Physiological responses during hypoglycaemia induced by regular human insulin or a novel human analogue, insulin glargine

S. Dagogo-Jack,[1] H. Askari,[2] B. Morrill,[3] L. L. Lehner,[2] B. Kim[3] and X. Sha[3]

[1] Department of Medicine (Endocrinology), University of Mississippi Medical Center, Jackson, MS, USA
[2] Division of Endocrinology, Diabetes and Metabolism, Washington University School of Medicine, St Louis, MO, USA
[3] Hoechst Marion Roussel, Inc., Bridgewater, NJ, USA

Aim: Glargine, a product of recombinant technology, has different structural and physico-chemical properties compared with native human insulin. We determined whether such differences are associated with alterations in the responses to hypoglycaemia induced by glargine.
Methods: Nineteen adults (six healthy and 13 with type 1 diabetes) underwent a 5-h hyperinsulinaemic (2 mU/kg/min^{-1}) stepped hypoglycaemic clamps (hourly targets of 4.7, 4.2, 3.6, 3.1 and 2.5 mmol/l, respectively) on two occasions using intravenous infusion of regular human insulin or glargine, in random sequence. Hypoglycaemic symptoms, counter-regulatory hormones and glucose disposal rates were assessed at intervals throughout the clamps. A 1-week 'wash out' period was observed between studies.
Results: The peak total symptoms scores (mean ± s.e.m.) at nadir blood glucose (2.5 mmol/l) were 18.83 ± 2.68 (healthy) and 17.46 ± 3.62 (diabetic) during regular insulin, and 18.50 ± 3.20 (healthy) and 19.08 ± 3.83 (diabetic) during glargine infusion. The peak epinephrine levels during hypoglycaemia were 767.8 ± 140.4 pg/ml (regular insulin) and 608.8 ± 129.9 pg/ml (glargine) among healthy subjects, and 332.5 ± 54.8 pg/ml (regular insulin) and 321.8 ± 67.4 pg/ml (glargine) in diabetic patients. Diabetic patients had blunted glucagon responses during hypoglycaemia with either insulin. Both insulins also elicited similar rates of glucose disposal.
Conclusions: We conclude that insulin glargine and regular human insulin elicit comparable symptomatic and counter-regulatory hormonal responses during hypoglycaemia in healthy or diabetic subjects, and induce similar rates of glucose disposal. Since glargine is designed for subcutaneous (s.c.) use, it is possible (though unlikely) that our findings obtained using an intravenous protocol could differ from responses to hypoglycaemia induced by the s.c. route.

Keywords: hypoglycaemia, symptoms, counter-regulation, insulin analogue, insulin glargine
Received 18 November 1999; returned for revision 12 January 2000; revised version accepted 25 February 2000

Introduction

Insulin glargine (HOE 901) is a human insulin analogue obtained through recombinant DNA modification of native human insulin. The molecular substitutions shift the isoelectric point from pH 5.4 in native insulin to 7.0 in insulin glargine, and result in an insulin analogue that is absorbed more slowly from subcutaneous sites, compared with native insulin [1]. The objective of the present study was to compare the physiological symptoms and counter-regulatory hormones during hypoglycaemia induced by either regular human insulin or

Correspondence:
Samuel Dagogo-Jack, MD, Department of Medicine (Endocrinology), University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA
E-mail:
sdagogojack@medicine.umsmed.edu

insulin glargine in healthy subjects and in patients with type 1 diabetes mellitus.

Unanticipated reports of altered perception of hypoglycaemic symptoms appeared shortly after human insulin was introduced to replace animal insulin preparations for the treatment of diabetes [2–5]. The collective data indicate that human insulin, as compared with animal insulins, may be associated with reduced [2–5] or similar [6–8] counter-regulatory hormone responses, and reduced [9] or similar [10] intensity of symptoms during experimental hypoglycaemia in type 1 diabetes patients. Some workers have reported more robust counter-regulatory hormone responses (including glucagon, cortisol and growth hormone) during hypoglycaemia with human insulin than with animal insulin in nondiabetic volunteers [11,12].

Although human insulin is absorbed more rapidly from subcutaneous (s.c.) sites than animal insulins, and is less lipophilic (and thus less able to penetrate the central nervous system), no coherent mechanism has been advanced to explain the observed differences between human and animal insulins in the responses to hypoglycaemia. Nonetheless, because the molecular modifications of native human insulin that yielded insulin glargine are more extensive than the single amino acid difference between human and porcine insulin, we investigated the integrity of symptomatic and neuroendocrine responses to hypoglycaemia induced by the novel insulin glargine. Although glargine is designed for s.c. use, we have chosen to conduct the present studies using intravenous (i.v.) infusion (glucose clamp methodology), which provides a more controllable hypoglycaemic stimulus than s.c. insulin.

Research Design and Methods

Study Subjects

A total of six healthy subjects and 13 patients with type 1 diabetes, whose characteristics are summarized in table 1, completed the study. The healthy subjects were nonsmokers who had no significant abnormalities in their medical history, had normal findings on physical examination, and normal haemotological values, serum chemistry, and electrocardiogram. Female subjects had negative pregnancy tests and were using contraceptives during the period of study. None of the healthy subjects was taking any routine medications. The diabetes patients completed a screening questionnaire [13] on hypoglycaemic symptoms prior to enrolment. Patients whose responses suggested the presence of hypoglycaemia unawareness were excluded from participation in the study. Additional inclusion criteria were a history of type 1 diabetes for at least 1 year, C-peptide deficiency, haemoglobin A1c level between 7.5% and 9.5%, and absence of active diabetic complications or other concurrent illness.

Table 1 Characteristics of the study subjects

	Diabetic	Healthy
No.	13	6
Male : Female	4 : 9	3 : 3
Age (years)	29.3 ± 1.9	25.8 ± 2.2
Body mass index (kg/m^2)	26.9 ± 0.9	24.5 ± 1.8
HbA_{1c} (%)	8.6 ± 0.2	5.3 ± 0.3
Diabetes duration (years)	15.7 ± 1.9	N/A

Values are mean ± s.e.m.
N/A, not applicable

Study Design

The study was a single-dose, double-blind, randomized, two-way cross-over trial in six healthy subjects and 13 type 1 diabetes patients. The study consisted of four visits–screening visit (visit 1), treatment visits (visits 2 and 3) and follow-up visit (visit 4). After obtaining a written informed consent from the subjects, a screening visit was arranged during which a medical history, physical examination and baseline laboratory tests were performed. During visits 2 and 3, a specific intervention (stepped hypoglycaemic clamp) was performed using either insulin glargine or regular human insulin. Visit 2 was performed within 14 days of visit 1, and a minimum of 7 days elapsed between visits 2 and 3. During the follow-up visit (visit 4), which was conducted within 7 days after visit 3, study subjects underwent a repeat physical examination and blood sampling for routine haematological and chemical analyses.

