type I Diabetes, Type II Diabetes, Insulin Resistance (Type A and Type B), Insulin Antibodies, Insulin Allergy, and "Brittle" Diabetes

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Thirty-eight insulin-dependent diabetic subjects were treated for periods ranging from 1 to 14 mo with human insulin (recombinant DNA) in order to investigate the clinical effects of human insulin in comparison with pork insulin. Human insulin was well tolerated and no side effects were detected. The following differences between human and pork insulin were observed: reduced blood glucose oscillations associated with a reduction in hypoglycemic symptoms in patients with "brittle" diabetes and type I diabetes, decreased concentrations of antibodies against pork insulin related to a reduction of insulin requirement of ~15%, increased specific receptor binding in patients with type I diabetes and insulin resistance: type A, possibility for treating patients with pork insulin allergy, and an increased biologic activity in a patient with polyclonal antireceptor antibodies. No difference was detected between pork and human insulin treatment in patients with type II diabetes and in a patient with insulin resistance: type B with monoclonal antireceptor antibodies. Human insulin was used safely and successfully in the treatment of diabetic patients. DIABETES CARE 5 (SUPPL. 2): 152–160, 1982.

The administration of homologous insulin is a natural target in the treatment of diabetes. Diem and Teuscher[1] found the same biologic activity for human insulin (recombinant DNA) and pork insulin in diabetic patients. Since then many in vitro and in vivo results have been reported for semisynthetic and human insulin (recombinant DNA).[2–10] In vivo, significant differences were found between human insulin and pork insulin of the same formula.[11–18] These studies have, however, been based on short-term treatment and mainly with regular human insulin, whereas long-term studies and experience with NPH human insulin have not been reported.

We now report a year's experience of human insulin treatment in patients with insulin resistance, insulin antibodies, insulin allergy, "brittle" diabetes, type I, type II, and newly diagnosed diabetes mellitus.

MATERIALS AND METHODS

Patients. The clinical data of the 38 insulin-dependent diabetic subjects participating in the study are shown in Table 1. They are grouped according to type of diabetes. No patient had any other chronic disease or was taking steroids during the study. Informed consent for human insulin treatment was given by all patients.

Determinations. Before using human insulin in patients, an intradermal skin test was performed. The 20-min, 24-h, and 36-h reaction to the intradermal skin test with 0.01 U/0.05 ml, 0.1 U/0.05 ml, and 1.0 U/0.05 ml of human insulin was compared with the negative reaction to 0.9% NaCl and positive reaction to histamine (1:10,000/0.05 ml).

Plasma glucose was determined with a Beckman Glucose Analyzer (GOD method) in our laboratory or the blood glucose was measured by the patient himself with the Dextrometer (Miles, USA) at the following times: fasting, 1 h after breakfast, before supper, and before the last intake.

Serum insulin was determined by radioimmunoassay (PhadebasInsulintest, Pharmacia Diagnostics, Uppsala, Sweden) after treatment with 25% polyethylene glycol to remove endogenous antibodies.[19] The inter- and intraassay coefficients of variation were 15% and less than 5%, respectively.

Radioimmunoassay was also used to determine serum C-peptide (Riamat, C- peptide assay, Byk-Mallinckrodt, Dietzenbach, FRG). Fasting glycosylated hemoglobin (HbA_{1c})

TABLE 1
Entry clinical data of patients

Patient no.	Age (yr)	Sex	Body weight (kg)	Body height (cm)	Duration of diabetes (yr)	Insulin regimen (U/day)	Fasting HbA_{1c} (%)	Fasting insulin (μU/ml)	Fasting C-peptide (ng/ml)	Clinical types of diabetes
1	19	M	54	166	—	—	14.3	4.5	0.56	Newly diagnosed type I
2	29	F	53	165	—	—	9.8	7.8	1.51	"
3	13	F	57	170	—	—	13.6	11.3	2.18	"
4	27	M	75	180	—	—	12.9	5.7	0.95	"
5	22	M	61	181	—	—	13.0	8.3	0.38	"
6	24	M	141	170	—	—	14.5	42.8	3.75	Newly diagnosed type II
7	56	F	65	164	—	—	14.7	6.5	3.53	"
8	68	M	78	179	—	—	14.1	13.7	2.58	"
9	23	F	68	176	15	50	16.1	7.6	0.05	Type I diabetes
10	38	M	78	182	12	64	11.8	7.0	0.10	"
11	25	M	74	179	9	30	9.8	14.0	0.05	"
12	20	M	60	176	3	52	10.8	2.2	0.01	"
13	21	F	68	168	8	40	14.8	6.0	0.02	"
14	41	F	50	158	17	32	13.8	7.0	0.03	"
15	31	M	70	181	6	55	8.7	5.2	0.02	"
16	26	M	65	179	14	65	10.6	6.0	0.02	"
17	53	M	71	176	18	42	10.8	7.3	0.08	"
18	72	F	64	158	5	85	9.6	36.4	3.91	Type II diabetes
19	63	F	70	155	6	70	12.3	31.6	2.96	"
20	49	M	74	175	5	48	10.1	21.6	5.80	"
21	61	F	92	164	8	90	10.7	19.7	0.92	"
22	67	F	76	151	6	125	12.4	32.6	2.14	"
23	45	F	64	166	2	38	8.1	4.1	1.92	Insulin allergy, immediate type
24	65	F	73	164	11	40	9.0	2.9	2.31	"
25	48	F	65	170	2	32	—	—	—	"
26	42	F	59	159	9	40	—	—	—	"
27	48	F	66	162	3	28	—	—	—	Insulin allergy, delayed type
28	24	F	57	165	2	24	9.7	4.7	1.42	"
29	74	F	60	165	7	55	16.4	8.9	1.62	"
30	39	F	34	134	17	130	9.8	204.9	1.58	Insulin resistance: Type A
31	68	M	50	167	4	200	10.4	111.3	6.03	Insulin resistance: Type B
32	62	F	77	162	6	150	—	56.7	1.81	"
33	62	M	79	172	21	120	9.7	25.5	2.19	Insulin antibody
34	72	M	91	168	17	110	11.2	12.6	2.75	"
35	44	M	68	167	14	37	12.4	4.1	0.01	"Brittle" diabetes
36	56	M	70	174	12	48	10.7	3.1	0.12	"
37	67	M	71	172	40	40	7.7	7.8	0.18	"
38	41	M	69	171	2	32	10.9	1.1	0.01	Pancreatectomy

