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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Running title: Counterregulatory responses with insulin detemir in humans

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ABSTRACT

Objective: The acylated long-acting insulin analog detemir is more lipophilic than human insulin (HI) and likely crosses the blood-to-brain barrier more easily than does HI. Aim of these studies was to assess the brain/hypothalamus responses to euglycemia and hypoglycemia in humans during i.v. infusion of equipotent doses of detemir and HI.

Research Design And Methods: Ten normal, non-diabetic subjects (6 males, age 36±7 years, BMI 22.9±2.6 kg/m2) were studied on 4 occasions at random, during i.v. infusion of either detemir or HI in euglycemia (plasma glucose, PG, 90 mg/dl) or during stepped hypoglycemia (PG 90, 78, 66, 54, and 42 mg/dl steps).

Results: Plasma counterregulatory hormone response to hypoglycemia did not differ between detemir and HI. The glycemic thresholds for adrenergic symptoms were higher with detemir (51±7.7) vs HI (56±7.8) (mg/dl, p=0.029). However, maximal responses were greater with detemir vs HI 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 vs HI (51±8.1 vs 47±3.6 mg/dl, p=0.031) and cognitive function was more deteriorated with detemir vs HI (p<0.05).

Conclusions: As compared to HI, responses to hypoglycemia with detemir resulted in higher glycemic thresholds for adrenergic symptoms, 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 mellitus.

Key Words: Hypoglycemia, counterregulation, symptoms, cognitive function, long-acting insulin analogs, insulin detemir.
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 which occur at specific glycemic thresholds (2-4).

It is important that new insulin formulations and/or insulin analogs available for treatment of subjects with diabetes mellitus are compared to the reference human insulin for thresholds of responses, as well as 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 vs human insulin. Regarding the long-acting insulin analog detemir, there are no systematic observations with the exception of two preliminary studies, one in normal, non-diabetic subjects (8) and the other in subjects with type 1 diabetes mellitus (9) with conflicting results.

Insulin detemir is a long acting soluble insulin analogue with a 14C fatty acid chain conferring lipophilicity, associated with free fatty acid binding sites on albumin (10). Because of these characteristics, insulin detemir’s responses 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 is, the higher is its concentration in the cerebrospinal fluid (13). Thus, it is possible that the lipophilic insulin detemir has access to brain tissues easier than regular human insulin. Indeed, in mice, there is greater insulin signaling with detemir as compared to human insulin in the hypothalamic region (phosphorilation of insulin receptor, Irs2 proteins and PI-3 kinase activity) (14). In addition, plasma concentrations of insulin detemir are greater than those of human insulin due to its binding to albumin (15) and to its higher (four time) molar concentration than human insulin (16). This might directly influence counterregulatory responses, since 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 standard hyperinsulinemic eu- and hypoglycemic studies, first, to prove equipotency, and, second, to measure physiologic 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 obtaining written informed consent by all subjects.

Subjects. Ten healthy non-obese volunteers (6 males, age 36±7 years, BMI 22.9±2.6 kg/m²), 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.

