Cerebral Blood Flow and Glucose Metabolism in Appetite-Related Brain Regions in Type 1 Diabetic Patients After Treatment With Insulin Detemir and NPH Insulin

A randomized controlled crossover trial

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OBJECTIVE—To test the hypothesis that insulin detemir, which is associated with less weight gain than other basal insulin formulations, exerts its weight-modulating effects by acting on brain regions involved in appetite regulation, as represented by altered cerebral blood flow (CBF) or cerebral glucose metabolism (CMR_{glu}).

RESEARCH DESIGN AND METHODS—Twenty-eight male type 1 diabetic patients (age 36.9 ± 9.7 years, BMI 24.9 ± 2.7 kg/m^2, A1C 7.5 ± 0.6%) successfully completed a randomized crossover study, consisting of two periods of 12-week treatment with either insulin detemir or NPH insulin, both in combination with prandial insulin aspart. After each treatment period, patients underwent positron emission tomography scans to measure regional CBF and CMR_{glu}.

RESULTS—After 12 weeks, A1C, daily insulin doses, fasting insulin, and blood glucose levels were similar between treatments. Insulin detemir resulted in body weight loss, whereas NPH insulin induced weight gain (between-treatment difference 1.3 kg; $P = 0.02$). After treatment with insulin detemir relative to NPH insulin, CBF was higher in brain regions involved in appetite regulation, whereas no significant difference in CMR_{glu} was observed.

CONCLUSIONS—Treatment with insulin detemir versus NPH insulin resulted in weight loss, paralleled by increased CBF in appetite-related brain regions in the resting state, in men with well-controlled type 1 diabetes. These findings lend support to the hypothesis that a differential effect on the brain may contribute to the consistently observed weight-sparing effect of insulin detemir.

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Intensive insulin therapy in type 1 diabetes helps patients attain normoglycemia and improve long-term diabetes outcome. These benefits, however, may be offset by increased risk of hypoglycemia and body weight gain. Insulin detemir is a basal insulin analog that has weight-sparing effects compared with other basal insulin formulations in both type 1 and type 2 diabetes (1), but to date the exact mechanisms underlying these effects have not been elucidated.

In contrast to its anabolic effects in peripheral tissues in the brain, insulin acts as a satiety signal. These central effects have been established mainly in rodent studies, in which insulin was administered intracerebroventricularly (2,3). Effects of insulin on the human brain have been studied by intranasal insulin administration, which results in direct brain insulin uptake without systemic effects (4). A single dose of intranasal insulin intensified postmeal satiety in women (5) and decreased food intake in men (6), whereas 8-week intranasal insulin administration was associated with weight loss in men only (7).

It has been hypothesized that, in comparison with other insulin formulations, insulin detemir enters the brain more easily owing to the fatty acid attached to the insulin molecule (8). Furthermore, insulin detemir is suggested to have stronger effects on brain functions than other basal insulin therapies: insulin detemir infusion in mice and healthy humans resulted in enhanced cortical activity compared with human insulin (as measured with electroencephalography and magnetoencephalography) and decreased food intake (9–11). These results suggest the existence of tissue-specific kinetics of insulin detemir in the brain.

In addition to methods such as electroencephalography and magnetoencephalography, both of which measure neuronal activity in cortical areas only, positron emission tomography (PET) can be used to quantify metabolic effects of insulin within the whole brain. Using [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and PET, it has been shown that the brain is sensitive to insulin with respect to its action on glucose uptake and metabolism (12,13). Also, based on the observed blunting of the effect of insulin on cerebral glucose metabolism (CMR_{glu})

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in obese men with peripheral insulin resistance compared with lean insulin sensitive men, the existence of central insulin resistance in humans was postulated (14). CMR_{glu} is known to be closely linked to cerebral blood flow (CBF). The gold standard to obtain regional CBF in humans is $[^{15}O]H_2O$ PET. Regional CBF (measured using single-photon emission computed tomography) and CBF velocity (measured by transcranial Doppler) were found to have a negative association with BMI in humans (15,16). In rats, topically applied insulin increased cortical blood flow (17), but in a small study acute hyperinsulinemia during a euglycemic clamp was not associated with an effect on CBF in healthy and impaired glucose tolerant subjects (13).