concentrations were estimated using prepacked microcolumns (Bio-Rad Laboratories, Richmond, California).

Insulin antibodies against pork and beef insulin were determined with the cellulose adsorption method, as described elsewhere.[20]

Insulin binding to erythrocytes and monocytes. Insulin binding to monocytes was determined as described previously.[21] The blood for the binding studies was drawn in the morning after 12-h fasting and before insulin injection. At the same time heparinized blood samples (8 ml) were drawn for the determination of insulin receptor binding to erythrocytes. The cells were diluted 1:1 with PBS, pH 7.4, layered onto Ficoll-Hypaque gradients, and centrifuged at 350 × *g* for 25 min. The mononuclear cells and granulocytes were aspirated and the sedimented erythrocytes-reticulocytes were washed in Krebs-Ringer phosphate buffer, pH 7.4, 0.25% BSA, and resuspended in a concentration of 1.2×10^9 cells. The reticulocyte concentration in the final cell preparation was 12 ± 3 mono-^{125}I-(Tyr A14)-human insulin (Lot J84-02N-166 to Lot J84-02N-276 and Lot J84-2H1-14 to Lot J84-2H1-90, Eli Lilly and Company, Indianapolis, Indiana) was used for all binding studies at a concentration between 50 and 100 pM (spec. act. ~320 μCi/μg). Tracer was given to all samples, and unlabeled human insulin (Lot 615-2H2-86, Lot 615-70N-174-10, Lilly) was added in concentrations increasing from 0–4000 ng/ml. The cells were incubated (40 min,

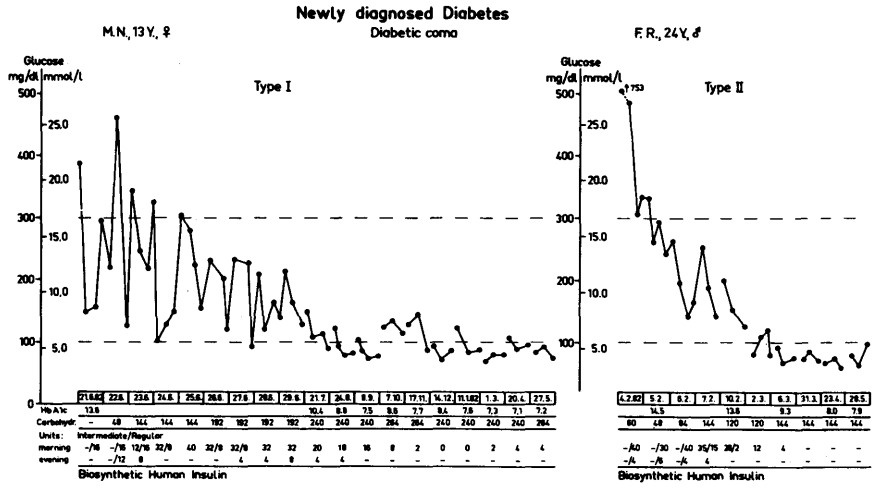

FIG. 1. Plasma glucose (mg/dl), HbA_{1c} (%), carbohydrate intake (g/day), and insulin requirement (U/day) during diabetic coma and human insulin treatment in a patient (no. 3) with newly diagnosed type I diabetes and a patient (no. 6) with newly diagnosed type II diabetes.

37°C) and slowly agitated in a total volume of 1.3 ml. The separation of free and bound insulin has been described previously.[21]

Nonspecific binding was defined in the presence of 4000 ng/ml unlabeled insulin and subtracted from each point. Degradation was 1.1 ± 0.4% after the incubation (precipitation with 12% TCA).