In-Vivo Methods

The healthy subjects were admitted to the Washington University School of Medicine General Clinical Research Center (GCRC) at 7 a.m. on the day of study, after an overnight fast. An indwelling i.v. line was placed in an antecubital vein for infusion of insulin and dextrose. Another line was inserted in a contralateral hand vein and the hand was warmed in a thermoregulated (60 °C) chamber to enable arterialized blood sampling. Two weeks prior to admission, the diabetic patients performed self-monitoring of blood glucose levels four times daily (before each meal and at bedtime). The home blood glucose results were obtained daily from each

patient and adjustments were made to insulin regimens as necessary to avoid hypoglycaemia. Treatment with ultralente insulin was discontinued 2 days before admission and intermediate-acting insulin was stopped 1 day before entry; each patient was then treated with a conservative regimen of 3–4 injections of regular insulin designed to avoid hypoglycaemia. The diabetes patients were admitted to the GCRC at 5 p.m. on the eve of the study; their regular insulin regimens were continued; and capillary blood glucose levels were monitored every 2 h, and maintained between 100 mg/dl and 200 mg/dl by adjusting insulin doses and caloric intake. The patients ate an evening meal consistent with their usual diet plan, and had nothing to eat or drink from midnight. At 5 a.m. on the day of study, indwelling i.v. lines were placed in an antecubital vein for infusion of insulin and dextrose and in a contralateral hand vein for arterialized blood sampling. Between 5 a.m. and 7 a.m., i.v. regular insulin was infused at a variable rate to achieve a plasma glucose level of ≈100 mg/dl by 7 a.m. in all the diabetic patients. Baseline blood specimens were obtained from study subjects at 7.30 a.m. (−30 min), 7.45 (−15 min), and again at 8 a.m. (0 min), just before commencement of the hypoglycaemic clamp.

Hypoglycaemic Clamp

We used the hyperinsulinaemic, stepped hypoglycaemic clamp technique with glycaemic targets of 85, 75, 65, 55, and 45 mg/dl (4.7, 4.2, 3.6, 3.1 and 2.5 mmol/l, respectively), 1 h at each step [14,15]. A continuous infusion (2 mU/kg/min^{-1}) of either regular human insulin (Eli Lilly, Indianapolis, IN, USA) or insulin glargine (Hoechst Marion Roussel, Inc. Kansas City, MO, USA) was administered, in a random, double-blind fashion, and dextrose (20%) was infused at a variable rate to maintain blood glucose targets. The subjects' electrocardiogram and heart rate were monitored continuously and blood pressure measured every 20 min during the clamp. Total, neurogenic, and neuroglycopaenic symptom scores were obtained every 30 min, using a visual analogue scale [15,16]. Six neurogenic symptoms (sweaty, heart pounding, shaky, hungry, nervous, and tingling) and six neuroglycopaenic symptoms (difficulty thinking, tired, dizzy, faint, weak, and blurred vision) were scored by the subjects on a scale of 0 (none at all) to 6 (severe), and the combined scores constituted the total symptom score.

Plasma glucose was measured by the bedside every 10 min, and blood specimens were obtained every 30 min for measurement of free insulin, glucagon, C-peptide, pancreatic polypeptide, epinephrine, norepinephrine, nonesterified fatty acids (NEFA), lactate, alanine, β-hydroxybutyrate, growth hormone and cortisol levels. At the end of 1 h at the 45 mg/dl (2.5 mmol/l) step, the clamp procedure was terminated by stopping the insulin infusion, and dextrose (10%) was infused to restore euglycaemia. Before discharge from the GCRC, a return appointment for a repeat clamp study using the alternative insulin was scheduled, after a 1-week 'washout' period.

Analytical Methods

Plasma-free insulin and C-peptide levels were measured with in-house radioimmunoassays (lower limit of quantification: plasma free insulin 5.0 μU/ml; C-peptide 0.15 ng/ml) at Hoechst Marion Roussel, Inc. laboratories. The specimens were stored at −20 °C prior to shipment to the Hoechst Marion Roussel, Inc. laboratories, where they were thawed, treated with polyethylene glycol, and analysed [17]. Immunoreactive insulin was measured using an antibody to regular insulin with 56% cross-reactivity to insulin glargine and its active metabolites. Glucagon [18], cortisol [19] and growth hormone [20] were measured by specific radioimmunoassays; and epinephrine and norepinephrine were measured with a single isotope (radioenzymatic) method [21]. Plasma glucose was measured with a glucose oxidase method (Glucose Analyser 2, Beckman Instruments, Fullerton, CA, USA), NEFA with a colourimetric method [22], and β-hydroxybutyrate [23], lactate [24] and alanine [25] with microfluorimetric methods.

Statistical Analysis

Results are expressed as mean ± s.e.m.. Symptomatic and biochemical measurements during the two randomized treatments were analysed using anova with terms for sequence, subject, period, and treatment. Baseline differences between defined groups were analysed using unpaired *t*-tests. The effect of hypoglycaemia was assessed by comparing responses at baseline (time 0 min) with those obtained during nadir blood glucose (time 300 min). Data from the healthy subjects were analysed separately from data from patients with type 1 diabetes.

Results

Plasma Glucose, Insulin, and C-Peptide Concentrations

The plasma glucose concentrations measured at 10-min intervals during the hypoglycaemic clamps are shown in