The purpose of the current study was to assess whether insulin detemir, compared with NPH insulin, alters CBF or CMR_{glu} in appetite-related brain regions in type 1 diabetic patients as a potential mechanism contributing to the reported differential effects on body weight.

RESEARCH DESIGN AND METHODS

From January 2009 until May 2011, patients were included in this randomized controlled crossover trial; the last follow-up visit was on 13 December 2011. Thirty-five patients with type 1 diabetes, aged 18–60 years and with a BMI of 18–35 kg/m^2, were included; they were recruited from the outpatient clinic of the VU University Medical Center (VUMC) and from neighboring hospitals. After giving written informed consent, all participants had a screening visit consisting of a medical history, physical examination, and fasting blood and urine analyses. Exclusion criteria were diabetes duration <1 year; A1C >8.5%; proliferative retinopathy; a history of recurrent severe hypoglycemia (defined as an episode that requires external assistance for recovery); a medical history of hypoglycemia unawareness; history of cardiovascular, renal, or liver disease or severe head trauma; any neurological or psychiatric disorder; endocrine diseases not well controlled for the last 3 months; inability to undergo magnetic resonance imaging (MRI) scanning; substance abuse; and the use of anticoagulants, oral steroids, or any centrally acting agent. Of all patients in analysis, one had microalbuminuria, four stable background retinopathy, and one peripheral neuropathy (Toronto score [18] of 9/19 and a vibration perception [19] threshold of >25 V at 5 of 12 locations). Three patients were treated with antihypertensive medication (one used an angiotensin II receptor antagonist [ARB], one an ACE inhibitor and an ARB, and one an ACE inhibitor and ARB, a diuretic, and a calcium antagonist). Three patients used cholesterol-lowering medication, and one used aspirin as well. Two patients had stable hypothyroidism treated with thyroxin, and one had stable ulcerative colitis treated with mesalazin. The study was approved by the Medical Ethics Review Committee of the VUMC and the Central Committee on Research involving Human Subjects. The study was conducted according to the Declaration of Helsinki.

The study was conducted in a randomized crossover design and was part of a larger trial (ClinicalTrials.gov, clinical trial reg. no. NTC00626080). Primary outcomes were CBF and CMR_{glu} after a 12-week treatment period, and change in body weight after this 12-week treatment was a secondary outcome measurement. After a run-in period of at least 4 weeks, during which the current insulin therapy was optimized, patients were randomly assigned to start with either insulin detemir or NPH insulin in the evening, both in combination with insulin aspart at mealtimes. Randomization (block design) was conducted by the Trial Pharmacy of the VUMC, and the assigned treatments were concealed by envelopes; a research physician (L.W.v.G.) enrolled patients in the study and assigned them to the intervention. After assignment, no blinding was applied, since NPH insulin needs to be mixed and visually inspected before injection. Weekly seven-point self-measured blood glucose curves were made, and all fasting blood glucose levels were reported. Where appropriate, basal insulin dose was adjusted to maintain a fasting glucose level of <7 mmol/L. Regular telephone contact was available for advice on basal and prandial insulin adjustments. After 12 weeks of treatment, patients switched from basal insulin.

On the day prior to the scan session, patients refrained from food, alcohol, and coffee intake from 2200 h onward. They were carefully instructed not to forget their basal insulin injection and, if possible, not to use any insulin aspart after their dinnertime injection. Telephone calls were made both on the night before and early in the morning of the day of the PET scan, i.e., before traveling to the hospital. In addition, a similar protocol was followed at the day of MRI scanning (a week prior to the PET scan), when patients had to arrive at the hospital at the same time in a fasting state, using the same basal insulin the night before. If necessary, the insulin regimen was adjusted after the MRI scan to improve fasting glucose levels on the day of the PET scan. Patients arrived at the hospital at 0715 h in the fasting state and remained fasted during the entire imaging procedure. Upon arrival, a catheter was placed in an antecubital vein for blood collection and tracer injection. Blood glucose levels were checked and corrected if necessary (when glucose was <4 mmol/L and falling or when glucose was >15 mmol/L). To prevent further rising of glucose during the remaining duration of the test visit, a low dose of the individual's basal insulin was injected subcutaneously. No insulin aspart was used to avoid interference with the PET measurements. After we check for collateral circulation and administration of local anesthesia using intradermal 1% lidocain, a radial artery was cannulated by an experienced anesthesiologist. Both cannulas were kept patent by a 3 IE/mL 0.9% NaCl heparin solution.