Insulin binding curves were analyzed by a Scatchard plot,[22] modified for two types of affinity (K_1, K_2: high and low affinity constants) as well as concentrations (R_1, R_2: high and low affinity concentration). Results are expressed as mean ± SEM. Student's *t* and Welch test were used to test significance.

RESULTS

Aside from the patients with allergic reactions against insulin, no patients showed a positive reaction to the various human insulin skin test doses. There were also no allergic reactions during human insulin treatment.

Newly diagnosed type I and type II diabetic patients. Five newly diagnosed patients (22.0 ± 1.3 [SEM] yr; 60.0 ± 1.8 kg; 172.4 ± 1.5 cm; fasting HbA_{1c} 12.7 ± 0.4%; fasting insulin 7.5 ± .0.5 μU/ml; fasting C-peptide 1.1 ± 0.2 ng/ml) and three newly diagnosed type II diabetic patients (49.3 ± 5.6 yr; 94.7 ± 8.1 kg; 171.0 ± 1.5 cm; fasting HbA_{1c} 14.1 ± 0.1%; fasting, insulin 21.0 + 6.4 μU/ml; fasting C-peptide 3.3 ± 0.2 ng/ml) were treated with NPH or a combination of NPH and regular human insulin. Figure 1 shows the treatment of two diabetic nonketoacidotic coma patients. In diabetic patients the initial remission appeared to be reached earlier than has been our exerience with pork insulin.

Significant increases of the insulin receptor affinity and receptor concentration in both newly diagnosed type I and type II diabetics were seen (Figure 2 and Table 2).

Type I diabetes. Nine type I diabetics (30.9 ± 1.2 yr; 67.1 ± 0.9 kg; 175.0 ±0.8 cm; fasting HbA_{1c} 11.9 ± 0.3%; fasting insulin 6.9 ± 0.4 μU/ml; fasting C-peptide 0.04 ± 0.003 ng/ml), who had been treated previously with pork insulin, were switched to human insulin. No change

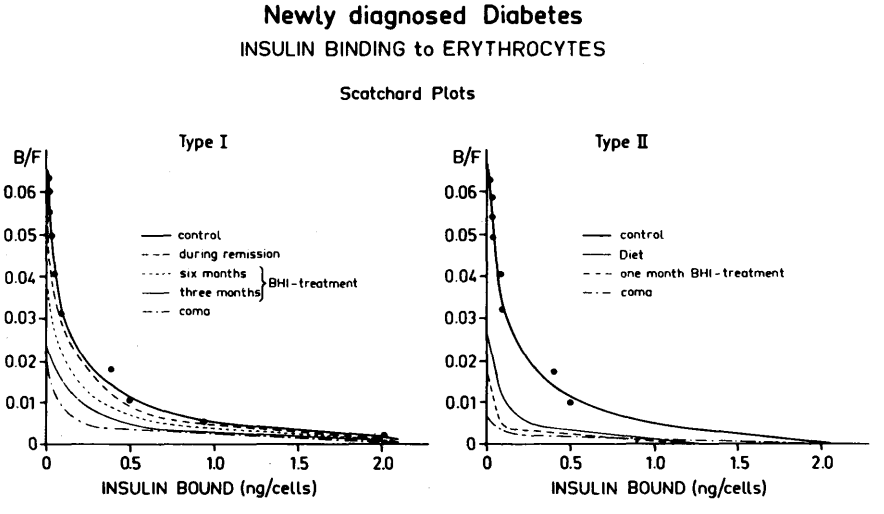

FIG. 2. Scatchard plots of insulin receptor binding to erythrocytes in five newly diagnosed type I (nos. 1–5) and three type II (nos. 6–8) diabetic patients during human insulin treatment. Mean values are plotted and the data are corrected for nonspecific binding.

TABLE 2
Insulin receptor binding data derived from individual binding curves from patients with type I (N = 5) and type II (N = 3) diabetes in diabetic coma and during human insulin treatment in comparison to healthy volunteers (mean ± SEM)

	K_1 (L/mol) $\times 10^{10}$	K_2 (L/mol) $\times 10^{8}$	R_1/cell $\times 10^{1}$	R_2/cell $\times 10^{2}$
Type I diabetes				
diabetic coma	0.44 ± 0.13	0.15 ± 0.09	0.10 ± 0.05	0.92 ± 0.47
3 mo	0.03 ± 0.01	0.09 ± 0.04	0.64 ± 0.33	1.82 ± 0.95
6 mo	1.04 ± 0.32	1.41 ± 0.50	0.31 ± 0.17	1.93 ± 0.84
remission	1.25 ± 0.52	0.97 ± 0.37	0.95 ± 0.51	0.94 ± 0.44
control	4.37 ± 1.89	1.03 ± 0.45	13.21 ± 7.13	3.94 ± 1.21
Type II diabetes				
diabetic coma	0.01 ± 0.01	0.09 ± 0.08	0.32 ± 0.18	0.84 ± 0.42
1 mo	0.36 ± 0.38	1.51 ± 0.40	0.57 ± 0.22	0.72 ± 0.41
during diet	0.95 ± 0.66	1.23 ± 0.73	0.41 ± 0.35	1.80 ± 1.47
control	5.48 ± 2.10	8.73 ± 3.10	9.50 ± 4.34	5.21 ± 2.74

was observed during the 12-mo period of the study in routine biochemical parameters, which were all in the normal range.

