Different Brain Responses to Hypoglycemia Induced by Equipotent Doses of the Long-Acting Insulin Analog Detemir and Human Regular Insulin in Humans

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OBJECTIVE—The acylated long-acting insulin analog detemir is more lipophilic than human insulin and likely crosses the blood-to-brain barrier more easily than does human insulin. The aim of these studies was to assess the brain/hypothalamus responses to euglycemia and hypoglycemia in humans during intravenous infusion of equipotent doses of detemir and human insulin.

RESEARCH DESIGN AND METHODS—Ten normal, nondiabetic subjects (six men, age 36±7 years, and BMI 22.9±2.6 kg/m^2) were studied on four occasions at random during intravenous infusion of either detemir or human insulin in euglycemia (plasma glucose 90 mg/dl) or during stepped hypoglycemia (plasma glucose 90, 78, 66, 54, and 42 mg/dl steps).

RESULTS—Plasma counterregulatory hormone response to hypoglycemia did not differ between detemir and human insulin. The glycemic thresholds for adrenergic symptoms were higher with detemir (51 ± 7.7 mg/dl) versus human insulin (56 ± 7.8 mg/dl) ($P = 0.029$). However, maximal responses were greater with detemir versus human insulin for adrenergic (3 ± 2.5 vs. 2.4 ± 1.8) and neuroglycopenic (4 ± 3.9 vs. 2.7±2.5) symptoms (score, $P < 0.05$). Glycemic thresholds for onset of cognitive dysfunction were lower with detemir versus human insulin (51 ± 8.1 vs. 47 ± 3.6 mg/dl, $P = 0.031$), and cognitive function was more deteriorated with detemir versus human insulin ($P < 0.05$).

CONCLUSIONS—Compared with human insulin, responses to hypoglycemia with detemir resulted in higher glycemic thresholds for adrenergic symptoms and greater maximal responses for adrenergic and neuroglycopenic symptoms, with an earlier and greater impairment of cognitive function. Additional studies are needed to establish the effects of detemir on responses to hypoglycemia in subjects with diabetes. ***Diabetes*** **57:746–756, 2008**

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Received for publication 6 October 2007 and accepted in revised form 4 December 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 14 December 2007. DOI: 10.2337/db07-1433.

AUC, area under the curve; FFA, free fatty acid; GIR, glucose infusion rate; PASAT, paced auditorial serial addition test.

The physiology of glucose counterregulation to hypoglycemia in humans has been extensively studied (1). A progressive decline in plasma glucose induced by insulin triggers a well-established sequence of hierarchic responses that occur at specific glycemic thresholds (2–4).

It is important that new insulin formulations and/or insulin analogs available for treatment of diabetes are compared with the reference human insulin for thresholds of responses and overall responses to hypoglycemia to exclude additional risks of inducing hypoglycemia unawareness. The rapid-acting insulin analogs lispro (5) and aspart (6) and the long-acting analog glargine (7) have been shown to be no different in terms of responses to hypoglycemia versus human insulin. Regarding the long-acting insulin analog detemir, there are no systematic observations with the exception of two preliminary studies—one in normal, nondiabetic subjects (8) and the other in subjects with type 1 diabetes (9)—with conflicting results.

Insulin detemir is a long-acting soluble insulin analog with a ^{14}C fatty acid chain conferring lipophility, associated with free fatty acid (FFA) binding sites on albumin (10). Because of these characteristics, the responses of insulin detemir to hypoglycemia are of particular interest. Normally, circulating insulin crosses the blood-to-brain barrier and the blood-cerebrospinal fluid barrier via a saturable transport mechanism (11), whereas albumin may directly penetrate into the cerebrospinal fluid through the choroids plexus epithelial cells (12). There is evidence that the more lipophilic a molecule, the higher its concentration in the cerebrospinal fluid (13). Thus, it is possible that the lipophilic insulin detemir has easier access to brain tissues than regular human insulin. In mice, there is greater insulin signaling with detemir compared with human insulin in the hypothalamic region (phosphorylation of insulin receptor, Irs2 proteins, and phosphatidylinositol 3-kinase activity) (14). In addition, plasma concentrations of insulin detemir are greater than those of human insulin because of its binding to albumin (15) and because of its higher (four time) molar concentration than human insulin (16). This might directly influence counterregulatory responses because high plasma concentrations of insulin might modulate per se counterregulation to hypoglycemia (17–22).

The aim of the present study was to compare the physiological responses (counterregulatory hormones, symptoms, and impairment of cognitive function) to hypoglycemia induced by equipotent doses of insulin detemir and regular human insulin. Healthy subjects were studied during stan-

dard hyperinsulinemic-euglycemic and hypoglycemic studies: first, to prove equipotency, and, second, to measure physiological responses to hypoglycemia corrected for euglycemia.

RESEARCH DESIGN AND METHODS

The study (investigator-initiated trial, EudraCT-Nr: 2006-003744-33) was approved by the local ethics committee and carried out according to the Helsinki declaration after written informed consent was obtained from all subjects.

Ten healthy nonobese volunteers (six men, age 36 ± 7 years, and BMI 22.9 ± 2.6 kg/m^2) were recruited. Subjects had no family history of diabetes, had no medical problems, and maintained regular levels of physical activity. Subjects were not on any medication known to affect glucose metabolism.

Subjects were studied on four occasions in random order, a computer-generated sequence, using the hyperinsulinemic-euglycemic clamp technique and, on a separate occasion, the hypoglycemic glucose clamp technique (2,23). In an initial phase, because preliminary data from our laboratory suggested that the bioequivalence of insulin action between detemir and intravenoushuman insulin occurred at a molar ratio greater than the commercially available formulation (4:1), pilot studies were performed to establish the concentration of detemir infused intravenously needed to match the effects of human insulin. The ratio in molar concentration detemir:human insulin best resulting in bioequivalence of the two insulin infusions (as measured by the glucose infusion rate [GIR] in euglycemia) was 8:1 with an initial bolus of detemir twice as great as that of human insulin. In fact, lower detemir infusion concentrations resulted in biological activity that remained constantly behind that of human insulin, whereas higher concentrations and/or bolus produced overshooting of glucose infusions over the last 2–3 h of the clamp studies (data not shown). Subjects were studied in random order, 14–21 days apart, during intravenous infusion of either insulin detemir (1 unit = 24 nmol; Levemir; Novo Nordisk) or regular human insulin (1 unit = 6 nmol; Actrapid U 100; Novo Nordisk) in euglycemia or during stepped hypoglycemia. To ensure the double-blind design of the study project, a qualified person not otherwise involved in the study was in charge of preparing the insulin infusions, in accordance to the randomization list. To use the same rate of infusion with both regular human insulin and detemir insulin, insulin solutions were diluted to final concentrations of 1 IU/ml and 2 units/ml, respectively, in saline (0.9% [wt/vol] NaCl). Two milliliters of the respective subject's blood was added to each insulin solution to prevent adhesion of insulin to plastic surfaces.