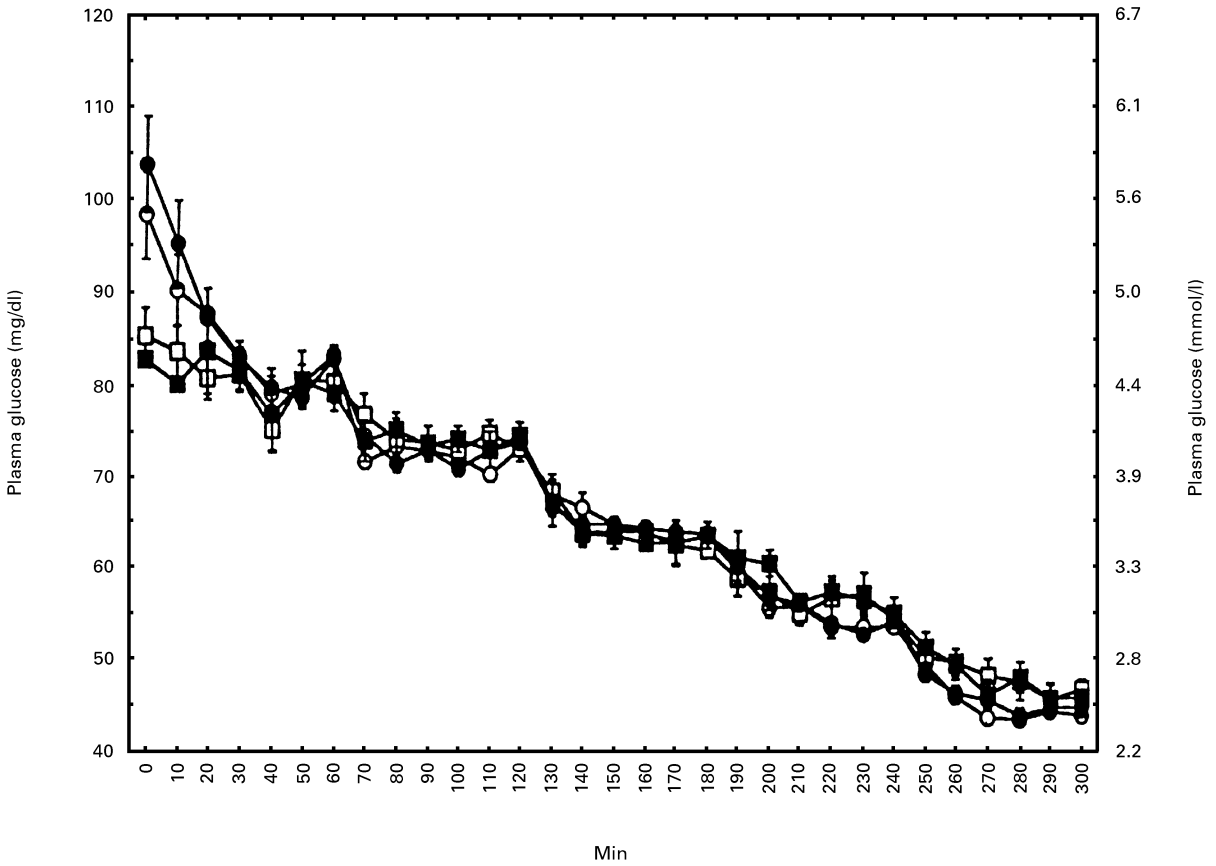

Fig 1. Plasma glucose levels in healthy subjects (□, ■) and type 1 diabetes patients (○, ●) during stepped hypoglycaemic clamps using either glargine (□, ○) or regular human insulin (■, ●).

Table 2 Heart rate, systolic and diastolic blood pressures during stepped hypoglycaemic clamp using regular human insulin or insulin glargine in healthy subjects

Study time (min)	**0**	**60**	**120**	**180**	**240**	**300**
BG target (mg/dl)	**100**	**85**	**75**	**65**	**55**	**45**
Heart rate (beats/min)						
Regular	68.50 ± 5.82	73.83 ± 7.40	70.67 ± 6.27	77.50 ± 8.05	73.33 ± 6.61	78.50 ± 4.42
Glargine	65.83 ± 5.34	67.50 ± 5.35	73.33 ± 5.56	72.00 ± 6.70	80.00 ± 4.42	77.17 ± 4.25
Systolic BP (mmHg)						
Regular	114.0 ± 4.51	14.0 ± 4.2	112.7 ± 4.3	112.8 ± 6.0	115.0 ± 4.8	119.2 ± 4.5
Glargine	114.5 ± 3.7	113.8 ± 3.5	116.5 ± 4.1	113.5 ± 4.8	117.3 ± 6.7	121.5 ± 8.1
Diastolic BP (mmHg)						
Regular	64.3 ± 3.5	60.2 ± 4.7	59.3 ± 4.4	61.5 ± 2.9	54.3 ± 3.8	57.7 ± 3.3
Glargine	70.3 ± 4.0	64.2 ± 2.6	62.8 ± 3.9	60.5 ± 2.5	56.7 ± 4.1	54.8 ± 3.0

BG = blood glucose; BP = blood pressure.

figure 1. Baseline mean plasma glucose levels were higher in diabetes patients than in healthy subjects ($p < 0.001$) but were not significantly different in either group on the two clamp study occasions (figure 1). The hourly glycaemic targets attained in each of the study groups were similar. Steady-state plasma insulin levels were reached within 60 min of initiation of the clamp procedure on both occasions in all subject groups. The peak plasma concentration (C_{max}) of immunoreactive-free insulin was 145.83 ± 19.33 μU/ml for regular human insulin and 98.40 ± 12.56 μU/ml) for insulin glargine in healthy subjects and 136.59 ± 10.76 μU/ml for regular human insulin

Table 3 Heart rate, systolic and diastolic blood pressures during stepped hypoglycaemic clamp using regular human insulin or insulin glargine in diabetes patients

Study time (min)	**0**	**60**	**120**	**180**	**240**	**300**
BG target (mg/dl)	**100**	**85**	**75**	**65**	**55**	**45**
Heart rate (beats/min)						
Regular	74.08 ± 2.74	75.62 ± 3.30	78.00 ± 3.40	79.46 ± 3.97	79.85 ± 4.10	79.00 ± 3.63
Glargine	74.23 ± 2.99	80.46 ± 3.84	77.62 ± 3.45	81.31 ± 3.67	78.54 ± 3.17	85.69 ± 3.01
Systolic BP (mmHg)						
Regular	116.2 ± 2.5	117.5 ± 3.6	118.1 ± 2.4	117.1 ± 2.9	119.2 ± 3.4	122.5 ± 2.5
Glargine	112.5 ± 2.6	116.3 ± 3.4	116.4 ± 3.0	114.2 ± 3.1	114.2 ± 2.4	119.4 ± 2.8
Diastolic BP (mmHg)						
Regular	65.7 ± 1.5	63.5 ± 1.6	63.5 ± 1.6	61.4 ± 1.4	59.3 ± 2.3	62.7 ± 2.4
Glargine	63.2 ± 1.8	63.9 ± 3.0	61.8 ± 2.4	60.6 ± 1.8	60.1 ± 1.9	59.8 ± 2.0

BG = blood glucose; BP = blood pressure.