Before and immediately after scanning, patients completed a questionnaire, scoring their hunger ("How hungry are you right now?"), fullness ("How full are you at this moment?"), appetite ("How much do you feel like eating right now?"), prospective consumption ("How much could you eat right now?"), desire to eat ("How strong is your desire to eat right now?"), and thoughts of eating ("How much do you think about food right now?") on a 10-point Likert scale. Furthermore, patients scored their insulin treatment satisfaction using the Diabetes Treatment Satisfaction Questionnaire, which measures satisfaction with treatment regimen, perceived frequency of hyperglycemia, and perceived frequency of hypoglycemia over the past few weeks (20).

Data acquisition

Three-dimensional structural MRI images were acquired on a 3.0 T GE Signa HDxt scanner (General Electric, Milwaukee, WI), using a T1-weighted fast Spoiled Gradient echo sequence. PET scans were acquired with a High Resolution Research Tomograph (HRRT) (Siemens/CTI, Knoxville, TN) PET scanner. The scanning protocol consisted of a $[^{15}O]H_2O$ scan to measure CBF and an $[^{18}F]$FDG scan to measure CMR_{glu}. Details on scan protocol have previously been published

(21). During both scans, arterial concentrations were monitored continuously, and in addition, manual samples were taken for cross-calibration of the measured input function. Samples obtained during the [^{18}F]FDG scan (15, 35, and 55 min postinjection) were also used to measure arterial plasma glucose levels. All scans were performed between 0930 and 1200 h to minimize diurnal variations.

Data analyses

List mode emission data were histogrammed into multiframe sinograms, which subsequently were normalized, and corrected for randoms, dead time, decay, scatter, and attenuation. Fully corrected sinograms were reconstructed using the standard 3D Ordinary Poisson Ordered-Subsets Expectation Maximization (OP-OSEM) reconstruction algorithm (22), resulting in 207 image planes with 256 × 256 voxels and a voxel size of 1.22 × 1.22 × 1.22 mm^3 (21). The effective spatial resolution of the reconstructed images was ~3 mm.

MRI and PET images were coregistered using the software package VINCI (23). PET images were rebinned, and PET and MRI images were cropped into a 128 × 128 × 126 matrix (21). Regions of interest (ROIs) were delineated on the MRI scan using the template defined in PVElab (24). Subsequently, all ROIs were projected onto the dynamic PET images, generating time activity curves (TACs) for the following 16 left and right regions: orbitofrontal cortex, anterior and posterior cingulate cortex, thalamus, insula, caudate nucleus, putamen, medial inferior frontal cortex, superior temporal cortex, parietal cortex, medial inferior temporal cortex, superior frontal cortex, occipital cortex, sensorimotor cortex, cerebellum, hippocampus, a single white matter region, a total gray matter region, and striatum (putamen and caudate nucleus combined). Of these ROIs, the first seven were of specific interest, as these are involved in appetite regulation and reward.

With use of standard nonlinear regression (NLR), appropriately weighted [^{15}O]H_2O TACs were fitted to the standard one-tissue compartment model (25) to obtain regional CBF values. In addition, parametric (voxel-wise) CBF images were generated from 6-mm full-width-at-half-maximum Gaussian smoothed dynamic [^{15}O]H_2O images using a basis function method (BFM) implementation of the same model (26).