Subjects were admitted to the General Clinical Research Center of the University of Perugia, Perugia, Italy, at 0730 h on the day of study, after an overnight fast. One intravenous cannula was placed into an antecubital vein of the nondominant forearm for infusion of insulin, glucose, and saline. A second cannula was inserted retrogradely into a dorsal vein of the ipsilateral hand and kept in a thermoregulated box at about 65°C to obtain arterialized-venous blood (24). This second cannula was used for intermittent blood sampling. After not less than 1 h for equilibration and baseline testing, at time 0 min of study, an intravenous bolus of either 10 mU/kg (0.01 ml/kg) human insulin or 20 mU/kg (0.01 ml/kg) detemir insulin was given. Subsequently, a continuous intravenous infusion of 1 mU · kg^{-1} · min^{-1} (6 pmol · kg^{-1} · min^{-1}) human insulin or 2 mU · kg^{-1} · min^{-1} (48 pmol · kg^{-1} · min^{-1}) detemir insulin was initiated and remained unchanged until time 240 min of study, after which infusion rates were doubled for the last 60 min of study (time 240–300 min).

Immediately after the insulin bolus, a variable infusion of 20% glucose was initiated by means of a syringe pump (Harvard Apparatus, Ealing, South Natick, MA) and continued at variable rate according to the principle of the euglycemic-hypoglycemic glucose clamp technique on all four study occasions. On two occasions, plasma glucose was maintained at the target value of 90 mg/dl (human insulin and detemir insulin euglycemic clamps, respectively), whereas on the two other occasions plasma glucose was clamped at sequential target glucose concentrations of 90, 78, 66, 54, and 42 mg/dl (human insulin and detemir insulin hypoglycemic clamps). Each step consisted of 60 min, with the initial 30 min used to reach the desired plasma glucose target and the subsequent 30 min to maintain the plasma glucose plateau for measurement of variables.

At time 300 min, the clamp procedure was terminated, the insulin infusion was withdrawn, and the glucose infusion increased to quickly restore euglycemia. Subjects were given a meal and observed until their plasma glucose was consistently euglycemic for at least 1 h without glucose infusion, after which they were discharged.

In all studies, blood samples were drawn at 5- to 10-min intervals for plasma glucose measurements and at 30-min intervals for measurements of plasma insulin, C-peptide, counterregulatory hormones, and nonglucose substrates (see below). A semiquantitative symptom questionnaire (2,25) was administered every 30 min. Subjects were asked to score from 0 (none) to 5 (severe) on each of the following symptoms: seven autonomic/neurogenic (adrenergic: heart pounding, tremor, anxiety, and irritability; cholinergic: sweating, hunger, and tingling), five neuroglycopenic (difficulty in thinking, weakness, dizziness, blurred vision, and drowsiness), and three nonspecific (thirst, nausea, and headache) (2,25). The sum of each of these constituted the total symptom score.

In addition, at baseline, during each of the plateaus (indicated as time 0, time 60, time 120, time 180, time 240, and time 300 min) and at similar times during the euglycemic studies, cognitive function was assessed by applying a battery of hypoglycemia-sensitive tests: trail-making A and B tests (26); verbal fluency (27); verbal memory test (27); backward digit span (28); Stroop word, color, and color-word (interference) subtests (29); and paced auditorial serial addition test (PASAT 3 s) (30), with tests always performed in this order. The whole battery took 20 min to complete and was, therefore, suitable for repeated administration. All tests presented were paper based except PASAT, which was presented on a audiocassette tape to control the rate of stimulus presentation. Each subject practiced these tasks on each study occasion, i.e., before the commencement of the glucose clamp, until stable performance was achieved; in addition, to prevent any learning/practice effects, six alternate forms of each test were prepared and used.

Cognitive domains were assessed using composite scores, grouping selected tests together on conceptual and clinical grounds (31). Composites were created by transforming raw scores to z scores, according to the following formula: $z = (x - M)/\mathrm{SD}$, where x is the original score, and M and SD are the mean and SD, respectively, of the x scores at baseline (2) as follows: *1*) memory (composite of verbal memory and backward digit span); *2*) speed of information processing (composite of trail-making A, Stroop word and color subtests, and PASAT); *3*) attention and executive function (composite of trail-making B and Stroop color-word subtest); and *4*) fluency (verbal fluency alone).

Analytical methods. Bedside plasma glucose was measured using a Beckman Glucose Analyzer (Beckman Instruments, Palo Alto, CA). Plasma C-peptide was measured by radioimmunoassay (Linco Research, St. Charles, MO). Plasma insulin was measured using a two-site sandwich chemiluminescent immunoassay for human insulin (MLT, Cardiff, U.K.). Glucagon, growth hormone, cortisol, adrenaline and norepinephrine, plasma glycerol, β-hydroxybutyrate, lactate, and alanine were measured by previously described assays (32). Plasma FFA and pancreatic polypeptide concentrations were measured using commercial kits (NEFA C test kit; Wako Chemicals, Neuss, Germany) (Human Pancreatic Polypeptide; Linco Research).

Statistical analysis. All data were subjected to repeated-measures ANOVA with Huynh-Feldt adjustment for nonsphericity (33). The ANOVA model included the sequence of studies as a between-subjects factor, whereas test condition (euglycemic versus hypoglycemic) and time were the within-subjects factors. Subjects were entered in the model as random factors. If there were significant differences between baseline values, these were used as covariates. In this way, the data over the serial time points could be adjusted for any differences in baseline values (33). Post hoc comparisons (Newman-Keuls test) were carried out to pinpoint specific differences on significant interaction terms. The area under the curve (AUC) for plasma insulin concentrations was calculated according to the trapezoidal rule.

A modified Bonferroni procedure (34) for multiple cognitive test adjustments was used to maintain an overall type 1 error rate of 5% ($\alpha = 0.05$). Glycemic thresholds for counterregulatory hormones release, symptoms initiation, and cognitive dysfunction were calculated as the plasma glucose level at which a given response first exceeded the 95% confidence limit observed for that parameter at the corresponding time point in euglycemic control experiments, after the adjustment of experimental and control baseline data to 0 (2,4,35). Glycemic threshold for initiation of cognitive impairment was calculated on the average z score for all cognitive tests.

Data are given as means ± SD in the text and tables, but for the sake of clarity, SE bars are shown in the figures. We considered differences to be statistically significant if the P value was 0.05 or less. We conducted the statistical analyses by using NCSS 2007 software (NCSS , Kaysville, UT) (36) and Statistica software, version 6.0 (StatSoft, Tulsa, OK).

RESULTS

Plasma glucose, GIR, plasma C-peptide, and insulin concentrations. Plasma glucose was maintained at the preselected plateaus, without any significant difference between human insulin and detemir either in euglycemic and in hypoglycemic studies (Fig. 1).

GIR was higher in euglycemia compared with hypoglycemia, with both detemir and human insulin ($P < 0.001$).