Design of studies. Subjects were studied on four occasions in random order, computer-generated sequence, using the hyperinsulinemic euglycemic and, on a separate occasion, the hypoglycemic glucose clamp technique (2, 23). In an initial phase, since preliminary data from our laboratory
suggested that the bioequivalence of insulin action between detemir and human insulin i.v. occurred at a molar ratio greater than the commercially available formulation (4:1), pilot studies were performed to establish the concentration of detemir infused i.v. 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 in euglycemia) was 8:1 with an initial bolus of detemir twice as great than that of human insulin. In fact, lower detemir infusion concentrations resulted in biological activity which remained constantly behind that of human insulin, whereas higher concentrations and/or bolus produced overshooting of glucose infusions over the last two-three hours of the clamp studies (data not shown). Subjects were studied in random order, 14-21 days apart, during i.v. infusion of either insulin detemir (Levemir®), Novo Nordisk, 1 U = 24 nmol, purchased by Apostato Apotheke, Frankfurt, Germany) or regular human insulin (henceforth indicated as HI) (Actrapid® U 100, Novo Nordisk, 1 U = 6 nmol) 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. In order to use the same rate of infusion with both regular HI and detemir insulin, insulin solutions were diluted to final concentrations of 1 IU/ml and 2 U/ml, respectively, in saline [0.9% (w/v) 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 07:30 h on the day of study, after an overnight fast. One intravenous 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 i.v. bolus of either 10 mU/kg (0.01ml/kg) of HI or 20 mU/kg (0.01ml/kg) of detemir insulin, was given. Subsequently, a continuous intravenous infusion of 1 mU/Kg/min (6 pmol/kg/min) of HI or 2 mU/kg/min (48 pmol/kg/min) of detemir insulin was initiated and maintained 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, Mass., USA), and continued at variable rate according to the principle of the eu- and hypoglycemic glucose clamp technique on all four study occasions. On two occasions plasma glucose was maintained at the target value of 90 mg/dl (euglycemic clamps, henceforth indicated as Eu-HI and Eu-Det, human insulin and detemir insulin, respectively), whereas on the two other occasions plasma glucose was clamped at sequential target glucose concentrations of 90, 78, 66, 54, 42 mg/dl (hypoglycemic clamps, indicated as Hypo-HI and Hypo-Det). 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 withdrawn, and the glucose infusion increased to quickly restore euglycemia. Subjects were given a meal and observed until their plasma
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Glucose was consistently euglycemic for at least one hour without glucose infusion, after which they were discharged.

In all studies blood samples were drawn at 5-10 min intervals for plasma glucose measurements and at 30-min intervals for measurements of plasma insulin, C-peptide, counterregulatory hormones and non-glucose 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, drowsiness) and three non-specific (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’, ‘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), Paced Auditory Serial Addition Test (PASAT 3 sec) (30), with tests being 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 but PASAT which was presented on an audiocassette tape to control the rate of stimulus presentation. Each subject practiced these tasks on each study occasion, i.e., prior to 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: \( Z = \frac{x - M}{SD} \), where \( x \) is the original score, and \( M \) and \( SD \) are the mean and standard deviation 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, PASAT); 3) attention and executive function (composite of Trail Making B and Stroop color-word subtest); 4) fluency (Verbal Fluency alone).

Analytical methods. Bedside plasma glucose was measured using a Beckman Glucose Analyzer (Beckman Instruments, Palo Alto, CA, USA). Plasma C-peptide was measured by RIA (Linco Research, St. Charles, MO, USA). Plasma insulin was measured using a two-site sandwich chemiluminescent immunoassay for HI (MLT Ltd, Cardiff, UK). Glucagon, growth hormone, cortisol, adrenaline and noradrenaline, plasma glycerol, \( \beta \)-hydroxybutyrate, lactate, and alanine were measured by previously described assays (32). Plasma free fatty acid and pancreatic polypeptide concentrations were measured using commercial kits (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany; Human Pancreatic Polypeptide, Linco Research, St. Charles, MO, USA).

Statistical Analysis. All data were subjected to repeated measures analysis of variance (ANOVA) with Huynh-Feldt adjustment for nonsphericity (33). The ANOVA model included the sequence of studies as between-subjects factor, whereas test condition (Eu vs Hypo) 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 for plasma insulin concentrations was calculated according to the trapezoidal rule.

A modified Bonferroni procedure (34) for multiple cognitive test adjustments was used in order 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 zero (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±SDs 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 (Kaysville, UT, USA) (36) and Statistica software, version 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS

**Plasma Glucose, Glucose infusion rate, plasma C-Peptide and insulin concentrations.** (Figure 1) Plasma glucose (PG) was maintained at the pre-selected plateaus, without any significant difference between HI and detemir either in euglycemic and in hypoglycemic studies.

Glucose infusion rate (GIR) was higher in eu- as compared to hypoglycemia, with both detemir and HI ($p<0.001$). With detemir, GIR was lower vs HI from time 30 to 105 min ($p<0.05$) in the euglycemic studies, and from time 30 min 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 HI, 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 as compared to HI, both in euglycemic and hypoglycemic studies ($p<0.05$).