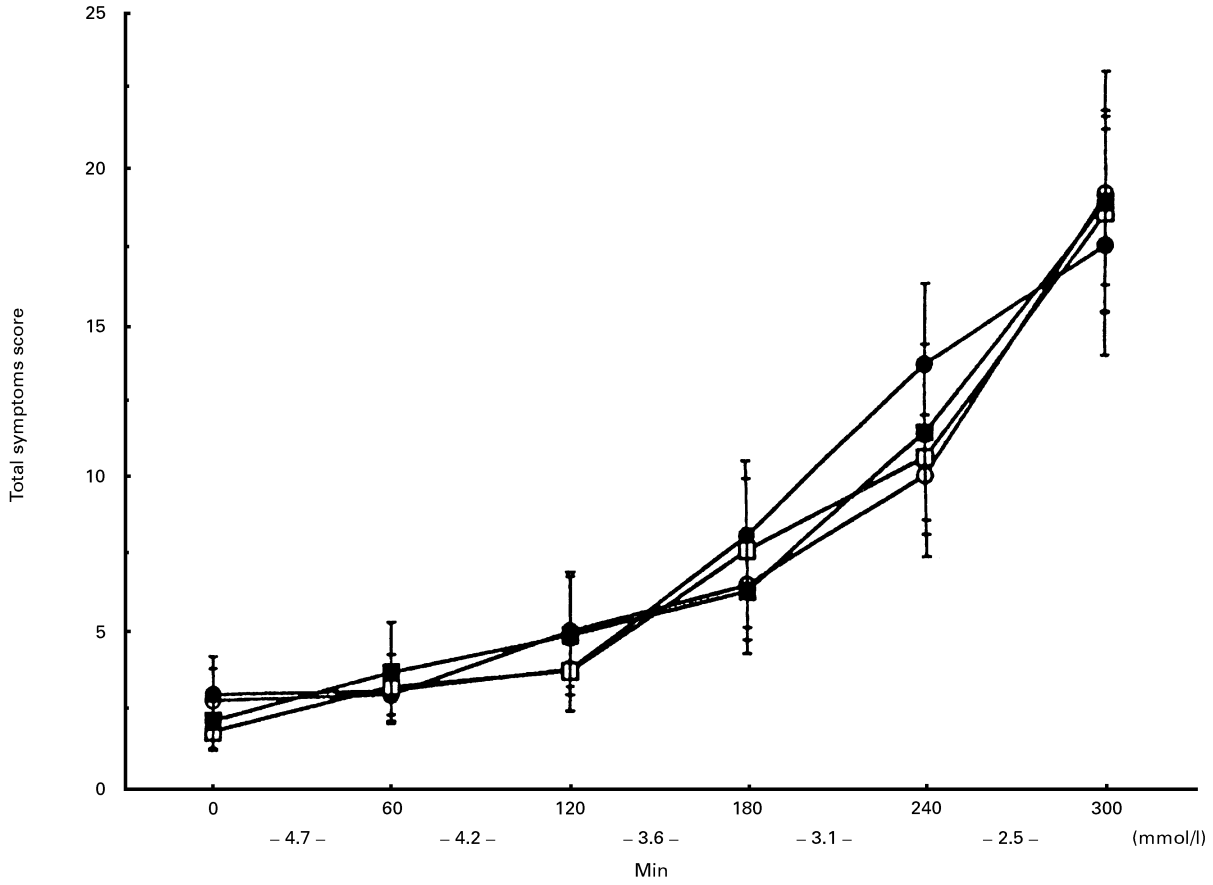

Fig 2. Total Symptoms Scores in healthy subjects (□, ■) and type 1 diabetes patients (○, ●) during stepped hypoglycaemic clamps using either glargine (□, ○) or regular human insulin (■, ●).

and 99.95 ± 10.47 μU/ml for insulin glargine in the diabetic patients. The area-under-the-curve for plasma insulin concentration (μU/ml × min) was 36515 ± 4498 for regular human insulin and 25040 ± 3058 for insulin glargine in healthy subjects and 34560 ± 2760 for regular human insulin and 23828 ± 2014 for insulin glargine in the diabetic patients. Both the C_{max} ($p < 0.02$) and the area-under-the-curve ($p < 0.05$) were higher during infusion of regular human insulin as compared with glargine. These differences were due to incomplete cross-reactivity (56%)

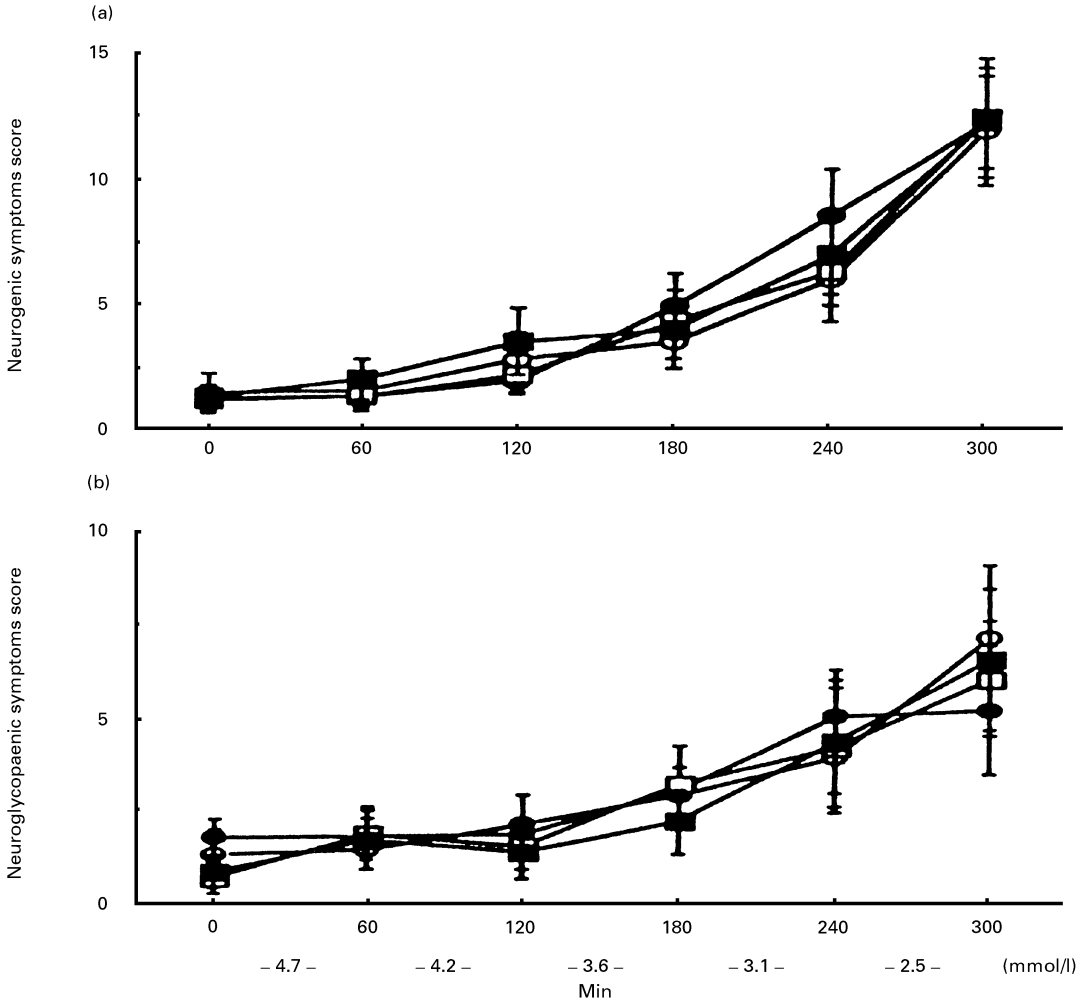

Fig 3. (a) Neurogenic and (b) neuroglycopaenic symptoms scores in healthy subjects (□, ■) and type 1 diabetes patients (○, ●) during stepped hypoglycaemic clamps using either glargine (□, ○) or regular human insulin (■, ●).

of insulin glargine in the human insulin radioimmunoassay at Hoechst Marion Roussel, Inc. laboratories. The time to attainment of peak plasma insulin concentrations, however, did not differ significantly by insulin species in healthy or diabetic subjects. The patients with type 1 diabetes were C-peptide deficient (< 0.1 ng/ml) prior to enrolment except for one patient in the 'honey moon' phase of diabetes who had detectable plasma C-peptide levels of 1.07 ng/ml and 1.28 ng/ml on the insulin glargine and regular human insulin study days, respectively. Among the healthy subjects, mean plasma C-peptide levels decreased from 2.3 ± 0.7 ng/ml at baseline to a nadir of 0.08 ± 0.05 ng/ml during infusion of regular human insulin and from 2.0 ± 0.6 ng/ml to 0.08 ± 0.05 ng/ml during infusion of insulin glargine.