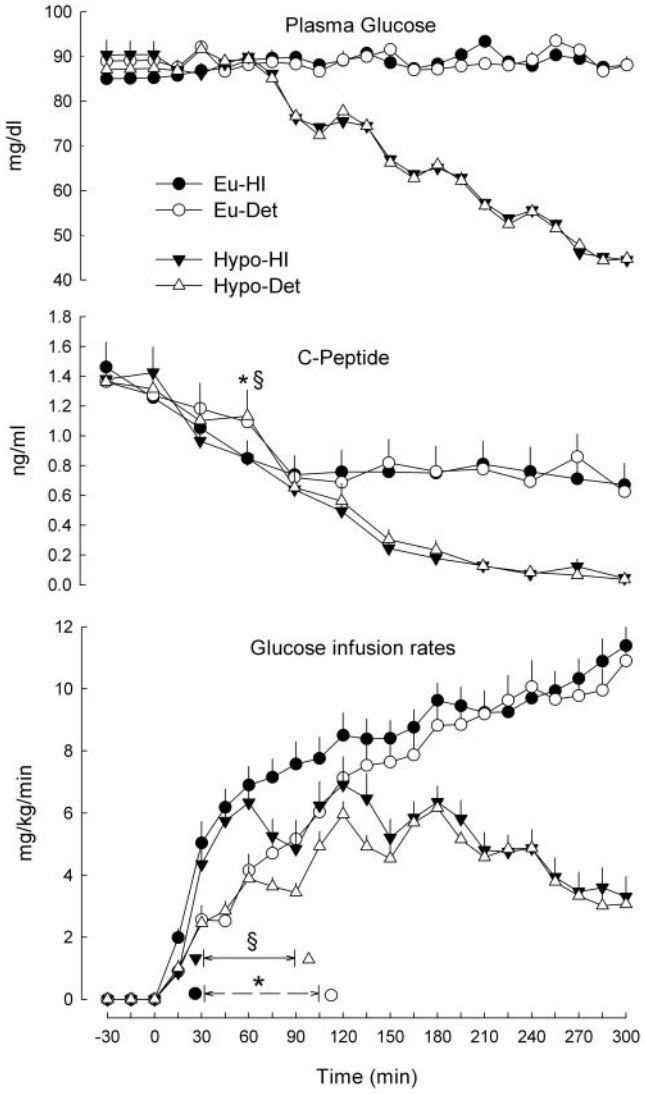

FIG. 1. GIRs, plasma glucose, and C-peptide concentrations during euglycemia and hypoglycemia studies (means ± SE). Dashed and solid arrows indicate temporal intervals of statistically significant differences between studies; *detemir insulin euglycemic clamp (Eu-Det) ≠ human insulin euglycemic clamp (Eu-HI), §detemir insulin hypoglycemic clamp (Hypo-Det) ≠ human insulin hypoglycemic clamp (Hypo-HI), $P < 0.05$.

With detemir, GIR was lower versus that with human insulin from time 30 to 105 min ($P < 0.05$) in the euglycemic studies and from time 30 to 75 min ($P < 0.05$) in the hypoglycemic studies, but it was not different thereafter.

C-peptide was similarly suppressed with both detemir insulin and human insulin, the degree of suppression being greater in hypoglycemia (95%) than euglycemia (55%) ($P < 0.001$). However, at time 60 min, C-peptide resulted slightly but significantly less suppressed with detemir compared with human insulin both in euglycemic and hypoglycemic studies ($P < 0.05$).

Plasma insulin concentrations were greater in the detemir compared with the human insulin studies, without any difference between euglycemia and hypoglycemia (AUC 845 ± 90 and 857 ± 143 $\mu U \cdot ml^{-1} \cdot min^{-1}$ detemir studies, 83 ± 20 and 80 ± 13 $\mu U \cdot ml^{-1} \cdot min^{-1}$ human insulin studies for euglycemia and hypoglycemia, respectively). Plasma detemir concentrations were on average 11 times greater than human insulin (95% CI 8.8–13.2). No formal statistics were applied to insulin levels given the expected higher concentrations of insulin detemir compared with human insulin. The time to reach plasma insulin plateau, however, was similar for both insulins (8.5 ± 6.1 vs. 9 ± 6.3 min for detemir and human insulin, respectively, $P > 0.2$).

Plasma counterregulatory hormone concentrations. In euglycemic control studies, there were no significant changes in plasma concentrations of any of the counterregulatory hormones both with detemir and human insulin, with the exception of glucagon. In fact, plasma glucagon levels decreased significantly from baseline to the end of studies without differences between the two insulins (from 62 ± 21 to 36 ± 16 and from 61 ± 9 to 34 ± 21 pg/ml, detemir and human insulin, respectively, both $P < 0.05$) (Fig. 2).

In hypoglycemic studies, all counterregulatory hormones increased significantly compared with the control euglycemic studies, both with detemir and human insulin. In the detemir insulin hypoglycemic clamp study, the time course and magnitude of counterregulatory hormone response was nearly identical to those in the human insulin hypoglycemic clamp study. However, in the detemir insulin hypoglycemic clamp study, the growth hormone concentration tended to increase more than in the human insulin hypoglycemic clamp study ($AUC_{150-300\ min}$ 3.2 ± 2 mg/ml detemir insulin hypoglycemic clamp vs. 2.7 ± 1.5 mg/ml human insulin hypoglycemic clamp, $P = 0.08$)

Symptom scores. Symptoms scores increased significantly during hypoglycemia compared with euglycemia, with both detemir and human insulin. The mean total symptoms score was greater in the detemir insulin hypoglycemic clamp than in the human insulin hypoglycemic clamp study (5.5 ± 3.3 vs. 4.4 ± 1.8, respectively), although the difference was not statistically significant. However, the maximal response (time 300 min) was significantly greater in the detemir insulin hypoglycemic clamp compared with the human insulin hypoglycemic clamp study (16 ± 11.8 vs. 11.8 ± 6.5, respectively, $P < 0.05$). This was the result of greater maximal responses of both autonomic and neuroglycopenic symptoms with detemir (8.2 ± 5.1 vs. 7.1 ± 3.8 and 4.0 ± 3.9 vs. 2.7 ± 2.5, detemir insulin vs. human insulin hypoglycemic clamp, respectively, $P < 0.05$) (Fig. 3). Adrenergic symptoms accounted for the greater maximal autonomic symptoms score (3 ± 3.4 vs. 2.4 ± 1.8, detemir insulin vs. human insulin hypoglycemic clamp, respectively, $P < 0.05$), whereas no difference was seen in cholinergic symptoms (Fig. 3).

Cognitive function. Raw scores for each cognitive test at baseline and during each plateau period of euglycemic and hypoglycemic studies are given in Table 1. All tests except digit span backward, and trail-making A and B deteriorated significantly during hypoglycemia compared with euglycemic control studies ($P < 0.05$). During hypoglycemia, PASAT and Stroop word subtest were different between detemir and human insulin. In fact, performance deterioration in the PASAT and Stroop word subtest was greater in the detemir insulin hypoglycemic clamp study compared with the human insulin hypoglycemic clamp study.