With use of a standard NLR algorithm, appropriately weighted [^{18}F]FDG TACs were fitted to an irreversible two-tissue compartment model with three rate constants and blood volume as fit parameters. Next, the net rate of influx K_i was calculated as $K_1 \cdot k_3/(k_2+k_3)$, where K_1 is the rate of transport from blood to brain, k_2 the rate of transport from brain to blood, and k_3 the rate of phosphorylation by hexokinase. Finally, K_i was multiplied with the plasma glucose concentration and divided by a lumped constant (LC) of 0.81 (27) to obtain regional CMR_{glu} values. In addition, parametric CMR_{glu} images were generated using Patlak linearization (28).

Biochemical analyses

Capillary blood glucose (patient monitoring) was measured using a blood glucose meter (OneTouch UltraEasy; LifeScan, Milpitas, CA). Arterial glucose samples (to determine CMR_{glu}) were measured using the hexokinase method (Glucoquant; Roche Diagnostics, Mannheim, Germany). A1C was measured by cation-exchange chromatography (reference values 4.3–6.1%; Menarini Diagnostics, Florence, Italy). Serum insulin concentrations were quantified using immunometric assays (Centaur; Siemens Diagnostics, Deerfield, IL); insulin detemir levels were divided by four to compensate for the difference in molar dose ratio relative to NPH insulin. Urine microalbumin was quantified using immunonephelometry (Immage 800; Beckman Coulter, Brea, CA).

Statistical analysis

Data are expressed as mean ± SD. Skewed data and ordinal values are expressed as median and interquartile (IQ) range. Differences between both insulin treatments were tested by repeated-measures analysis or the Wilcoxon signed rank test (insulin detemir vs. NPH insulin). Analyses were performed using SPSS for Windows, version 20.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

Parametric images were analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, U.K.). Parametric images were smoothed using a 6-mm full-width-at-half-maximum Gaussian kernel, coregistered to corresponding T1-weighted MRI images and normalized to Montreal Neurological Institute space. Paired *t* tests were performed (insulin detemir vs. NPH insulin).

With use of data of 18 paired H_2O PET measurements and an expected difference in total gray matter CBF of 15% (0.046 ± 0.05 mL · cm^{-3} · min^{-1}), our study had a power of 96% (α 0.05) to detect differences between treatment with insulin detemir and NPH insulin. With use of 24 paired FDG PET data and an expected difference in total gray matter CMR_{glu} of 7.5% (0.011 ± 0.02 μmol · cm^{-3} · min^{-1}), our study had a power of 73% to detect differences between treatments.

RESULTS—During the study, one patient dropped out during his first treatment period (because of NPH insulin schedule difficulties) and one in the second period (because of a hip fracture). Owing to technical problems (n = 2) and patient movement (n = 2), combined [^{18}F]FDG and [^{15}O]H_2O data were discarded for these four subjects. [^{15}O]H_2O was not available for one patient on both occasions and for three patients on one occasion. After quality control of the remaining scans, paired CMR_{glu} data were available in 24 patients and paired CBF measurements in 18 patients.

Subject characteristics of all 28 patients included in the analyses are listed in Table 1. Of all patients included in the analyses (n = 28), 15 patients started with NPH insulin and 13 with insulin detemir. Of patients starting with NPH insulin, 5 had used insulin detemir and

Table 1—*Patient characteristics*

n	28
Age (years)	36.9 ± 9.7
Diabetes duration (years)	12.8 (6.0–17.0)
Pretrial insulin detemir	9 (32)
Pretrial NPH insulin	1 (4)
Pretrial insulin glargine	18 (64)
Body weight (kg)	82.4 ± 12.7
BMI (kg/m^2)	24.9 ± 2.7
Systolic blood pressure (mmHg)	117 ± 9
Diastolic blood pressure (mmHg)	78 ± 7
A1C (%)	7.5 ± 0.6
Total cholesterol (mmol/L)	4.5 ± 0.6
HDL cholesterol (mmol/L)	1.4 ± 0.4
LDL cholesterol (mmol/L)	2.5 ± 0.6
Triglycerides (mmol/L)	1.1 ± 0.5
Urine albumin-to-creatinine ratio (mmol/mg)	1.1 ± 2.9

Data are mean ± SD, median (IQ range), or *n* (%) unless otherwise indicated.