Cardiovascular Responses

The mean heart rate and systolic and diastolic blood pressures at each glycaemic step in healthy subjects are shown in table 2 and those for diabetes patients are shown in table 3. There were similar heart rate and blood pressure changes in each of the two insulins, and none of the study subjects developed an abnormal electrocardiographic pattern during hypoglycaemic clamp using regular human insulin or insulin glargine.

Symptom Scores

The total (figure 2), neurogenic, and neuroglycopaenic symptoms (figure 3) increased significantly in healthy

Table 4 Plasma levels of counter-regulatory hormones and substrates during stepped hypoglycaemic clamp using regular human insulin or insulin glargine in healthy subjects

Study time (min)	**0**	**60**	**120**	**180**	**240**	**300**
BG target (mg/dl)	**100**	**85**	**75**	**65**	**55**	**45**
Glucagon (pg/ml)						
Regular	53.5 ± 6.3	45.9 ± 4.7	49.7 ± 4.8	56.3 ± 8.1	89.1 ± 9.4	105.6 ± 24.2
Glargine	55.6 ± 4.7	48.2 ± 4.9	51.5 ± 6.2	57.1 ± 7.9	87.5 ± 15.4	82.3 ± 11.1
Epinephrine (pg/ml)						
Regular	17.7 ± 3.2	18.2 ± 3.7	27.5 ± 3.0	105.0 ± 33.4	404.8 ± 129.8	767.8 ± 140.4
Glargine	22.2 ± 7.9	19.7 ± 4.8	41.2 ± 10.3	95.7 ± 39.7	357.2 ± 51.6	603.8 ± 129.9
Norepinephrine (pg/ml)						
Regular	124.3 ± 24.9	131.8 ± 16.0	143.3 ± 16.7	170.7 ± 22.7	214.3 ± 34.9	283.0 ± 37.2
Glargine	174.5 ± 42.6	163.5 ± 27.1	152.3 ± 21.8	196.7 ± 29.7	229.2 ± 25.2	332.3 ± 31.6
NEFA (μM/l)						
Regular	339.8 ± 91.7	105.8 ± 44.2	55.2 ± 7.0	53.0 ± 12.7	58.7 ± 14.6	91.8 ± 21.5
Glargine	299.3 ± 56.2	88.7 ± 31.5	51.9 ± 16.5	42.4 ± 16.7	48.8 ± 11.6	65.8 ± 22.9
Lactate (μM/l)						
Regular	901.2 ± 215.2	1260.8 ± 182.1	1149.0 ± 211.9	1126.2 ± 170.7	1242.8 ± 105.3	1896.7 ± 216.9
Glargine 1628.7 ± 633.1	1383.2 ± 239.6	952.0 ± 103.3	1036.0 ± 120.3	1284.3 ± 124.1	2043.0 ± 266.2	
Alanine (μM/l)						
Regular	470.0 ± 110.9	404.2 ± 29.8	386.8 ± 78.2	304.7 ± 31.3	275.5 ± 43.6	256.8 ± 48.1
Glargine	448.3 ± 83.0	456.7 ± 77.0	303.7 ± 71.7	282.8 ± 63.3	273.8 ± 43.2	312.7 ± 42.9
βOH-butyrate (μM/l)						
Regular	76.0 ± 22.3	76.0 ± 21.4	70.0 ± 20.2	63.3 ± 23.4	52.7 ± 10.2	80.8 ± 29.3
Glargine	119.2 ± 40.4	77.3 ± 14.3	86.5 ± 30.3	55.7 ± 9.0	49.0 ± 13.2	41.0 ± 7.8
Growth hormone (ng/ml)						
Regular	1.8 ± 0.6	0.8 ± 0.3	0.6 ± 0.1	1.3 ± 0.4	6.1 ± 1.7	19.4 ± 7.8
Glargine	2.5 ± 1.0	0.7 ± 0.2	0.5 ± 0.0	1.6 ± 1.0	5.1 ± 1.4	16.9 ± 7.6
Cortisol (μg/dl)						
Regular	14.6 ± 2.2	13.1 ± 2.1	11.2 ± 1.0	12.7 ± 2.4	18.0 ± 3.0	24.3 ± 3.4
Glargine	11.1 ± 1.3	9.1 ± 0.7	7.6 ± 1.1	10.7 ± 2.5	16.9 ± 0.8	23.0 ± 1.8

BG = blood glucose

subjects during hypoglycaemia induced by either regular human insulin ($p = 0.002$, 0.001 and 0.041, respectively) or insulin glargine ($p = 0.006$, 0.005 and 0.037, respectively). The total and neurogenic symptoms increased significantly in diabetic subjects during hypoglycaemia induced by either regular human insulin ($p = 0.002$ and < 0.001, respectively) or insulin glargine ($p = 0.002$ and < 0.001, respectively). The neuroglycopaenic symptoms increased significantly in diabetic subjects during hypoglycaemia induced by insulin glargine ($p = 0.012$) but not regular human insulin ($p = 0.086$). The total symptom scores observed during stepped hypoglycaemia were similar in healthy subjects and diabetes patients (figure 2). Evaluation of total symptom scores in each study group demonstrated comparable scores at each glycaemic step on the two study days using either insulin glargine or regular human insulin (figure 2). A similar concordance was observed between the two insulins with regard to neurogenic and neuroglycopaenic symptom scores (figure 3).

Counter-Regulatory Hormones and Substrates

The counter-regulatory hormone and substrate responses during hypoglycaemia are summarized in tables 4 and 5. Plasma epinephrine levels increased significantly in healthy subjects during hypoglycaemia induced by either regular human insulin ($p = 0.003$) or insulin glargine ($p = 0.008$) as well as in the diabetic patients during hypoglycaemia induced by either regular human insulin ($p < 0.001$) or insulin glargine ($p < 0.001$). The peak epinephrine response during hypoglycaemia was reduced by ≈50% in diabetic patients compared with healthy subjects (figure 4), as has been reported previously [15]. There were no significant differences between the two insulin species with regard to plasma epinephrine, norepinephrine, or cortisol levels during hypoglycaemia in healthy or diabetic subjects. Growth hormone responses were significantly increased during hypoglycaemia induced by insulin glargine and regular human insulin in

Table 5 Plasma levels of counter-regulatory hormones and substrates during stepped hypoglycaemic clamp using regular human insulin or insulin glargine in diabetes patients