Mean of overall z scores (0.1 ± 0.5 vs. −0.1 ± 0.5, detemir insulin and human insulin euglycemic clamp, respectively) and composites for each prespecified cognitive domain (Fig. 4) showed no difference between the two insulins in euglycemia studies ($P > 0.2$). In hypoglycemia studies, attention and information processing

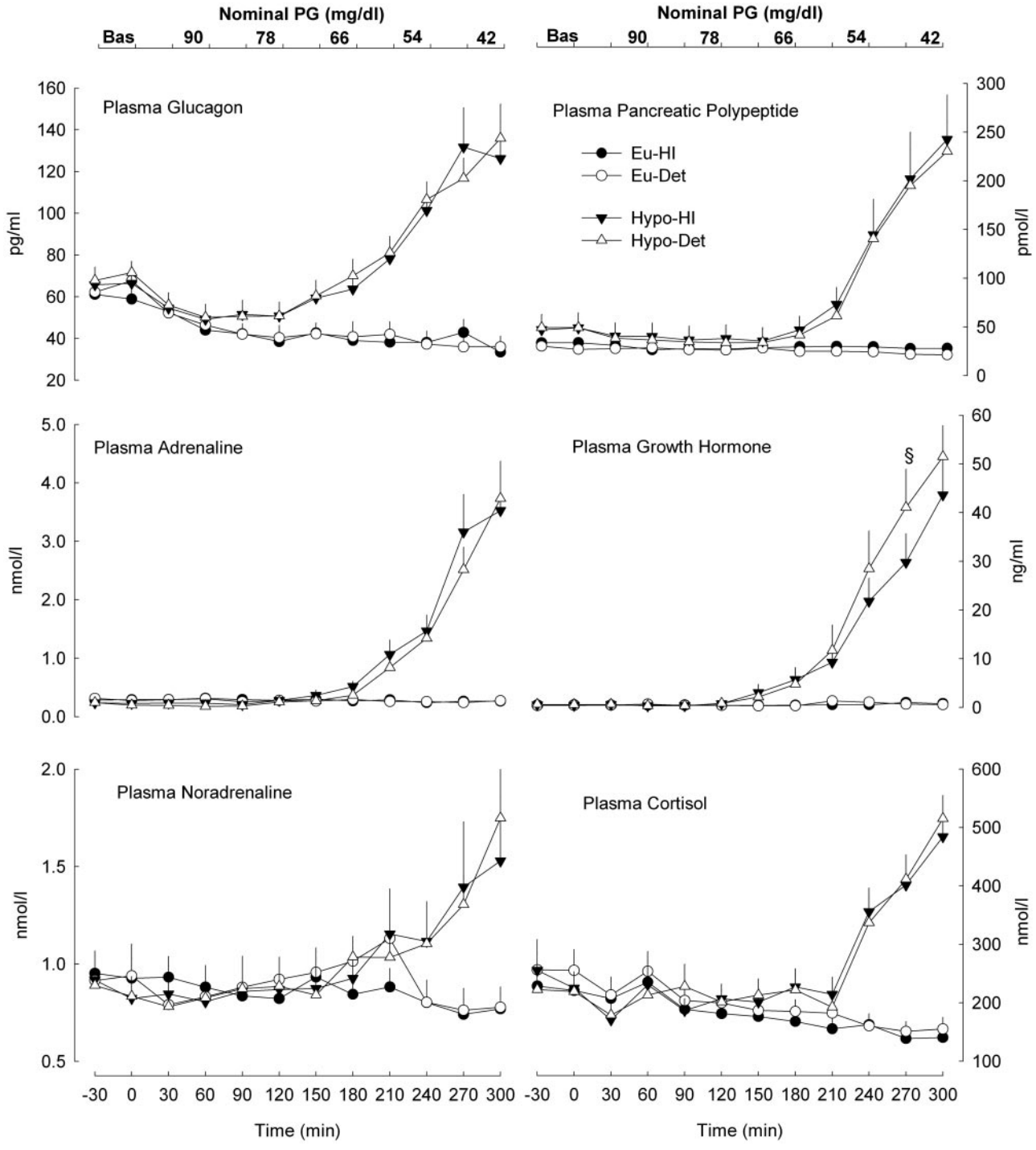

FIG. 2. Plasma counterregulatory hormones concentrations during euglycemia and hypoglycemia studies (means ± SE). All responses to hypoglycemia were significantly higher compared with euglycemia ($P < 0.05$). No differences were observed in euglycemia between detemir and human insulin. §Detemir insulin hypoglycemic clamp (Hypo-Det) ≠ human insulin hypoglycemic clamp (Hypo-HI), $P < 0.05$.

deteriorated both with detemir and human insulin ($P < 0.05$ vs. euglycemia). However, the degree of deterioration was greater with detemir compared with human insulin ($P < 0.05$). Memory was not affected in the human insulin hypoglycemic clamp study, whereas it was significantly impaired in the detemir insulin hypoglycemic clamp study compared with all other studies ($P < 0.05$). In contrast, fluency deteriorated only during the last plateau of the hypoglycemic studies, without differences between detemir and human insulin ($P > 0.2$).

Plasma FFA, β-hydroxybutyrate, glycerol, alanine, and lactate concentrations. Plasma FFA concentrations decreased significantly in euglycemia and hypoglycemia with both detemir and human insulin. However, at time 30 min, FFAs were significantly less suppressed with detemir compared with human insulin, both in euglycemic and hypoglycemic studies. β-Hydroxybutyrate concentrations followed a pattern similar to FFAs, without any difference between detemir and human insulin studies. Glycerol and alanine concentrations did not change significantly throughout the studies, whereas