Plasma insulin concentrations were greater in the detemir as compared to the HI studies, without any difference between eu- and hypoglycemia (AUCs: 845±90 µU/ml/min and 857±143 µU/ml/min detemir studies, 83±20 µU/ml/min and 80±13 µU/ml/min HI studies, eu- and hypoglycemia, respectively). Indeed, plasma detemir concentrations were on average 11 times greater than HI (95% CI 8.8-13.2). No formal statistics were applied to insulin levels given the expected higher concentrations of insulin detemir as compared to HI. The time to reach plasma insulin plateau, however, was similar for both insulins (8.5± 6.1 vs 9± 6.3 min, detemir and HI, respectively, $p=0.2$).

**Plasma counterregulatory hormone concentrations.** (Figure 2) In euglycemic control studies, there were no significant changes in plasma concentrations of any of the counterregulatory hormones both with detemir and HI, 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 HI, respectively, both $p<0.05$).

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

**Symptom scores.** (Figure 3) Symptom scores increased significantly during hypoglycemia as compared to euglycemia, with both detemir and HI. The mean total symptom score was greater in the Hypo-Det than in the Hypo-HI 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 Hypo-Det as compared to the Hypo-HI 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, Hypo-Det vs Hypo-HI, respectively, p<0.05) (Figure 3). Adrenergic symptoms accounted for the greater maximal autonomic symptoms score (3±3.4 vs 2.4±1.8, Hypo-Det vs Hypo-HI, respectively, p<0.05), whereas no difference was seen in cholinergic symptoms (Figure 3).

**Cognitive function.** (Figure 4, Table 1). Raw scores for each cognitive test at baseline and during each plateau period of eu- and hypoglycemic studies, are given in Table 1. All tests but Digit Span backword and Trail Making A and B, deteriorated significantly during hypoglycemia as compared to euglycemic control studies (p<0.05). During hypoglycemia, PASAT and Stroop word subtest were different between detemir and HI. In fact, performance deterioration in the PASAT and Stroop word subtest was greater in the Hypo-Det study as compared to Hypo-HI study.

Mean of overall z scores (0.1±0.5 vs -0.1±0.5, Eu-Det and Eu-HI, respectively) and composites for each pre-specified cognitive domain (Figure 4) showed no difference between the two insulins in euglycemia studies (p>0.2). In hypoglycemia studies, attention and information processing deteriorated both with detemir and HI (p<0.05 vs euglycemia). However, the degree of deterioration was greater with detemir as compared to HI (p<0.05). Memory was not affected in the Hypo-HI study, whereas it was significantly impaired in the Hypo-Det study as compared to all other studies (p<0.05). In contrast, fluency deteriorated only during the last plateau of the hypoglycemic studies, without differences between detemir and HI (p>0.2).

**Plasma free fatty acid, β-hydroxybutyrate, glycerol, alanine, and lactate concentrations.** (Figure 5) Plasma FFA concentrations decreased significantly in eu- and hypoglycemia with both detemir and HI. However, at time 30 min, FFAs were significantly less suppressed with detemir as compared to HI, both in eu- and hypoglycemic studies. Beta-hydroxybutyrate concentrations followed a pattern similar to FFAs, without any difference between detemir and HI studies. Glycerol and alanine concentrations did not change significantly throughout the studies, whereas lactate concentrations increased significantly in hypoglycemia as compared to euglycemia, without differences between detemir and HI (p>0.2).

**Glycemic thresholds (Table 2).** 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 thresholds for counterregulatory hormone responses. Thresholds for initiation of adrenergic symptoms were significantly higher (i.e. occurred at lower plasma glucose
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concentrations) in the Hypo-Det study as compared to the Hypo-HI study (Table 2, Figure 3). Thresholds for initiation of cognitive dysfunction, resulted significantly lower (occurred at higher plasma glucose concentrations) in the Hypo-Det study as compared to the Hypo-HI study (Table 2).

**DISCUSSION**

The present study indicates that the responses to hypoglycemia induced by i.v. equipotent doses of the long-acting insulin detemir and regular HI in humans differ. Although the response of counterregulatory hormones was no different, other responses indicate unequivocal differences between detemir and HI. 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 vs HI. Notably, these results have been obtained with rates of insulin infusion resulting in plasma HI concentrations in the physiological range of the post-prandial 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 to HI.