The mean insulin requirements were 47.8 ± 1.4 U/day before and were significantly reduced after the 9-mo human insulin treatment (40.5 ± 1.1 U/day; $P < 0.05$, paired comparison). The plasma glucose profiles (fasting, 1-hr post-breakfast plasma glucose) remained virtually unchanged throughout human insulin treatment. In contrast, fasting HbA_{1c} was significantly reduced (before human insulin treatment 11.6 ± 0.4% versus 10.6 ± 0.2% after 9 mo, $P < 0.05$). The circulating antibodies against pork insulin were also significantly ($P < 0.05$) reduced after human insulin treatment (Mean antibody binding was 10.6 ± 1.5 μU/ml before the switch and dropped to 5.0 ± 0.8 μU/ml after 9 mo).

Five of the nine type I diabetic patients had hypoglycemic episodes (3–4 times/wk) during treatment with pork insulin. In these patients the frequency and severity of hypoglycemia was markedly reduced with human insulin treatment. Sweating and palpitations were rare.

Type II diabetic patients. Five type II diabetic patients (62.4 ± 1.7 yr; 75.2 ± 2.1 kg; 160.6 ± 1.9 cm; fasting HbA_{1c} 11.0 ± 0.3%; fasting insulin 28.4 ± 1.5 μU/ml; fasting C-peptide 3.2 ± 0.4 ng/ml) were treated with pork insulin and switched over to human insulin. Again fasting plasma glucose remained in the same range (before: 215.0 ± 35.1 mg/dl versus 9 mo: 208.3 ± 34.2 mg/dl). Glycosylated hemoglobin was reduced from 11.5 ± 1.4% before to 10.3 ± 1.2% at 9 mo (n.s.). The insulin requirement was also unchanged (83.6 ± 5.7 U/day versus 81.7 ± 6.1 U/day).

The insulin receptor binding as represented by competitive

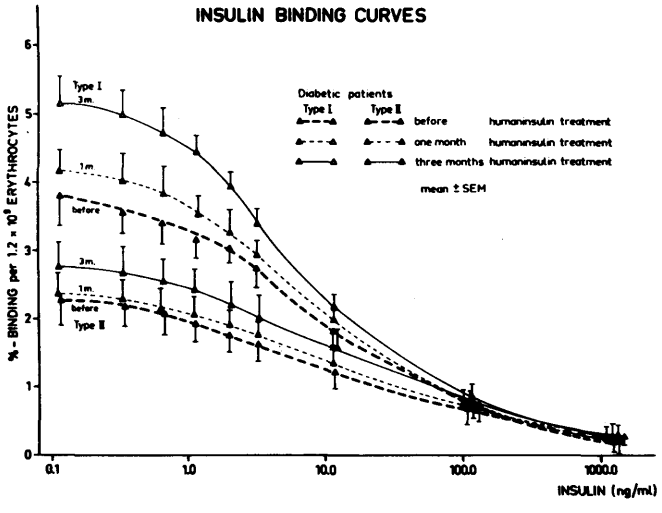

FIG. 3. Competitive curves of insulin binding to human erythrocytes for type I (nos. 9–17) and type II (nos. 18–22) diabetic patients before and during human insulin treatment. The mean values of specific binding (% of specific binding per 1.2 × 10⁹ erythrocytes) are plotted as a function of total insulin concentration.

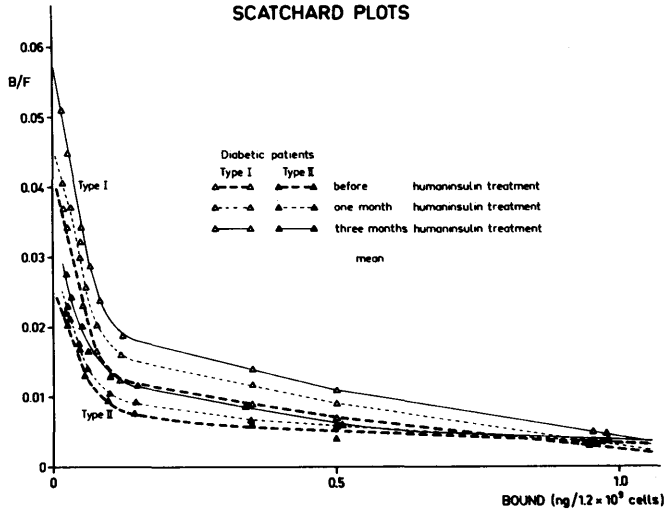

FIG. 4. Scatchard plots of insulin binding to human erythrocytes for type I and type II diabetic patients before and during human insulin treatment. The computer program analyzes two types of affinity together with the concentration of receptors and nonspecific binding. Affinities are calculated without nonspecific binding, which is shown in the plots.