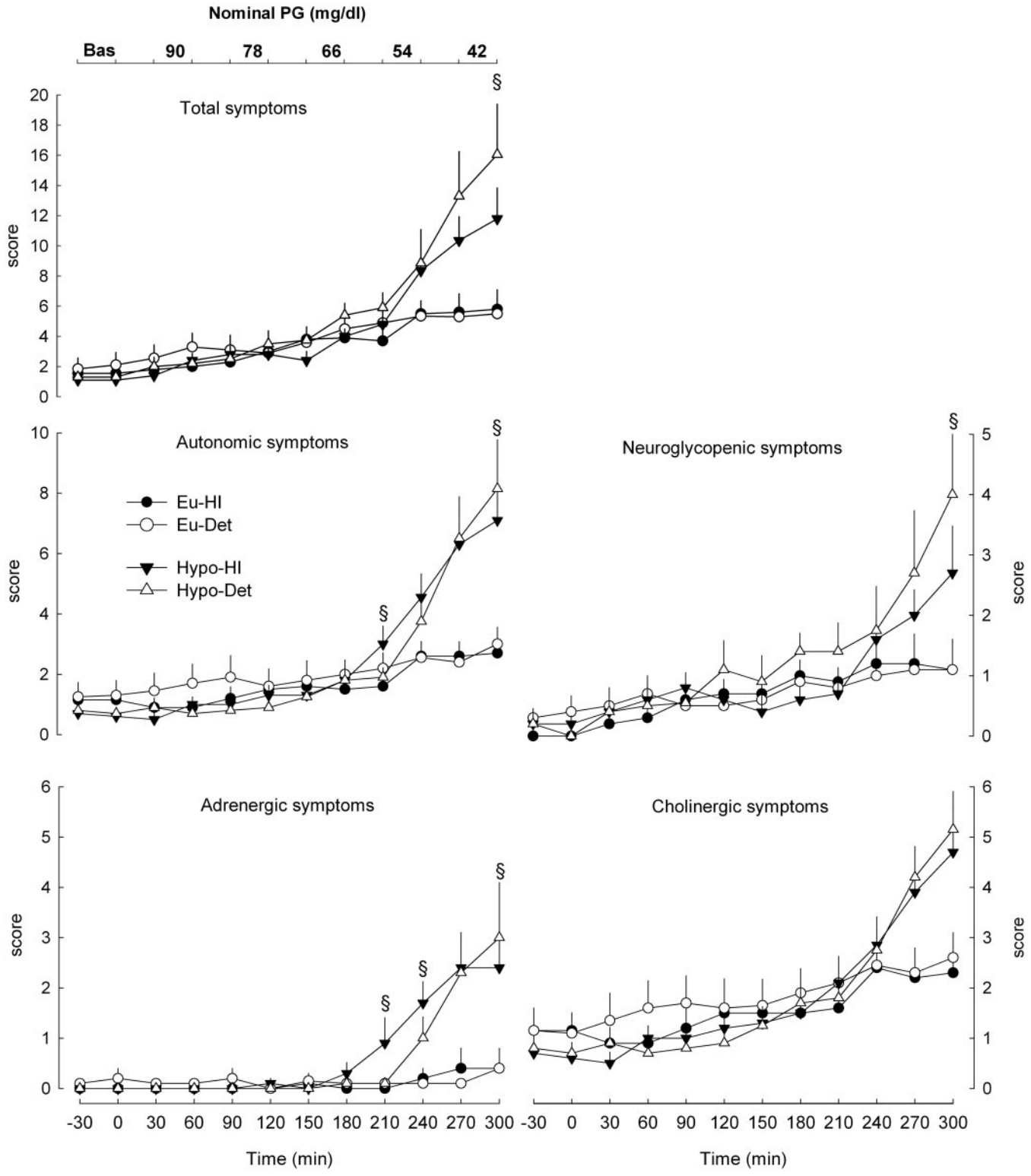

FIG. 3. All symptoms scores increased significantly in hypoglycemia compared with euglycemia studies, both with detemir and human insulin (means ± SE) ($P < 0.05$). No differences were observed in euglycemia between detemir and human insulin. During hypoglycemia, maximal total, autonomic, and neuroglycopenic symptoms responses were greater in the detemir insulin hypoglycemic clamp study. Adrenergic symptoms accounted for the higher maximal autonomic symptoms score in the detemir insulin hypoglycemic clamp study, although the response appeared delayed compared with the human insulin hypoglycemic clamp study. §Detemir insulin hypoglycemic clamp (Hypo-Det) ≠ human insulin hypoglycemic clamp (Hypo-HI), $P < 0.05$.

lactate concentrations increased significantly in hypoglycemia compared with euglycemia, without differences between detemir and human insulin ($P > 0.2$) (Fig. 5).

Glycemic thresholds. Glycemic thresholds for activation of counterregulatory hormones, initiation of symptoms, and onset of deterioration in cerebral function are shown in Table 2. No difference was observed in glycemic thresh-

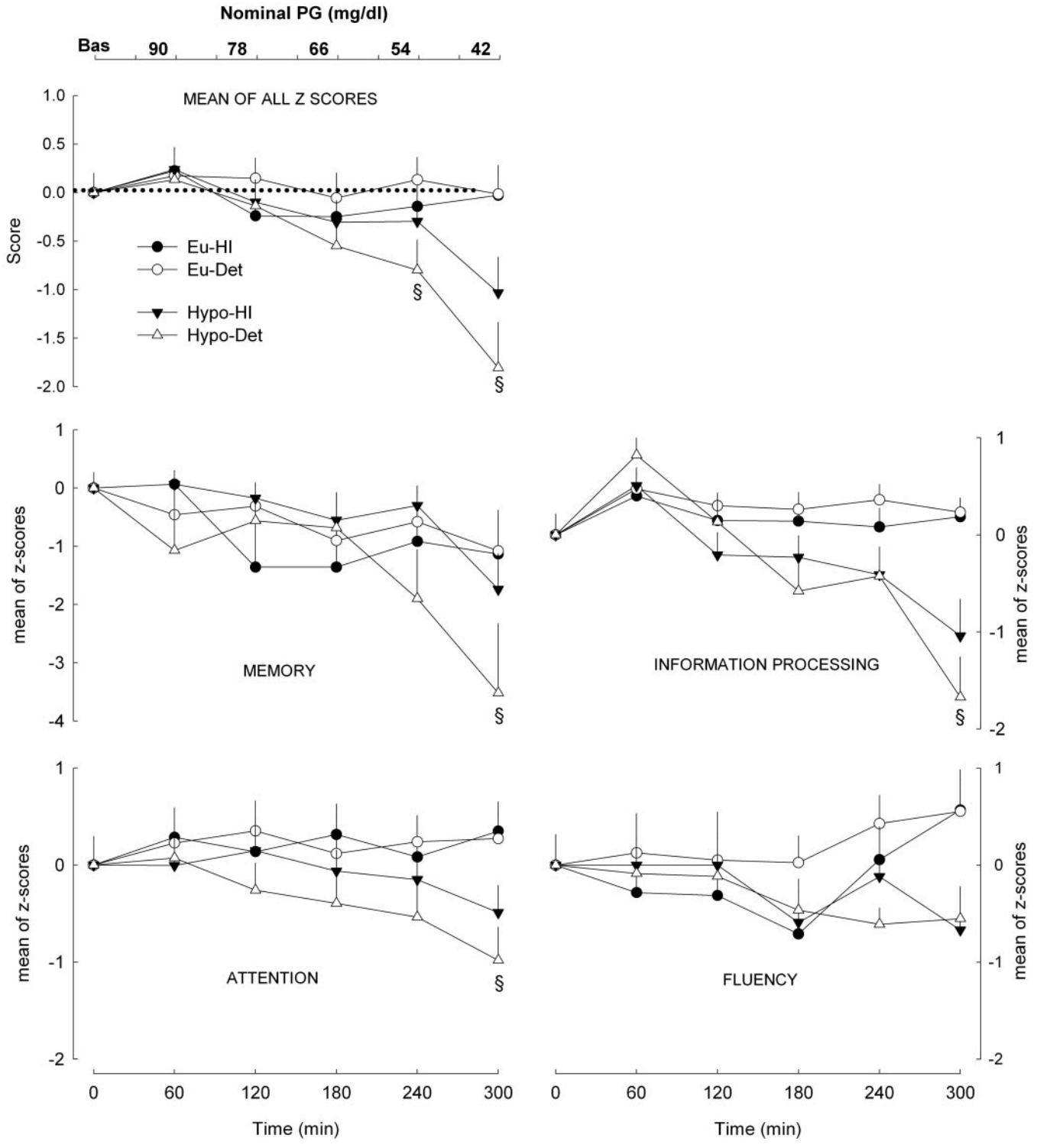

FIG. 4. Means ± SE of overall *z* scores and scores for each prespecified cognitive domain (see text). No differences were observed in euglycemia between detemir and human insulin. Maximal impairment of cognitive function during hypoglycemia resulted greater with detemir in all domains with the exception of fluency. §Detemir insulin hypoglycemic clamp (Hypo-Det) ≠ human insulin hypoglycemic clamp (Hypo-HI), $P < 0.05$.

olds for counterregulatory hormone responses. Thresholds for initiation of adrenergic symptoms were significantly higher (i.e., occurred at lower plasma glucose concentrations) in the detemir insulin hypoglycemic clamp study compared with the human insulin hypoglycemic clamp study (Table 2; Fig. 3). Thresholds for initiation of cognitive dysfunction resulted significantly lower (occurred at higher plasma glucose concentrations) in the detemir insulin hypoglycemic clamp study compared with the human insulin hypoglycemic clamp study (Table 2).