The reasons for the differences in response to hypoglycemia observed in the present study are not known. It is possible, however, that detemir and HI 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 HI, 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 BBB in proportion to plasma insulin levels via a receptor-mediated transport 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 (44). 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. Indeed, 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, 45-47) aiming at evaluating the effect of different plasma insulin concentrations have reported results which 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 as compared to the present study (19, 45-47). Thus, a difference between the present and other studies involving hyperinsulinemic clamp procedures (8, 9, 19,
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45-47), 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 (48), were similar in euglycemia with both detemir and HI, further indicating the matched peripheral actions of both detemir and HI. In addition, changes in substrates levels such as lactate, whose levels increase during higher insulin infusions in relation to augmented catecholamine levels (46), might have affected brain responses, in particular causing improvement of cognitive function (47). 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 (49, 50). This is not the case in our study where 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 HI. In one hypoglycemic stepwise clamp study (8), responses were examined during hypoglycemia induced either with i.v. detemir (5 mU/Kg/min) or HI (2 mU/Kg/min) in normal subjects. In the other study (9), hypoglycemia was induced by s.c. injection of detemir (0.5 U/Kg) or NPH (0.5 U/Kg) insulin in subjects with type 1 diabetes mellitus. The former study found increased sweating with detemir as compared to HI. The latter study, did not indicate any significant difference between detemir and HI 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 HI 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 (51), must be taken into account in order 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 to NPH and glargine insulin (52). 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 symptom hunger either in eu- or in hypoglycemia with detemir as compared to HI. 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 HI 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 (53). Consequently, the increased maximal score and the higher threshold of autonomic/adrenergic symptoms with detemir as compared to HI 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 HI. This effect, however, may be dependent on the greater brain concentration of detemir insulin which enables it to suppress brain glucose utilization (54) and hence cause neuroglycopenia. The higher score of neuroglycopenic symptoms with detemir as compared to HI might be similarly interpreted. Alternatively, since insulin plays an important role in memory and other aspects of brain function, cerebral detemir-induced hyperinsulinemia might provoke synchronous increases of inflammatory markers and β-amyloid in the brain that may have deleterious effects on cognition (55).

We acknowledge that one should use considerable caution to extrapolate from the experimental situation of the present study which analyzes normal-non diabetic subjects with i.v. infusion of insulin detemir, to the clinical situation of diabetic subjects who receive therapeutic insulin doses of detemir as s.c. 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 towards a delayed perception of symptoms to hypoglycemia, and an earlier deterioration of cognitive function with detemir as compared to HI. 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 vs HI. On the other hand, insulin detemir has been shown to reduce hypoglycemia in type 1 and type 2 diabetes (52) and therefore detemir is expected to prevent/improve rather than inducing/deteriorating hypoglycemia unawareness. However, the present study disclosing subtle, but net differences between detemir and HI in normal, non-diabetic subjects, legitimates the need for additional studies in subjects with T1 and T2 diabetes mellitus to establish the effects of detemir on overall responses of counterregulatory hormones, symptoms and onset of cognitive dysfunction, after s.c. injection of therapeutic doses of this long-acting analog compared to other basal insulin formulations.

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REFERENCES


TABLE 1. Raw scores for each cognitive test at baseline and during each plateau period with either detemir (Det) or regular human insulin (HI) in eu- and hypoglycemic studies (indicated as Eu- and Hypo-).

<table>
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<tr>
<th>Time of test (min)</th>
<th>Basal</th>
<th>90/90</th>
<th>90/78</th>
<th>90/66</th>
<th>90/54</th>
<th>90/42 mg/dl</th>
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<td>Nominal PG(Eu/Hypo)</td>
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### 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
- **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
- **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
- **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
- **Eu-HI**: 12.8±3.5, 11.8±5.0, 11.7±8.8, 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, 11.6±3.4

### 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
- **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
- **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
- **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
- **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
- **Eu-HI**: 53.4±8.0, 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±10.5

Values are means±SD. * N. of responses, † Time in sec.; ‡ Hypo≠Eu, § Hypo-Det≠Hypo-HI, p<0.05.
### TABLE 2. Glycemic thresholds of response of counterregulatory hormones, onset of deterioration of brain function and initiation of symptoms.