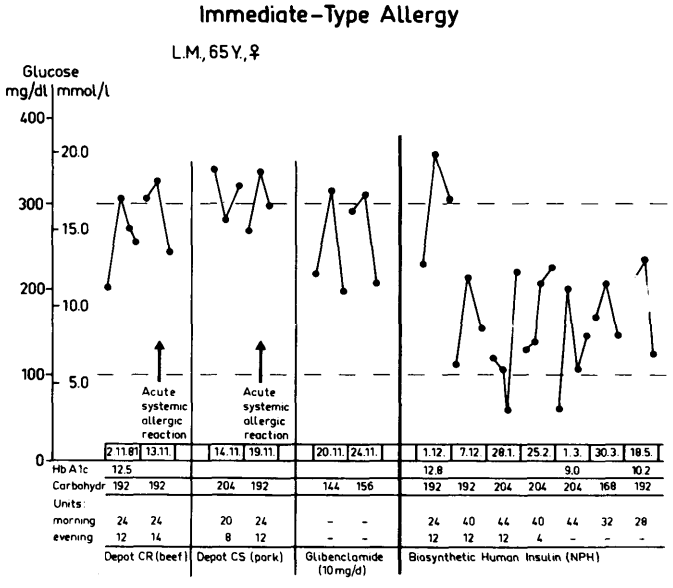

FIG. 5. Plasma glucose (mg/dl), HbA_{1c} (%), carbohydrate intake (g/day), and insulin requirement (U/day) in a patient (no. 24) with immediate type allergy during heterologous and human insulin treatment. For 10 days the patient was treated with glibenclamide (10 mg/day).

curves (Figure 3) and Scatchard plots (Figure 4) increased significantly in type I diabetic subjects (before: K_1 1.7 ± 0.5 × 10^{10} L/mol to K_1' 5.1 ± 0.6 × 10^{10} L/mol after 9-mo human insulin treatment. Because of the hyperinsulinemia the insulin binding is significantly lower in type II diabetes during both pork and human insulin treatment. In contrast to patients with type I, the type II diabetic patients showed no change in insulin receptor affinity and receptor concentration after switching to human insulin.

Insulin Allergy. Four female patients (nos. 23–26) with immediate type I allergy (urticaria) against purified beef and pork insulin were treated after an intradermal skin test with human insulin. Three of the diabetic patients showed a local allergic skin reaction 10–20 min following the insulin injection with beef (CR, Hoechst), pork (Actrapid, Novo), semisynthetic human insulin (Actrapid HM, Novo), and human insulin. Since, however, the allergic reaction to human insulin was weaker, it was decided to treat the patients for 4 wk with human insulin. During this time no change in the local allergic reaction was observed. An acute systemic allergic reaction did not occur. The fourth patient (no. 24) (Figure 5) had an acute systemic allergic reaction against both beef and pork insulin. The intradermal skin test with human insulin was negative, and during treatment with human insulin, no allergic reaction occurred. Glucose profiles and HbA_{1c} improved during this time (Figure 5).

In contrast three patients (nos. 27–29) with delayed type allergy against beef and pork insulin preparations showed no allergic reaction against human insulin. Two of them showed, however, a delayed allergic reaction against semisynthetic human insulin.

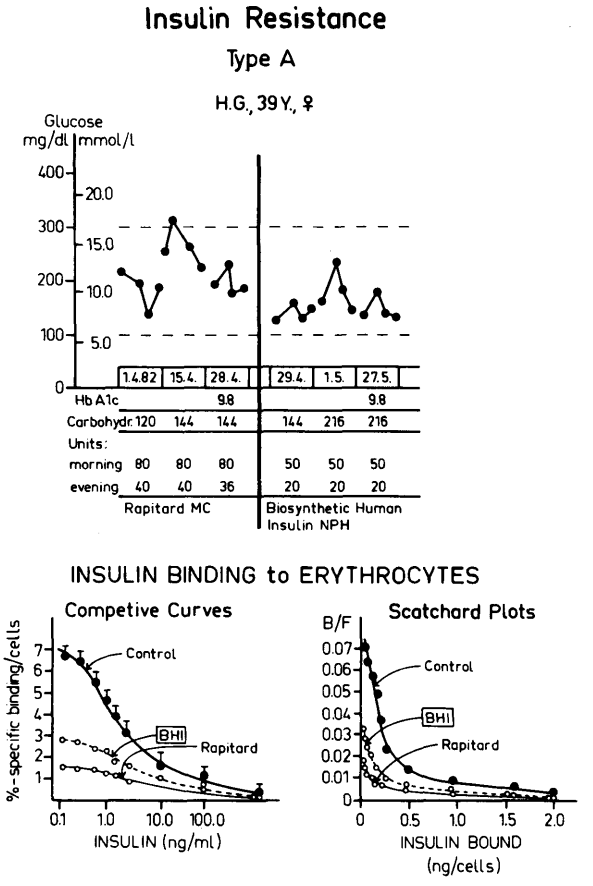

FIG. 6. Plasma glucose (md/dl), HbA_{1c} (%), carbohydrate intake (g/day), and insulin requirement (U/day) in a patient (no. 30) with insulin resistance type A before and during human insulin treatment (upper panel). Insulin binding to erythrocytes (competitive curves and Scatchard plots) before and during human insulin treatment (lower panel).