10 insulin glargine, while of those starting with insulin detemir, 4 had used insulin detemir, 1 NPH insulin, and 8 insulin glargine before the trial. At the end of the treatment period, daily insulin doses and A1C did not differ between treatment (Table 2). Insulin detemir decreased body weight by 0.7 kg, whereas NPH insulin increased weight by 0.6 kg (between-treatment difference 1.3 kg, $P = 0.02$) (Table 2). Perceived hyperglycemia and hypoglycemia did not differ significantly between treatments (Diabetes Treatment Satisfaction Questionnaire); patient satisfaction was significantly greater when with use of insulin detemir than NPH insulin ($P = 0.003$). Irrespective of the treatment arm, patients scored five of six items (hunger, appetite, prospective consumption, desire to eat, and thoughts of eating) significantly higher after the scan than before the scan ($P < 0.01$ for each item), indicating that appetite increased during the scanning period (all were fasting). When treated with insulin detemir, patients scored higher on the sixth item, i.e., fullness, after the PET scan than patients treated with NPH insulin (mean 4.0 [IQ range 3.0–5.0] vs. 3.0 [2.0–4.0], $P = 0.03$ for between-group difference).

For insulin detemir, on the day of the PET scan, three patients, of whom two were excluded afterward from the CBF analyses, required several dextrose tablets to prevent or resolve a mild hypoglycemia, whereas six patients, of whom one was excluded from the CBF analyses, received ~20 mL i.v. 20% glucose before the scan to prevent hypoglycemia. One patient received insulin detemir (12 IU s.c.) because glucose was rising upon arrival at the hospital. For NPH insulin, three patients, of whom two were excluded from the CBF analyses, required dextrose tablets because of a low or falling blood glucose level, whereas two patients, who were afterward excluded from the CBF analyses, received ~15 mL i.v. 20% glucose before the PET scan started. Three patients, who all were included in the CBF analyses, required insulin NPH insulin (14, 10, and 5 IU s.c.) at arrival in the hospital as a result of hyperglycemia. In all patients, average arterial glucose levels were stable within 10% and >5.0 mmol/L during data acquisition. For checking whether acute glucose manipulations had affected PET measurements of CBF and CMR_{glu}, a separate analysis was performed in which patients who had received glucose or insulin were excluded. Results of this additional analysis, however, were similar to those of the original analysis (data not shown).

NLR analysis showed that, after treatment with insulin detemir compared with treatment with NPH insulin, CBF was higher in all regions. This was statistically significant in most appetite-related brain regions—bilateral insula, bilateral putamen and right caudate nucleus, right thalamus, and bilateral anterior and right posterior cingulate cortices—when patients received insulin detemir versus NPH insulin (Table 3). In addition, higher CBF was observed in the right medial inferior frontal cortex, bilateral parietal cortex, and bilateral sensorimotor cortex (all $P < 0.05$) after treatment with insulin detemir versus NPH insulin. In all other brain regions investigated, CBF was similar for both treatments. Results were similar after exclusion of patients using antihypertensive medication ($n = 3$) and after exclusion of the one left-handed patient. After adjustment for A1C, glucose, and insulin levels, CBF differences in appetite-related regions remained unaltered (data not shown). No significant correlation between changes in CBF and changes in glucose, insulin, and A1C levels or body weight was found. Regional analyses of parametric images showed good correlation with regional NLR analyses (slope = 0.99,

Table 2—*Clinical characteristics before and at the end of each treatment period*

Patient characteristics ($n = 28$)	NPH insulin	Insulin detemir
Body weight, $t = 0$ weeks (kg)	82.7 ± 12.6	83.1 ± 12.6
Body weight, $t = 12$ weeks (kg)	83.4 ± 13.0	82.4 ± 12.4*
ΔBody weight (kg)	0.6 ± 1.9	−0.7 ± 1.8*
Systolic blood pressure (mmHg)	112 ± 10	113 ± 9
Diastolic blood pressure (mmHg)	75 ± 7	76 ± 5
A1C, $t = 0$ weeks (%)	7.3 ± 0.6	7.4 ± 0.6
A1C, $t = 12$ weeks (%)	7.4 ± 0.6	7.4 ± 0.6
Daily insulin dose, basal, 12 weeks (IU/day)	25.9 ± 11.0	26.5 ± 10.1
Daily insulin dose, aspart, 12 weeks (IU/day)	31.4 ± 11.8	31.0 ± 11.2
Serum insulin during PET (pmol/L)	75.6 (62.0–110.7)	85.6 (58.4–119.3)
Blood glucose during PET (mmol/L)	10.7 ± 2.9	9.9 ± 3.1

Data are mean ± SD or median (IQ range). *$P < 0.05$ for treatment effect.