DISCUSSION

The present study indicates that the responses to hypoglycemia induced by intravenous equipotent doses of the long-acting insulin detemir and regular human insulin in

TABLE 1
Raw scores for each cognitive test at baseline and during each plateau period with either detemir or regular human insulin in euglycemic and hypoglycemic studies

	Time of test (min)						*P*
	0	60	120	180	240	300	value
Nominal plasma glucose (mg/dl) (Eu/Hypo)	Basal	90/90	90/78	90/66	90/54	90/42	
Verbal M*							
Eu-Det	4.8 ± 0.4	4.6 ± 1.0	4.5 ± 0.7	4.0 ± 1.4	4.2 ± 0.9	4.0 ± 1.2	0.0021
Eu-HI	4.9 ± 0.3	4.9 ± 0.3	4.0 ± 1.7	4.0 ± 1.5	4.3 ± 0.8	4.1 ± 1.3	
Hypo-Det	4.9 ± 0.3	4.2 ± 1.3	4.5 ± 0.7	4.4 ± 1.1	3.7 ± 1.6	2.9 ± 2.0†	
Hypo-HI	4.8 ± 0.6	4.8 ± 0.4	4.5 ± 0.5	3.9 ± 1.3	4.3 ± 0.9	2.6 ± 2.0†	
Trail A‡							
Eu-Det	64.2 ± 28.9	37.3 ± 20.0	57.3 ± 16.4	62.9 ± 21.6	60.6 ± 20.7	69.4 ± 18.3	0.076
Eu-HI	64.7 ± 19.2	38.8 ± 26.2	61.8 ± 14.2	64.6 ± 11.1	65.4 ± 14.6	66.6 ± 10.9	
Hypo-Det	63.2 ± 11.3	27.4 ± 7.2	54.9 ± 10.1	76.6 ± 14.1	63.8 ± 14.1	74.0 ± 14.2	
Hypo-HI	61.6 ± 14.1	33.1 ± 13.7	61.5 ± 11.9	73.1 ± 20.4	58.4 ± 16.1	67.9 ± 18.2	
Trail B‡							
Eu-Det	53.6 ± 16.9	50.0 ± 21.3	48.0 ± 17.2	53.8 ± 16.6	51.1 ± 14.9	49.5 ± 15.8	0.1100
Eu-HI	53.3 ± 14.6	48.3 ± 16.6	52.8 ± 17.6	53.9 ± 17.0	49.9 ± 14.1	48.9 ± 13.4	
Hypo-Det	42.3 ± 12.9	41.9 ± 14.3	47.2 ± 12.9	49.8 ± 16.2	52.1 ± 17.4	54.4 ± 19.0	
Hypo-HI	50.2 ± 17.0	46.0 ± 18.6	45.8 ± 14.5	49.4 ± 15.7	50.5 ± 19.0	50.6 ± 19.2	
Verbal F*							
Eu-Det	12.4 ± 4.0	12.9 ± 4.4	12.6 ± 3.5	12.5 ± 3.5	14.1 ± 3.7	14.6 ± 4.0	0.0126
Eu-HI	12.8 ± 3.5	11.8 ± 5.0	11.7 ± 4.8	10.3 ± 2.3	13.0 ± 4.4	14.8 ± 4.6	
Hypo-Det	13.5 ± 3.4	13.2 ± 3.9	13.1 ± 4.3	11.9 ± 3.5	11.4 ± 1.8	11.6 ± 3.6†	
Hypo-HI	13.3 ± 2.5	13.3 ± 4.3	13.3 ± 4.4	11.8 ± 2.6	13.0 ± 4.1		
Digit SB*							
Eu-Det	5.2 ± 1.1	4.7 ± 1.7	5.3 ± 1.6	5.3 ± 1.7	5.5 ± 1.4	4.9 ± 1.6	0.0600
Eu-HI	4.9 ± 1.5	5.1 ± 1.7	5.1 ± 1.4	5.1 ± 1.5	5.0 ± 1.8	5.3 ± 1.6	
Hypo-Det	5.8 ± 1.4	5.9 ± 1.6	6.0 ± 1.2	6.1 ± 1.3	5.8 ± 1.3	4.8 ± 1.9	
Hypo-HI	5.4 ± 1.6	5.6 ± 1.7	5.6 ± 1.3	5.9 ± 1.4	5.7 ± 1.6	5.4 ± 1.6	
PASAT*							
Eu-Det	51.8 ± 8.8	56.1 ± 4.5	56.5 ± 4.3	56.9 ± 3.6	57.0 ± 4.4	58.1 ± 2.9	0.0002
Eu-HI	56.7 ± 6.1	57.0 ± 4.9	56.7 ± 3.1	58.0 ± 3.6	56.2 ± 4.3	57.0 ± 3.8	
Hypo-Det	58.4 ± 2.3	58.8 ± 1.6	58.3 ± 2.2	56.9 ± 3.5	57.6 ± 2.4	51.0 ± 8.7†§	
Hypo-HI	58.2 ± 2.9	58.0 ± 2.1	56.7 ± 3.0	59.0 ± 2.1	56.5 ± 3.2	55.1 ± 5.2	
Stroop word*							
Eu-Det	111.7 ± 18.7	118.9 ± 13.9	119.4 ± 12.8	117.7 ± 16.1	118.6 ± 13.8	116.1 ± 11.0	0.0000
Eu-HI	118.0 ± 18.8	116.1 ± 12.8	118.1 ± 15.2	116.8 ± 20.1	117.0 ± 15.1	120.7 ± 16.4	
Hypo-Det	120.2 ± 16.9	118.0 ± 18.1	118.8 ± 19.2	115.9 ± 18.4	107.8 ± 15.4	95.3 ± 17.8†§	
Hypo-HI	118.9 ± 13.6	118.5 ± 15.7	112.4 ± 12.5	116.6 ± 15.8	109.2 ± 11.8	102.8 ± 15.0†	
Stroop color*							
Eu-Det	82.4 ± 9.5	83.2 ± 7.6	82.5 ± 7.3	83.4 ± 9.3	85.8 ± 7.8	83.9 ± 9.0	0.0000
Eu-HI	79.3 ± 9.3	82.1 ± 11.8	83.3 ± 8.2	83.1 ± 11.3	83.9 ± 11.6	85.3 ± 9.9	
Hypo-Det	84.7 ± 15.4	85.9 ± 18.5	83.5 ± 18.1	81.3 ± 18.2	76.1 ± 10.0†	69.2 ± 11.2†	
Hypo-HI	82.6 ± 9.4	83.5 ± 11.2	84.0 ± 10.0	80.6 ± 9.5	77.1 ± 15.6†	68.7 ± 15.5†	
Stroop color-word*							
Eu-Det	52.9 ± 10.0	55.3 ± 10.3	56.6 ± 12.1	55.4 ± 9.0	56.2 ± 10.4	55.9 ± 10.3	0.0000
Eu-HI	53.4 ± 8.8	55.4 ± 9.2	55.5 ± 10.3	59.3 ± 10.2	52.8 ± 8.6	56.9 ± 11.3	
Hypo-Det	63.2 ± 11.4	64.5 ± 14.1	61.6 ± 10.4	60.8 ± 10.2	59.6 ± 11.2	51.5 ± 11.3†	
Hypo-HI	62.1 ± 13.8	58.5 ± 9.9	62.6 ± 11.6	59.7 ± 8.7	58.2 ± 11.9	51.3 ± 10.9†	