Study time (min)	0	60	120	180	240	300
BG target (mg/dl)	100	85	75	65	55	45
Glucagon(pg/ml)						
Regular	47.6 ± 9.7	44.8 ± 12.0	44.2 ± 10.2	42.4 ± 9.1	42.3 ± 9.3	43.3 ± 9.1
Glargine	43.2 ± 10.7	41.9 ± 10.8	41.3 ± 10.8	40.7 ± 10.5	42.7 ± 10.6	42.9 ± 9.6
Epinephrine(pg/ml)						
Regular	20.9 ± 4.0	37.3 ± 6.0	47.0 ± 8.1	94.5 ± 24.3	177.2 ± 36.6	332.5 ± 54.8
Glargine	24.9 ± 4.2	38.1 ± 12.1	41.6 ± 6.4	85.8 ± 19.5	192.1 ± 52.9	321.8 ± 67.4
Norepinephrine(pg/ml)						
Regular	182.5 ± 18.3	200.9 ± 17.3	212.5 ± 18.6	236.0 ± 15.8	259.6 ± 16.7	286.3 ± 22.7
Glargine	181.4 ± 22.6	218.2 ± 24.8	202.9 ± 16.6	236.5 ± 32.4	274.9 ± 37.1	278.1 ± 20.1
NEFA (μM/l)						
Regular	257.2 ± 76.8	50.8 ± 9.4	42.5 ± 10.5	69.8 ± 24.2	111.5 ± 44.2	185.5 ± 74.6
Glargine	171.2 ± 51.3	65.2 ± 11.7	55.2 ± 7.7	77.5 ± 23.7	113.9 ± 40.4	184.9 ± 64.8
Lactate (μM/l)						
Regular	1193.1 ± 238.3	1362.6 ± 123.0	1311.9 ± 85.3	1295.7 ± 119.9	1180.6 ± 96.3	1439.0 ± 145.6
Glargine	1098.3 ± 112.1	1256.6 ± 138.6	1227.3 ± 108.5	1107.2 ± 108.3	1094.7 ± 115.2	1263.8 ± 137.4
Alanine (μM/l)						
Regular	369.3 ± 50.5	360.9 ± 46.5	322.5 ± 34.1	299.4 ± 29.4	280.4 ± 42.4	266.4 ± 33.9
Glargine	327.2 ± 29.1	291.9 ± 29.9	315.1 ± 39.0	278.0 ± 38.7	235.2 ± 37.1	216.0 ± 39.2
βOH-butyrate(μM/l)						
Regular	220.4 ± 52.7	84.3 ± 13.7	69.8 ± 10.2	75.8 ± 15.4	119.6 ± 33.0	169.8 ± 58.0
Glargine	231.9 ± 76.7	83.2 ± 17.8	60.3 ± 11.7	115.8 ± 36.4	90.0 ± 19.5	139.0 ± 46.4
Growth hormone(ng/ml)						
Regular	3.4 ± 1.8	3.8 ± 1.1	6.6 ± 2.7	7.6 ± 2.4	10.8 ± 2.4	20.6 ± 4.9
Glargine	4.6 ± 2.0	7.6 ± 2.3	9.1 ± 3.9	9.0 ± 3.6	8.6 ± 2.2	23.2 ± 4.6
Cortisol (μg/dl)						
Regular	16.3 ± 2.0	14.0 ± 1.6	12.3 ± 1.4	13.5 ± 1.6	16.7 ± 2.5	23.9 ± 3.6
Glargine	16.8 ± 2.2	15.5 ± 1.5	13.7 ± 1.6	12.5 ± 1.4	15.8 ± 2.2	23.0 ± 3.0

BG = blood glucose

diabetes patients, but no such increase was observed in the healthy subjects. Plasma glucagon secretion in response to hypoglycaemia was blunted in the type 1 diabetes patients in the present study (figure 4), consistent with the known α-cell defect in type 1 diabetes [26]. The healthy subjects had glucagon secretory responses that reached statistical significance during hypoglycaemia induced by insulin glargine ($p = 0.012$) but not regular human insulin ($p = 0.059$). The plasma levels of NEFA, lactate, alanine, and β-hydroxybutyrate did not show statistically significant differences between glargine and regular human insulin (tables 4 and 5).

Glucose Infusion Rates

Because lower levels of assayable plasma immunoreactive free insulin were observed during infusion of insulin glargine as compared with regular human insulin, we analysed insulin-stimulated glucose disposal (glucose infusion rate, GIR) during the respective clamp studies. The results (table 6) showed that the GIR was similar at each glycaemic step during steady-state infusion of insulin glargine or regular human insulin in healthy subjects and diabetic patients. Because GIR was increased to ≈200 ml/h at study time 300 min in all subjects, the final GIR data used for calculations was that obtained at study time 240 min.

Discussion

Insulin glargine is an insulin analogue obtained through modification of the human insulin molecule by recombinant DNA technology. The molecular modification has shifted the isoelectric point for insulin glargine to a more neutral pH range compared with human insulin [1,27]. This alteration in physicochemical property has resulted in an insulin species that is absorbed more slowly from s.c. sites with a prolonged duration of action, compared with human insulin. In addition, there may be some differences between insulin glargine and human insulin with regard to their interaction with