Table 3—*Regional PET-measured CMR_{glu} and CBF at the end of each intervention period*

	CMR_{glu}			CBF		
	NPH	Detemir	*P*	NPH	Detemir	*P*
Total gray matter	0.15 ± 0.02	0.16 ± 0.02	0.2	0.31 ± 0.05	0.34 ± 0.05	0.06
Regions of interest						
OFC L	0.18 ± 0.03	0.18 ± 0.02	0.7	0.38 ± 0.06	0.40 ± 0.08	0.2
OFC R	0.18 ± 0.03	0.18 ± 0.02	0.7	0.39 ± 0.07	0.41 ± 0.08	0.3
Insula L	0.17 ± 0.03	0.18 ± 0.03	0.4	0.40 ± 0.07	0.44 ± 0.09	0.04
Insula R	0.17 ± 0.03	0.17 ± 0.03	0.8	0.39 ± 0.08	0.43 ± 0.08	0.05
Putamen L	0.21 ± 0.04	0.22 ± 0.03	0.3	0.40 ± 0.07	0.44 ± 0.09	0.04
Putamen R	0.21 ± 0.04	0.22 ± 0.03	0.3	0.40 ± 0.06	0.45 ± 0.09	0.02
Caudate L	0.19 ± 0.05	0.20 ± 0.04	0.6	0.34 ± 0.06	0.37 ± 0.08	0.08
Caudate R	0.19 ± 0.04	0.20 ± 0.03	0.2	0.31 ± 0.06	0.36 ± 0.09	0.02
Striatum	0.21 ± 0.04	0.22 ± 0.03	0.2	0.37 ± 0.06	0.42 ± 0.09	0.02
Thalamus L	0.18 ± 0.03	0.19 ± 0.03	0.4	0.39 ± 0.06	0.43 ± 0.07	0.07
Thalamus R	0.18 ± 0.03	0.19 ± 0.03	0.3	0.38 ± 0.06	0.43 ± 0.08	0.04
Cingulate ant L	0.16 ± 0.03	0.17 ± 0.03	0.4	0.36 ± 0.07	0.39 ± 0.09	0.03
Cingulate ant R	0.16 ± 0.02	0.17 ± 0.03	0.2	0.38 ± 0.07	0.41 ± 0.09	0.04
Cingulate post L	0.21 ± 0.03	0.22 ± 0.04	0.2	0.38 ± 0.06	0.41 ± 0.08	0.1
Cingulate post R	0.22 ± 0.03	0.22 ± 0.04	0.9	0.39 ± 0.06	0.43 ± 0.08	0.02

Data are mean ± SD unless otherwise indicated. CBF in $mL \cdot cm^{-3} \cdot min^{-1}$. CMR_{glu} in $\mu mol \cdot cm^{-3} \cdot min^{-1}$. Paired data, $n = 24$ for CMR_{glu} and $n = 18$ for CBF. ant, anterior; L, left; OFC, orbitofrontal cortex; post, posterior; R, right.

$R^2 = 0.93$, for $n = 5$ NPH and $n = 5$ insulin detemir, data not shown; similar to data obtained in healthy subjects [21]). These parametric analyses (voxel level) did not provide additional findings relative to regional NLR analyses.