Insulin resistance: type A. A young women (no. 30) with insulin resistance: Type A, characterized by insulin resistance, hyperinsulinemia, hirsutism, and polycystic ovaries, showed after human insulin treatment a significantly reduced insulin requirement (Figure 6). The patient was being treated with estrogen for her acanthosis nigricans and was in complete remission.

There was a marked decrease in the binding of ^{125}I-insulin to the erythrocytes of the type A patient. This can be seen from the competitive curves over the entire range of insulin concentrations. The Scatchard plot shows a nearly unchanged receptor affinity and a markedly reduced receptor concentration in comparison to controls. Human insulin treatment caused an increase in the insulin receptor concentration and a reduction in insulin requirement.

Insulin resistance: type B. In two patients (nos. 31 and 32), both with antireceptor antibodies, which in one was monoclonal, with insulin resistance type B, an insulin tolerance test (0.3 U/kg BW i.v.) was performed. A significant decrease

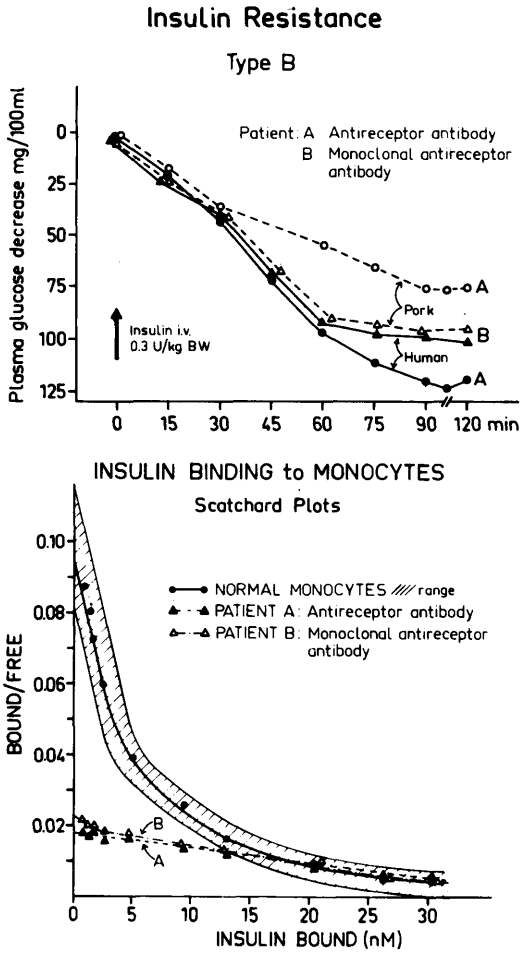

FIG. 7. Upper panel: Intravenous insulin tolerance test (0.3 U/kg body wt.) on two patients (nos. 31 and 32) with insulin resistance type B. Lower panel: Scatchard plots on insulin binding to monocytes in the same patients in comparison to monocytes from healthy volunteers.

of the plasma glucose could be observed in patient A after i.v. regular human insulin (Figure 7).

Both patients showed severe defects in insulin receptor binding (Figure 7, lower part) of human insulin to monocytes. The sera of the two patients inhibited the receptor binding to normal monocytes after 1-h preincubation.

Insulin antibody. Two patients (nos. 33 and 34) had insulin resistance caused by high insulin antibody titers (210 μl and 160 μl). After treatment (9 mo) with human insulin, glycosylated hemoglobin (HbA_{1c}: no. 33, 9.7–8.1%, No. 34, 11.2 to 9.1%) and insulin requirement (in one patient) were markedly reduced (No. 34, 110 U/day versus 76 U/day). This improvement was associated with a significant decrease of anti-insulin antibody concentration against pork insulin (no. 33: before, 210 μl; after 9-mo human insulin treatment, 420 μl; no. 36: 140 μl versus 365 μl). Figure 8 shows, in a patient (no. 34) with insulin resistance caused by a high antibody titer against pork insulin, improved blood glucose levels and