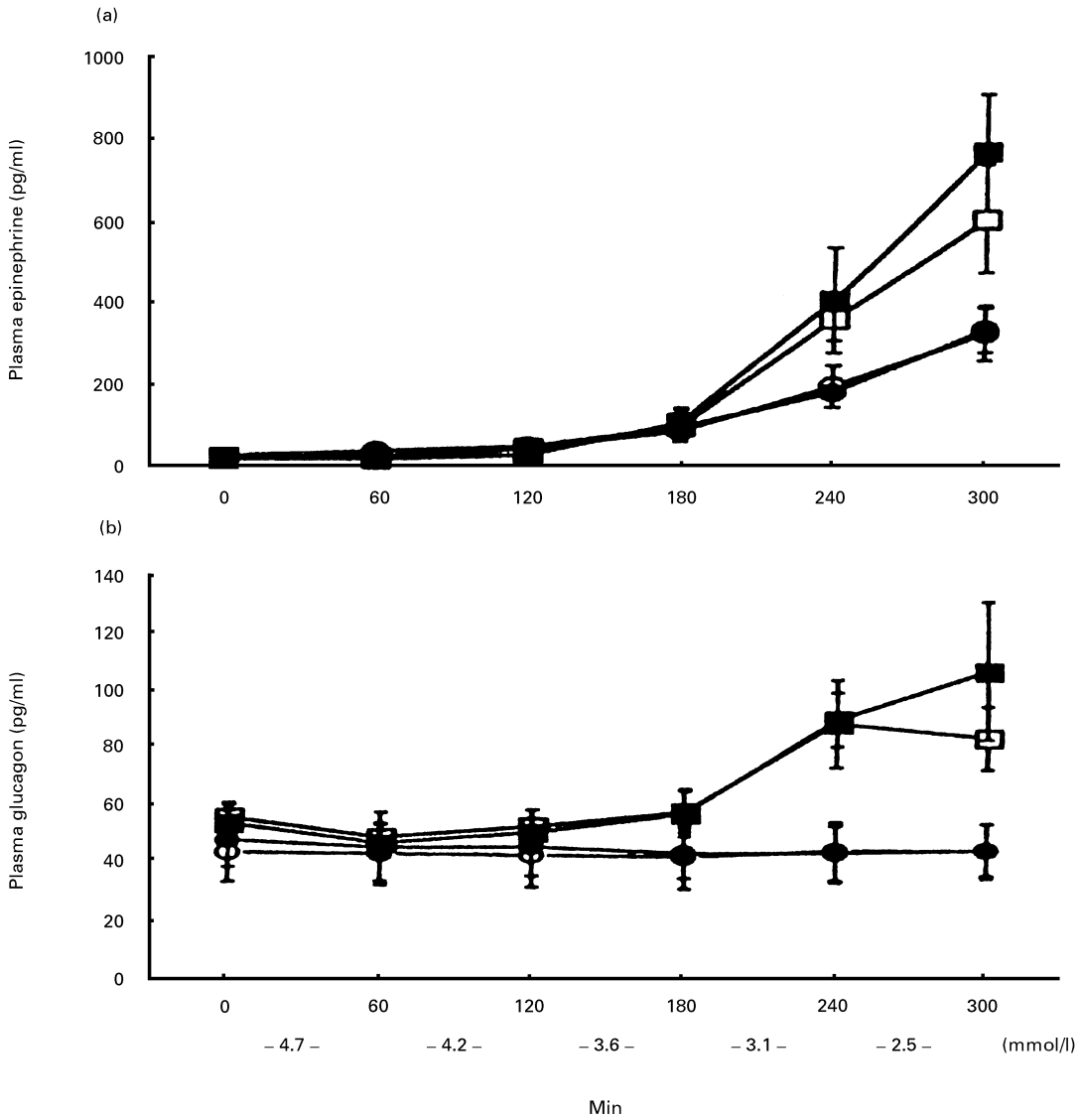

Fig 4. (a) Plasma epinephrine and (b) glucagon levels in healthy subjects (□, ■) and type 1 diabetes patients (○, ●) during stepped hypoglycaemic clamps using either glargine (□, ○) or regular human insulin (■, ●).

Table 6 Glucose infusion rates (GIR) during stepped hypoglycaemic clamp using regular human insulin or insulin glargine in healthy subjects and diabetes patients

Study time (min)	**60**	**120**	**180**	**240**
BG target (mg/dl)	**85**	**75**	**65**	**55**
GIR-healthy (mg/kg. min^{-1})				
Regular	6.90 ± 1.64	7.34 ± 1.53	7.45 ± 1.81	4.81 ± 1.35
Glargine	7.84 ± 2.05	7.18 ± 1.41	7.17 ± 1.56	5.44 ± 1.52
GIR-diabetes (mg/kg. min^{-1})				
Regular	4.43 ± 0.59	5.10 ± 0.50	4.80 ± 0.52	3.67 ± 0.52
Glargine	3.97 ± 0.45	4.87 ± 0.48	4.42 ± 0.51	3.62 ± 0.54

BG = blood glucose

insulin receptors and the IGF-1 receptors. Glargine binds insulin receptors with a lower affinity compared with native human insulin [1]. In contrast, studies in H9 cardiac myoblasts (a cell line expressing abundant IGF-1 receptors with undetectable insulin receptors) showed a higher IGF-1 binding affinity for glargine than regular human insulin [28]. However, glargine and regular human insulin are equipotent in their effects on mitogenesis [1,28,29].

The present study evaluated physiological symptoms, counter-regulatory hormones, and glucose disposal during hypoglycaemia induced by either regular human insulin or insulin glargine in healthy subjects and in

patients with type 1 diabetes mellitus. Thus, the study set out to determine whether the structural changes in insulin glargine molecule that alter its physicochemical properties could result in alterations in the physiological and neuroendocrine responses to insulin-induced hypoglycaemia. To ensure valid results the diabetic patients were selected for a history of consistent awareness of iatrogenic hypoglycaemia, as determined from their responses to a standard questionnaire [13]. In addition, blood glucose levels were closely monitored to prevent iatrogenic hypoglycaemia in the two weeks preceding study. Thus, our pre-study outpatient protocol allowed us to avoid the occurrence of hypoglycaemia-associated autonomic failure [16,30] as a confounding factor; the 2 weeks of scrupulous avoidance of hypoglycaemia also served to reverse [13,31] the effects of any recent antecedent hypoglycaemic episodes on perception of symptoms.

We found that continuous infusion of equivalent doses of insulin glargine or regular human insulin (Eli Lilly, Indianapolis, IN, USA) resulted in steady-state hyperinsulinaemia after ≈60 min, associated with progressive hypoglycaemia in healthy subjects and diabetic patients. The immunoreactive plasma free insulin levels measured during infusion of regular human insulin were higher than those measured during insulin glargine infusion because of incomplete cross-reactivity of glargine with the human insulin antibody; however, the plasma glucose targets and glucose infusion rates were similar during the clamp studies with either species of insulin. The latter finding indicates that insulin glargine and regular human insulin are equipotent with regard to a key biological property, namely, insulin-stimulated glucose disposal. Both insulin species also elicited similar changes in heart rate, systolic and diastolic blood pressures during hypoglycaemia.

Total, neurogenic, and neuroglycopaenic symptoms were perceived to equivalent degrees during hypoglycaemia induced by either regular human insulin or glargine. Neuroglycopaenic symptoms increased significantly with both insulins among healthy subjects, but in diabetic subjects the increase during hypoglycaemia induced by regular human insulin did not reach statistical significance. The latter finding, whose clinical import is doubtful, might be due in part to the higher baseline symptoms among the diabetes patients (figure 3a). Moreover, the secretory responses of the counter-regulatory hormones epinephrine, norepinephrine, growth hormone and cortisol did not differ significantly by insulin species. Plasma levels of NEFA and β-hydroxybutyrate reflect the antilipolytic and antiketogenic effects of insulin, respectively; lactate and alanine are gluconeogenic precursors. As with counter-regulatory hormones, plasma levels of these substrates were similar during infusion of either insulin glargine or regular human insulin, indicating equivalent anabolic effects of the two insulins.

In conclusion, the results of the present study demonstrate that healthy subjects and diabetic patients experience similar symptom scores, counter-regulatory hormonal responses, and glucose disposal during hypoglycaemia induced by either insulin glargine or regular human insulin. These findings indicate that the unique structural and physicochemical properties of insulin glargine are not associated with significant alteration in physiological or biochemical responses to hypoglycaemia induced by this novel insulin species, as compared with regular human insulin. Since glargine is designed for s.c. use, it is possible (though unlikely) that the present findings obtained using an i.v. protocol could differ from responses to hypoglycaemia from s.c. administration of glargine.

Acknowledgements

This study was supported in part by a research grant from Hoechst Marion Roussel, Inc. and by NIH grants MO1-RR00036 and 2P60-DK20579. Dr Dagogo-Jack is a recipient of a clinical research award from the American Diabetes Association.