Data are means ± SD. Det, detemir; HI, regular human insulin; Eu-, euglycemic studies; Hypo-, hypoglycemic studies. *Number of responses; ‡time in seconds; †Hypo≠Eu; §Hypo-Det≠Hypo-HI, $P < 0.05$.

humans differ. Although the response of counterregulatory hormones was no different, other responses indicate unequivocal differences between detemir and human insulin. With detemir, first, the response of autonomic-adrenergic symptoms occurred later (higher thresholds, i.e., responses occurred at lower plasma glucose concentration). Second, the maximal responses of autonomic-adrenergic as well as neuroglycopenic symptoms were greater. Third, the onset of cognitive dysfunction occurred earlier (lower threshold, i.e., responses occurred at higher plasma glucose concentration), and the cognitive tests exploring domains pertaining to memory, attention, information processing, and executive function were more deteriorated versus human insulin. Notably, these results have been obtained with rates of insulin infusion resulting in plasma human insulin concentrations in the physiological range of the postprandial condition in humans (~80 μU/ml), which corresponded to plasma detemir concentrations demonstrated to be equipotent in euglycemia. To the best of our knowledge, this is the first study reporting differences in responses to hypoglycemia with an insulin analog compared with human insulin.

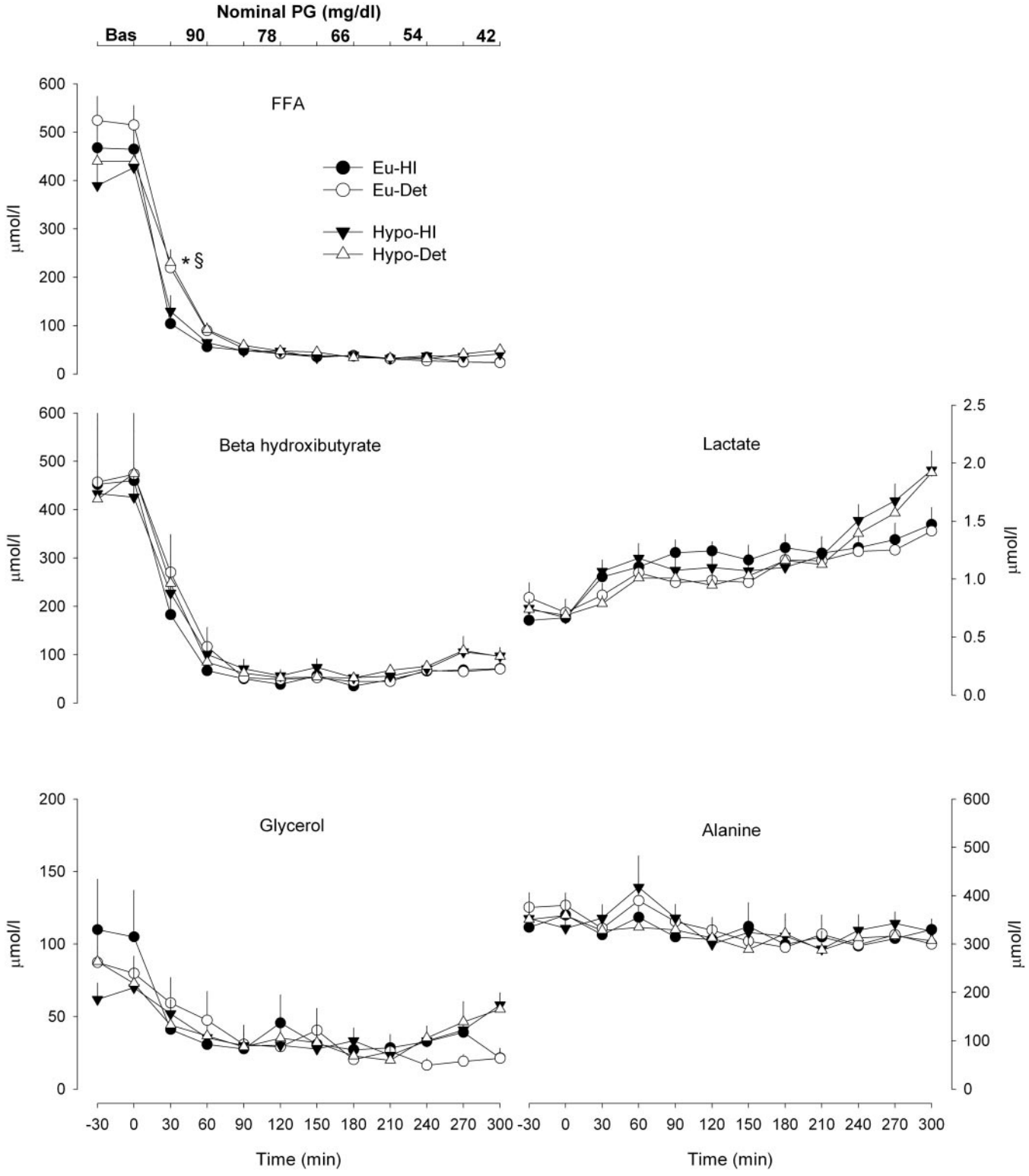

FIG. 5. FFA, β-hydroxibutyrate, glycerol, lactate, and alanine concentrations during euglycemia and hypoglycemia studies. No differences were observed between detemir and human insulin, with the exception of a lower FFA suppression at the beginning of the detemir studies. *Detemir insulin euglycemic clamp (Eu-Det) ≠ human insulin euglycemic clamp (Eu-HI), §detemir insulin hypoglycemic clamp (Hypo-Det) ≠ human insulin hypoglycemic clamp (Hypo-HI), $P < 0.05$.