During the [^{18}F]FDG scan, mean arterial plasma glucose levels did not differ between treatments; serum insulin levels were similar as well (Table 2). NLR analysis showed no significant differences in CMR_{glu} in appetite-related predefined (PVElab) regions (Table 3). No significant differences in transport parameters for total gray matter (K_i, K_1, k_2, and k_3), were observed (data not shown), and total gray matter CMR_{glu} did not differ significantly between treatments (0.15 ± 0.02 $\mu mol \cdot cm^{-3} \cdot min^{-1}$ after treatment with NPH insulin versus 0.16 ± 0.02 $\mu mol \cdot cm^{-3} \cdot min^{-1}$ after treatment with insulin detemir). Parametric analyses yielded similar results (data not shown).

CONCLUSIONS—The main finding of this study was that a relative loss in body weight in type 1 diabetic patients treated with insulin detemir was accompanied by an increase in CBF in insula, thalamus, anterior and posterior cingulate cortex, and striatum—regions that are involved in appetite regulation and reward. No significant differences in CMR_{glu} between groups were found.

Several studies have investigated the effects of body weight on CBF. Some of these studies suggest that changes in CBF are causal in the development of obesity. CBF responses in appetite-related brain regions to a meal in formerly obese persons were similar to those in obese persons but different from those in lean subjects (29), indicating a predisposition to obesity that may involve areas of the brain that control complex aspects of eating behavior. This is in line with the observed increase in CBF in appetite-regulating brain regions in response to meal consumption in successful dieters (30). In minipigs, however, diet-induced obesity resulted in a decrease in CBF in several of these brain regions, suggesting that the changes in CBF were the result of weight gain (31). From the current study, it is not possible to determine whether increases in CBF in patients treated with insulin detemir are cause or consequence of the observed weight loss. Previous studies in mice and healthy humans, however, showed cortical brain activation upon acute insulin detemir versus human insulin infusion with concomitant decrease in food intake (9–11). In addition, it was shown that insulin-induced glucose lowering in type 1 diabetic patients resulted in an increase in CBF (32,33). However, whether this was caused by increasing insulin or by decreasing glucose levels could not be determined in those studies. Still, a direct effect of insulin on the brain is supported by the acute effects of insulin on cerebrovascular responses in rats (17). The present CBF findings are in contrast with a study by Hirvonen et al. (13) in eight healthy volunteers and six individuals with impaired glucose tolerance, in which no between-group CBF differences were observed and no CBF effect of insulin. In their study, acute clamp-induced hyperinsulinemic (insulin levels 5–6 times higher compared with the current study) euglycemia was imposed, which is different from the insulin effect of two chronic 12-week treatment periods. In addition, fasted, elevated (glucose level 11 mmol/L) glucose levels during PET data acquisition were higher in the current study. In addition, Hirvonen et al. investigated two different subject groups, whereas we investigated only one group of individuals with type 1 diabetes and studied the effects of a chronic treatment in a crossover study design. Finally, Hirvonen et al. may not have observed the 10% difference owing to a lack of power (although insulin levels were higher, the number of subjects was much less than in the current study) or the lower signal-to-noise ratio of the PET scanner used.

In contrast to the differential effects on CBF, the two insulin treatments did not result in significant differences in CMR_{glu} in any of the regions investigated. Previous studies have shown an inverse association of CMR_{glu} and BMI (34) and increases in CMR_{glu} after stimulation with food pictures (35,36). Of note, the increase in CMR_{glu} in appetite-related brain regions after insulin infusion was blunted in insulin-resistant men compared with insulin-sensitive men (14), and it was associated with insulin resistance and overweight.

Previously, it was shown that in type 1 diabetes changes in k_3 are observed compared with healthy volunteers (37), without significant concomitant changes in CBF. Under the assumption of absence of between-group differences in phosphorylation (which were indeed absent in the present data), the relationship between CMR_{glu} and CBF is nonlinear [as CMR_{glu} and K_1 are linearly related via $CMR_{glu} = K_i \cdot \text{glucose/LC}$, where $K_i = K_1 \cdot k_3/(k_2 + k_3)$, and CMR_{glu} is linearly related to $E \cdot CBF$, where $E = 1 - \exp(-PS/CBF)$ (38,39)], and, especially at higher flow values, an increase in CBF will induce a smaller increase in CMR_{glu} (37), which is what was observed in the current study, although the latter was nonsignificant.