References

1 Home P. Insulin glargine: the first clinically useful extended-acting insulin in half a century? Exp Opin Invest Drugs 1999; **8:** 307–314.
2 Egger M, Smith GD, Teuscher AU, Teuscher A. Influence of human insulin on symptom and awareness of hypoglycaemia: a randomized double blind cross over trial. BMJ 1991; **303:** 1265–1268.
3 Schluter KJ, Petersen KG, Sontheimer J, Enzmann F, Kerp L. Different counter-regulatory responses to human insulin (recombinant DNA) and purified pork insulin. Diabetes Care 1982; **5** (Suppl)**:** 78–81.
4 Rosak C, Althoff PH, Enzmann F, Scoffling K. Comparative studies on intermediary metabolism and hormonal counterregulation following human insulin (recombinant DNA) and purified pork insulin in man. Diabetes Care 1982; **5** (Suppl)**:** 82–89.
5 Heine RJ, van der Heyden EAP, van der Veen EA. Responses to human and porcine insulin in healthy subjects. Lancet 1989; **2:** 946–949.
6 Petersen GK, Schluter KJ, Kerp L. Less pronounced changes in serum potassium and epinephrine during

hypoglycaemia induced by human insulin (recombinant DNA). Diabetes Care 1982; **5** (Suppl)**:** 90–92.
7 Owens DR, Vora JP, Tronier B, Keller V, Luzio S, Turks A. Hormonal counter-regulatory responses to human (semi-synthetic and recombinant) and porcine insulin induced hypoglycaemia. Diabetes Res 1988; **8:** 17–20.
8 Fisher BM, Gray CE, Beastall GH, Frier BM. Responses to acute insulin induced hypoglycaemia in diabetic patients: a comparison of short acting human and porcine insulins. Diabetes Res 1988; **8:** 1–9.
9 Patrick AW, Bodmer CW, Tieszen KL, White MC, Williams G. Human insulin and awareness of acute hypoglycaemic symptoms in insulin-dependent diabetes. Lancet 1991; **338:** 528–532.
10 Maran A, Lomas J, Archibald H, Macdonald IA, Gale EAM, Amiel SA. Double blind clinical and laboratory study of hypoglycaemia with human and porcine insulin in diabetic patients. BMJ 1993; **306:** 167–171.
11 Ferrer JP, Esmatjes E, Gonzalez-Clemente JM. *et al.* Symptomatic and hormonal hypoglycaemic responses to human and porcine insulin in patients with type 1 diabetes mellitus. Diabetic Med 1992; **9:** 522–527.
12 Raptis S, Karaiskos C, Enzmann F *et al.* Biological activities of biosynthetic human insulin in healthy volunteers and insulin-dependent diabetic patients monitored by the artificial endocrine pancreas. Diabetes Care 1981; **4:** 155–161.
13 Dagogo-Jack S, Rattarasarn C, Cryer PE. Reversal of hypoglycaemia unawareness, but not defective glucose counterregulation, in IDDM. Diabetes 1994; **43:** 1426–1434.
14 Schwartz NS, Clutter WE, Shah SD, Cryer PE. The glycaemic thresholds for activation of glucose counter-regulatory systems are higher than the thresholds for symptoms. J Clin Invest 1987; **79:** 777–781.
15 Dagogo-Jack SE, Craft C, Cryer PE. Hypoglycaemia-associated autonomic failure in insulin-dependent diabetes mellitus: recent antecedent hypoglycaemia reduces the autonomic responses to, symptoms of, and defense against subsequent hypoglycaemia. J Clin Invest 1993; **91:** 819–828.
16 Towler DA, Havlin CE, Craft S, Cryer PE. Mechanism of awareness of hypoglycaemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopaenic symptoms. Diabetes 1993; **42:** 1791–1798.
17 Kuzuya H, Blix P, Horwitz D, Steiner D, Rubenstein A.Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977; **26:** 22–29.
18 Ensinck J. Immunoassays for glucagon. In Lefebvre P, ed. Handbook of Experimental Pharmacology, Vol. 66. New York: Springer-Verlag, 1983: 203–221.
19 Farmer R, Pierce C. Plasma cortisol determination: radioimmunoassay and competitive binding compared. Clin Chem 1974; **20:** 411–414.
20 Schalch D, Parker M. A sensitive double antibody radioimmunoassay for growth hormone in plasma. Nature 1964; **203:** 1141–1142.
21 Shah SD, Clutter WE, Cryer PE. External and internal standards in the single isotope derivative (radioenzymatic) assay of plasma norepinephrine and epinephrine in normal humans and patients with diabetes mellitus or chronic renal failure. J Lab Clin Med 1985; **106:** 624–629.
22 Hosaka K, Kikuchi T, Mitsuhida N, Kawaguchi A. A new colorimetric method for the determination of free fatty acids with acyl-CoA synthetase and acyl-CoA oxidase. J Biochem 1982; **89:** 1799–1803.
23 Pinter J, Hayashi J, Watson J. Enzymatic assay of glycerol, dihydroxyacetone and glyceraldehyde. Arch Biochem Biophys 1967; **121:** 404–414.
24 Lowry O, Passoneau J, Hasselberger F, Schultz D. Effect of ischemia on known substrates and co-factors of glycolytic pathway of the brain. J Biol Chem 1964; **239:** 18–30.
25 Cahill G, Herrera M, Morgan A *et al.* Hormone-fuel interrelationships during fasting. J Clin Invest 1966; **45:** 1751–1769.
26 Gerich JE, Langlois M, Naocco C, Karam JM, Forsham PH. Lack of glucagon response to hypoglycaemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. Science 1973; **182:** 171–173.
27 Berti L, Kellerer B, Bossenmaier B, Seffer E, Seipke G, Haering HU. The long acting human insulin analog HOE 901: characteristics of insulin signaling in comparison to Asp (B10) and regular insulin. Horm Metab Res 1998; **30:** 123–129.
28 Liu L, Koenen M, Seipke G, Eckel J. IGF-1 receptor-mediated signaling of the human insulin analogues HOE901. Diabetologia 1997; **40** (Suppl)**:** A355.
29 Bahr M, Kolter T, Seike G, Eckel J. Growth promoting and metabolic activity of the human insulin analogue (GlyA21, ArgB31, ArgB32) insulin (HOE 901) in muscle cells. Eur J Pharmacol 1997; **320:** 259–265.
30 Rattarasarn C, Dagogo-Jack S, Zachwieja JJ, Cryer PE. Hypoglycaemia-induced autonomic failure in IDDM is specific for the stimulus of hypoglycaemia and is not attributable to prior autonomic activation. Diabetes 1994; **43:** 809–818.
31 Fanelli CG, Epifano L, Rambotti AM *et al.* Meticulous prevention of hypoglycaemia normalizes the glycaemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycaemia in intensively treated patients with short-term IDDM. Diabetes 1993; **42:** 1683–1689.