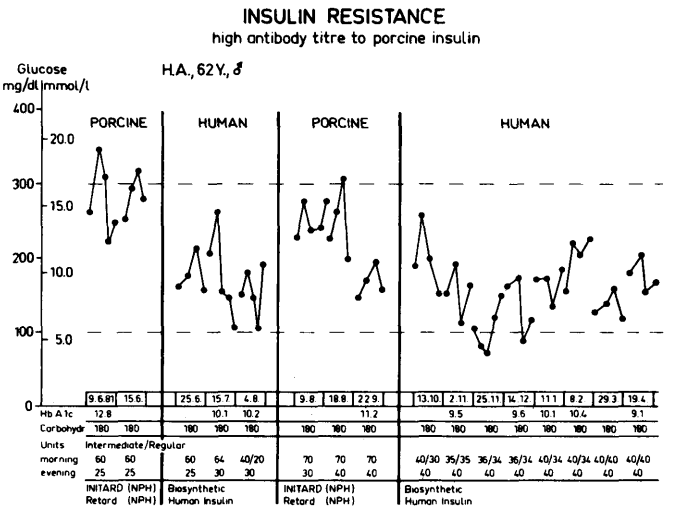

FIG. 8. Plasma glucose (mg/dl), HbA_{1c} (%), carbohydrate intake (g/day), and insulin requirement (U/day) in a patient (no. 34) with insulin resistance caused by high antibody titer to pork insulin during human insulin and pork insulin treatment.

a reduced glycosylated hemoglobin during human insulin treatment in comparison with pork insulin.

"Brittle" diabetes. Three patients (nos. 35–37) with brittle diabetes (non-insulin-secretors) were switched to human insulin. Figures 9 and 10 show that after human insulin treatment the plasma glucose oscillations were markedly reduced (patient no. 35: Figure 9; no. 36: Figure 10). Insulin re-

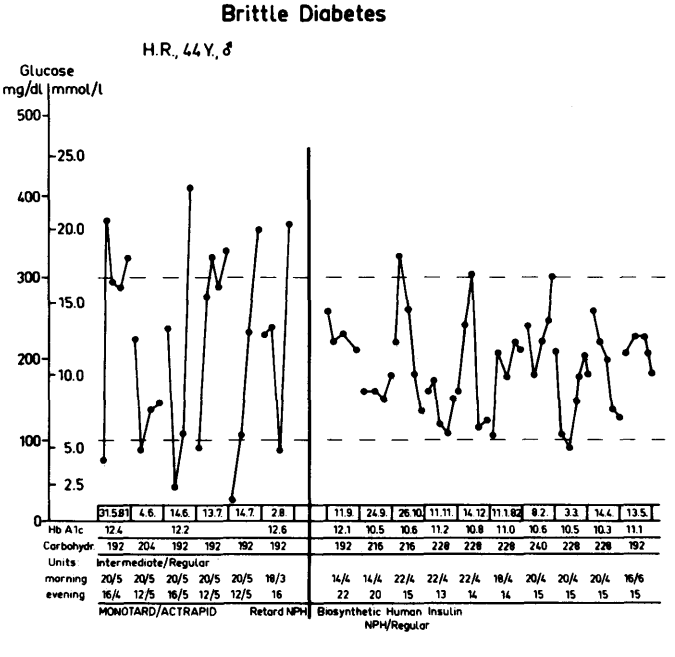

FIG. 9. Plasma glucose (mg/dl), HbA_{1c} (%), carbohydrate intake (g/day), insulin requirement (U/day) in a patient (no. 35) with "brittle" diabetes during pork and 9 mo of human insulin treatment.

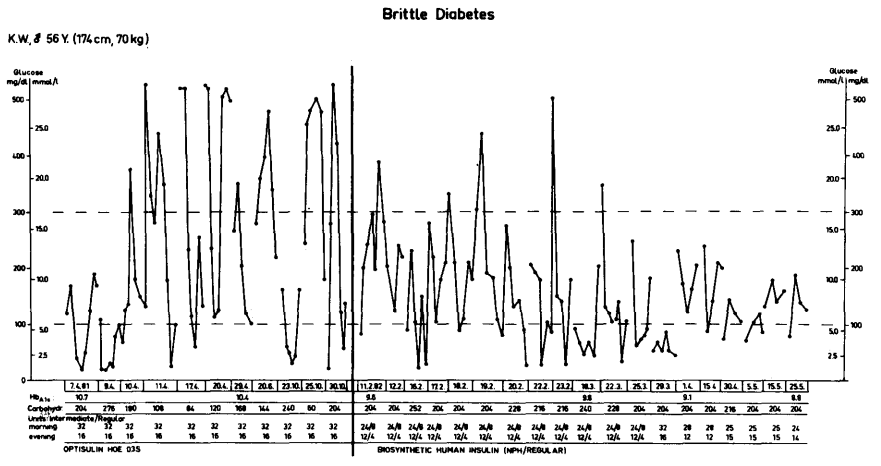

FIG. 10. Plasma glucose (mg/dl), HbA_{1c} (%), carbohydrate intake (g/day), insulin requirement (U/day) in a patient (no. 36) with "brittle" diabetes during pork and human insulin treatment.

quirement and glycosylated hemoglobin were reduced as well. During human insulin treatment the insulin receptor affinity was significantly increased in the three brittle diabetic patients (Figure 11). Episodes of hypoglycemia were less frequent and less severe in these patients during human insulin treatment, and they reported a remarkable improvement in their feeling of well-being. Similar results were observed in a patient (no. 38) with pancreatectomy.