Possible confounders that could have accounted for the differences in CBF include A1C or prevailing glucose and insulin levels. However, these parameters were not significantly different between treatments, and the insulin detemir–induced increase in CBF was similar after adjustment for A1C, glucose, and insulin levels.

Limitations of this study include its nonblinded nature owing to differences in insulin formulations. NPH insulin is a cloudy suspension that needs to be thoroughly stirred before injection, whereas insulin detemir is a clear, colorless solution that does not require stirring. Therefore, it was not possible to perform a double-blind study. Worldwide, however, NPH insulin is the standard (intermediate) long-acting human insulin and, therefore, the best active comparator. Moreover, even if patients were aware of the type of insulin treatment, it is unlikely that this will have had an effect on the present findings. It should be noted that not all patients in the study were insulin detemir naïve, i.e., five and six patients starting with NPH insulin and insulin detemir, respectively, already used insulin detemir before the start of the study. As insulin detemir–naïve patients and insulin detemir users were equally distributed between treatment groups, it is unlikely that medication prior to the study has affected the results, especially since PET scans were performed after 12 weeks of exposure to the test insulin.

Differences in CMR_{glu} between insulin detemir and NPH insulin were not statistically significant. Data in the current study were obtained during a resting and fasting condition. In future studies, it may be of interest to investigate responses to (visual) food stimuli in appetite regulating brain regions after both treatments. However, due to radiation exposure and practical reasons (small inner diameter of the HRRT scanner, making it difficult to present visual stimuli), this was not possible in the current study. In addition, for detection of changes in brain

activation using [^{18}F]FDG PET, two separate sessions are required to test stimulated versus nonstimulated conditions (35,36).

Some patients required glucose or (basal) insulin to prevent emerging hypo- or hyperglycemia, respectively. In six patients on insulin detemir versus one on NPH insulin, glucose was necessary to prevent low or falling blood glucose levels, which could have biased results, as hypoglycemia increases CBF (32,33). As three patients in the insulin detemir versus only one in the NPH insulin group required additional basal insulin to avoid hyperglycemia, one could argue that if acute injection of basal insulin would have affected CBF, this would have attenuated the difference in CBF between the groups. More importantly, the increase in CBF in the detemir versus NPH group remained unchanged after exclusion of patients who had received insulin or glucose.

Although weight gain associated with insulin treatment is relevant for type 1 diabetic patients, it is especially important for patients with type 2 diabetes. It is tempting to generalize the present findings to type 2 diabetes, but further studies in these patients are needed, especially since central insulin resistance possibly plays a role in type 2 diabetes.

The current study focused on insulin detemir action in the brain. It should be noted, however, that other mechanisms have been proposed to explain its weight-reducing effect. These include less defensive eating due to less hypoglycemia, increased energy expenditure, and higher insulin levels in the liver compared with peripheral tissue, although none of these could be firmly established (40–43). In the current study, no significant differences in perceived hypoglycemia frequency were found between treatments.

In conclusion, the present findings support the hypothesis that a differential effect on CBF, measured during a resting, fasting condition, may contribute to the consistently observed weight-sparing effect of insulin detemir treatment.

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L.W.v.G. participated in the design of the study; performed the study, PET analyses, and statistical analyses; drafted the manuscript; edited the text; and made crucial revisions to the manuscript. R.G.I. clinically supervised the study, clinically commented on the manuscript, edited the text, and made crucial revisions to the manuscript. M.C.H. supervised the PET analyses, critically commented on the manuscript, edited the text, and made crucial revisions to the manuscript. J.F.H. clinically supervised the study, critically commented on the manuscript, edited the text, and made crucial revisions to the manuscript. R.P.H. was involved with patient recruitment, edited the text, and made crucial revisions to the manuscript. M.L.D. participated in the design of the study, edited the text, and made crucial revisions to the manuscript. A.A.L. participated in the design of the study, supervised PET analyses, critically commented on the manuscript, edited the text, and made crucial revisions to the manuscript. M.D. participated in the design of the study, edited the text, and made crucial revisions to the manuscript. R.G.I., M.C.H., A.A.L., and M.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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