<table>
<thead>
<tr>
<th>Glycemic thresholds mean±SD (mg/dl)</th>
<th>Detemir</th>
<th>HI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Peptide</td>
<td>78±9.5</td>
<td>83±7.6</td>
<td>0.316</td>
</tr>
<tr>
<td>Glucagon</td>
<td>64±6.8</td>
<td>66±7.7</td>
<td>0.586</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>62.5±3.9</td>
<td>64±3.4</td>
<td>0.339</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>54±4.9</td>
<td>55±5.6</td>
<td>0.138</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>61±8.4</td>
<td>63±8.5</td>
<td>0.600</td>
</tr>
<tr>
<td>Cortisol</td>
<td>57±5.9</td>
<td>55±7.6</td>
<td>0.531</td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>57±6.6</td>
<td>56±10.1</td>
<td>0.823</td>
</tr>
<tr>
<td>Autonomic symptoms</td>
<td>55±8.6</td>
<td>57±7.4</td>
<td>0.892</td>
</tr>
<tr>
<td>Adrenergic</td>
<td>51±7.7</td>
<td>56±7.8</td>
<td>0.029</td>
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<tr>
<td>Colinergic</td>
<td>54±9.2</td>
<td>53±9.2</td>
<td>0.798</td>
</tr>
<tr>
<td>Neuroglycopenic symptoms</td>
<td>49±6.6</td>
<td>47±4.5</td>
<td>0.432</td>
</tr>
<tr>
<td>Cognitive dysfunction</td>
<td>51±8.1</td>
<td>47±3.6</td>
<td>0.031</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Figure 1:** Glucose infusion rates, plasma glucose and C-peptide concentrations during eu- and hypoglycemia studies (mean±SE). Dashed and solid arrows indicate temporal intervals of statistically significant differences between studies; * Eu-Det ≠ Eu-HI, § Hypo-Det≠Hypo-HI, p<0.05.

**Figure 2:** Plasma counterregulatory hormones concentrations during eu- and hypoglycemia studies (mean±SE). All responses to hypo- were significantly higher as compared to euglycemia (p<0.05). No differences were observed in euglycemia between detemir and HI. § Hypo-Det≠Hypo-HI, p<0.05.

**Figure 3:** All symptoms scores increased significantly in hypoglycemia as compared to euglycemia studies, both with detemir and HI (mean±SE) (p<0.05). No differences were observed in euglycemia between detemir and HI. During hypoglycemia, maximal total, autonomic and neuroglycopenic symptoms responses were greater in the Hypo-Det study. Adrenergic symptoms accounted for the higher maximal autonomic symptoms score in the Hypo-Det study, although the response appeared delayed as compared to the Hypo-HI study. § Hypo-Det≠Hypo-HI, p<0.05.

**Figure 4:** Mean±SE of overall Z scores and scores for each pre-specified cognitive domain (see text). No differences were observed in euglycemia between detemir and HI. Maximal impairment of cognitive function during hypoglycemia resulted greater with detemir in all domains with the exception of fluency. § Hypo-Det≠Hypo-HI, p<0.05.

**Figure 5:** FFA, beta-hydroxybutyrate, glycerol, lactate and alanine concentrations during eu- and hypoglycemia studies. No differences were observed between detemir and HI, with the exception of a lower FFA suppression at the beginning of the detemir studies. * Eu-Det ≠ Eu-HI, § Hypo-Det≠Hypo-HI, p<0.05.
FIGURE 1

Counterregulatory responses with insulin detemir in humans

Plasma Glucose

C-Peptide

Glucose infusion rates

Time (min)
FIGURE 3

Counterregulatory responses with insulin detemir in humans
FIGURE 4

Counterregulatory responses with insulin detemir in humans
FIGURE 5

Counterregulatory responses with insulin detemir in humans