DISCUSSION

Thirty-eight insulin-dependent diabetic patients with different types of diabetes were treated with human insulin. No patient dropped out during the 14-mo treatment period since the insulin, except in the 3 patients with acute allergic reactions, was well tolerated and no side effects were observed. In earlier studies no differences were found for the receptor binding[3,4,8,9] and the biologic activity[7,10] of human insulin in comparison to pork insulin. Other investigations have reported a quicker resorption after s.c. injection,[11-13] greater biologic activity at low doses,[14,15] diminished response of counterregulatory hormones,[17] less inhibition of endogenous insulin secretion,[23] and higher glucose requirement during clamp studies.[16-18]

In this long-term study on diabetic patients, differences were found between human insulin and pork insulin which seem to verify earlier findings of differences.

For one group of patients, however, there seem to be no differences between human and pork treatment; the four patients with type II diabetes showed no metabolic changes and no alterations in insulin receptor binding. This might be explained by their high basal endogenous insulin concentrations, which are associated with a reduction in insulin binding. Their previous treatment with pork insulin had been well tolerated and successful.

It was in problem diabetic patients that the greatest differences between pork and human insulin were observed. Extreme glucose oscillation observed in "brittle" patients under pork insulin treatment were reduced by human insulin administration. This difference was also observed in patients with type I diabetes and in the patient with pancreatectomy. The reason for this improvement might be the earlier re-

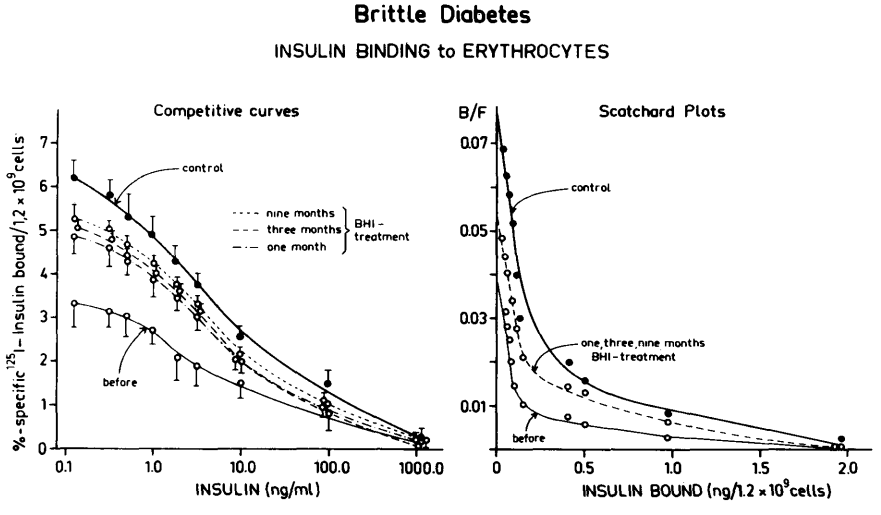

FIG. 11. Insulin binding to erythrocytes (competitive curves and Scatchard plots) before and during human insulin treatment in patients with "brittle" diabetes.

ported reduced output of counterregulatory hormones, especially epinephrine.[15] In these patients the hypoglycemic symptoms were greatly reduced. Their state of well-being was reported to be much improved.

The improvement in the hypoglycemic symptoms observed in patients with newly diagnosed diabetes was also noteworthy. In addition these patients showed a more rapid remission than is usually observed with pork insulin. This might be explained by a reduced inhibition of the beta-cell insulin secretion as reported previously in healthy subjects.[23] It was relatively easy with human insulin to achieve a euglycemic state in these patients. This good metabolic control appears to be related to a preservation of endogenous insulin secretion.[24] The effect of insulin antibodies against human insulin on endogenous insulin secretion will be reported later.

On the basis of the earlier short-term studies no conclusion concerning the insulin-neutralizing antibodies can be drawn. The insulin antibody binding in patients with type I diabetes and with high insulin antibody titer against pork insulin decreased under human insulin treatment, which led in some patients to a significant reduction in the insulin requirement.

In 1 patient with acute allergic reaction to pork insulin, human insulin treatment was successfully used. In three other patients who also had acute insulin allergy, no improvement was found. Human insulin had no antigenic effect in patients with delayed-type allergy to pork insulin.

Under long-term therapy with human insulin the insulin receptor sensitivity seems to increase, as was seen in patients with type I diabetes. This increased receptor binding was also observed in a patient with insulin resistance: type A, although a primary receptor defect seems likely.

Increased biologic activity was observed in a patient with insulin resistance: type B characterized by polyclonal antireceptor antibodies; this was not seen in a patient with monoclonal antireceptor antibodies. This might indicate that the receptor binding of human and pork insulin differs in the presence of receptor antibodies.

This report is dedicated to Prof. Dr. Georg Wilhelm Löhr on the occasion of his sixtieth birthday.

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