The reasons for the differences in response to hypoglycemia observed in the present study are not known. It is possible, however, that detemir and human insulin exert their differential actions in the brain where responses to hypoglycemia are generated (37,38). As a hypothesis, this might result from the different concentrations in the brain of insulin detemir and human insulin, with detemir having easier access to brain tissue, as demonstrated in mice (14). It is now established that insulin acting in the brain is peripherally derived (39). In fact, peripheral insulin enters the brain by crossing the blood-brain barrier in proportion to plasma insulin levels via a receptor-mediated transport

TABLE 2
Glycemic thresholds of response of counterregulatory hormones, onset of deterioration of brain function, and initiation of symptoms

	Glycemic thresholds (mg/dl)		
	Detemir	Human insulin	*P* value
C-peptide	78 ± 9.5	83 ± 7.6	0.316
Glucagon	64 ± 6.8	66 ± 7.7	0.586
Adrenaline	62.5 ± 3.9	64 ± 3.4	0.339
Noradrenaline	54 ± 4.9	55 ± 5.6	0.138
Growth hormone	61 ± 8.4	63 ± 8.5	0.600
Cortisol	57 ± 5.9	55 ± 7.6	0.531
Pancreatic polypetide	57 ± 6.6	56 ± 10.1	0.823
Autonomic symptoms	55 ± 8.6	57 ± 7.4	0.892
Adrenergic	51 ± 7.7	56 ± 7.8	0.029
Colinergic	54 ± 9.2	53 ± 9.2	0.798
Neuroglicopenic symptoms	49 ± 6.6	47 ± 4.5	0.432
Cognitive dysfunction	51 ± 8.1	47 ± 3.6	0.031

Data are means ± SD.

process located on the microvasculature (40–42). Once within the brain, insulin, alone or in interaction with other peptides, regulates processes such as energy homeostasis, satiety, counterregulation to hypoglycemia, cognitive function, and neuronal survival, among others (43). With regard to responses to hypoglycemia, the evidence, although not uniformly in agreement, indicates that higher plasma insulin concentrations, which imply higher cerebral insulin concentrations, may elicit greater counterregulatory responses, increased symptoms, and deterioration of cognitive function to insulin-induced hypoglycemia (20). The best evidence for such conclusions is provided by Lingenfelser et al. (19), who performed hyperinsulinemic stepped hypoglycemic clamps in subjects with type 1 diabetes. The results of our study, which might be related to a higher concentration of insulin detemir in the brain, fit well within this framework. However, the results of other studies (18,44–46) aiming at evaluating the effect of different plasma insulin concentrations have reported results that are at variance with those of Lingenfelser et al. (19) and with the results of our own study. Notably, such discrepancies may in part be due to and explained by the supraphysiological insulin levels in those studies associated with greater peripheral insulin action requiring higher glucose infusions compared with the present study (19,44–46). Thus, a difference between the present and other studies involving hyperinsulinemic clamp procedures (8,9,19,44–46), which may be critical to the interpretation of results, is that in the present study the peripheral action of both detemir and human insulin were matched. It is of note that in our study, plasma concentrations of glucagon, which are known to be exquisitely sensitive to the inhibitory effect of peripheral insulin levels (47), were similar in euglycemia with both detemir and human insulin, further indicating the matched peripheral actions of both detemir and human insulin. In addition, changes in substrates levels such as lactate, whose levels increase during higher insulin infusions in relation to augmented catecholamine levels (45), might have affected brain responses, in particular causing improvement of cognitive function (46). In fact, it is well known that lactate can be used by the brain as an alternative fuel to glucose during hypoglycemia when its plasma concentration increases (48,49). This is not the case in our study, in which plasma substrate concentrations were the same in the hypoglycemic clamps with the two insulins.

We are aware of only two studies (8,9) comparing responses to hypoglycemia induced with detemir and human insulin. In one hypoglycemic stepwise clamp study (8), responses were examined during hypoglycemia induced either with intravenous detemir ($5\ mU \cdot kg^{-1} \cdot min^{-1}$) or human insulin ($2\ mU \cdot kg^{-1} \cdot min^{-1}$) in normal subjects. In the other study (9), hypoglycemia was induced by subcutaneous injection of 0.5 unit/kg detemir or 0.5 unit/kg NPH insulin in subjects with type 1 diabetes. The former study found increased sweating with detemir compared with human insulin. The latter study did not indicate any significant difference between detemir and human insulin on counterregulatory, symptomatic, and cognitive responses, but responses were examined only at one single plateau of hypoglycemia, and thresholds of responses could not be measured. In some respects, the results of those studies, if confirmed, are at variance with the results of the present study. However, in those studies, a small battery of cognitive tests was used (8,9). Most importantly, the lack of a euglycemic study control renders the interpretation of those results difficult because doses of detemir and human insulin were not tested for equipotency. In addition, it is well known that several factors, such as fatigue, stress, and learning occurring over time in the setting of clamp procedures (50), must be taken into account to appropriately analyze responses to hypoglycemia and calculate glycemic thresholds of responses.

In animals, delivery of insulin to the brain causes anorexigenic effects, resulting in a reduction in body weight (43). Interestingly, in clinical trials, the use of insulin detemir has resulted in a lower increase in body weight compared with that associated with NPH and glargine insulin (51). Whether the lower weight gain associated with insulin detemir is dependent on the anorexigenic effects related to its higher brain insulin levels is not clear. However, we did not find any difference in the threshold and magnitude of the hunger symptom either in euglycemia or in hypoglycemia with detemir compared with human insulin. Therefore, most likely, other mechanisms rather than detemir-induced hypophagia are involved and remain to be established.

An interesting finding of this study is that the different autonomic/adrenergic symptom responses observed with detemir compared with human insulin were associated with comparable catecholamine concentrations. However, it should be kept in mind that autonomic symptoms are largely mediated by sympathetic neural, rather than adrenomedullary, activation (52). Consequently, the increased maximal score and the higher threshold of autonomic/adrenergic symptoms with detemir compared with human insulin may occur despite similar increases in plasma catecholamines concentrations.

From our study, it is not possible to determine the mechanisms that caused deterioration of cognitive function to be greater with detemir insulin than human insulin. This effect, however, may be dependent on the greater brain concentration of detemir insulin, which enables it to suppress brain glucose utilization (53) and, hence, cause neuroglycopenia. The higher score of neuroglycopenic symptoms with detemir compared with human insulin might be similarly interpreted. Alternatively, because insulin plays an important role in memory and other aspects of brain function, cerebral detemir-induced hyperinsulinemia might provoke synchronous increases of inflamma-

tory markers and β-amyloid in the brain that may have deleterious effects on cognition (54).

We acknowledge that one should use considerable caution to extrapolate from the experimental situation of the present study, which analyzes normal, nondiabetic subjects with intravenous infusion of insulin detemir, to the clinical situation of diabetic subjects who receive therapeutic insulin doses of detemir as subcutaneous injections. Therefore, from our data, it is not possible to assume similar findings in individuals with diabetes. However, keeping this premise in mind, data of the present study point toward a delayed perception of symptoms to hypoglycemia and an earlier deterioration of cognitive function with detemir compared with human insulin. Although during a more profound hypoglycemia, the magnitude of symptoms response was greater, the "higher" thresholds of response of adrenergic symptoms might potentially lead to delayed perception of hypoglycemia with insulin detemir versus human insulin. On the other hand, insulin detemir has been shown to reduce hypoglycemia in type 1 and type 2 diabetes (51), and therefore detemir is expected to prevent/improve rather than induce/deteriorate hypoglycemia unawareness. However, the present study disclosing subtle but net differences between detemir and human insulin in normal, nondiabetic subjects legitimates the need for additional studies in subjects with type 1 and type 2 diabetes to establish the effects of detemir on overall responses of counterregulatory hormones, symptoms, and onset of cognitive dysfunction after subcutaneous injection of therapeutic doses of this long-acting analog compared with other basal insulin formulations.

ACKNOWLEDGMENTS

This study has received Juvenile Diabetes Research Foundation Grant 1-2005-176. This is an investigator-initiated trial performed without financial support from drug companies.

We thank Dr. Steve Luzio (University of Wales College of Medicine, Cardiff, U.K.) for measuring plasma insulin concentrations.

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