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Insulin Detemir Is Transported From Blood to Cerebrospinal Fluid and Has Prolonged Central Anorectic Action Relative to NPH Insulin

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Insulin detemir (DET) reduces glycemia comparably to other long-acting insulin formulations but causes less weight gain. Insulin signaling in the brain is catabolic, reducing food intake. We hypothesized that DET reduces weight gain, relative to other insulins, owing to increased transport into the central nervous system and/or increased catabolic action within the brain. Transport of DET and NPH insulin into the cerebrospinal fluid (CSF) was compared over several hours and after the administration of different doses peripherally in rats. DET and NPH had comparable saturable, receptor-mediated transport into the CSF. CSF insulin remained elevated significantly longer after intraperitoneal DET than after NPH. When administered acutely into the 3rd cerebral ventricle, both DET and NPH insulin reduced food intake and body weight at 24 h, and both food intake and body weight remained lower after DET than after NPH after 48 h. In direct comparison with another long-acting insulin, insulin glargine (GLAR), DET led to more prolonged increases in CSF insulin despite a shorter plasma half-life in both rats and mice. Additionally, peripheral DET administration reduced weight gain and increased CSF insulin compared with saline or GLAR in mice. Overall, these data support the hypothesis that DET has distinct effects on energy balance through enhanced and prolonged centrally mediated reduction of food intake.

Insulin is secreted almost exclusively from pancreatic β -cells in response to increases in ambient glucose, circulating incretin hormones, and parasympathetic nervous system signaling (1). Insulin mediates a range of physiologic actions through an endocrine mechanism, and

the insulin receptor (IR) is widely distributed throughout peripheral tissues and brain (2,3). Given that insulin is a large peptide, active transport is required for it to cross the blood-brain barrier (BBB) in significant quantities (4). Once transported into brain interstitial fluid, insulin acts upon IR in diverse brain regions including the hypothalamus and hippocampus (5–7). The current model of insulin transport to the central nervous system (CNS) posits that insulin enters brain interstitial fluid from the plasma via receptor-mediated transport across brain endothelial cells where it can access IR on neurons and glial cells; from the brain interstitial fluid, insulin is collected into the cerebrospinal fluid (CSF), where it is cleared by IR back into the plasma. Hence, the normal movement of insulin is plasma-to-brain interstitial fluid to CSF (8,9).

Insulin detemir (DET) has a covalently attached fatty acid that promotes binding to albumin, extending the plasma half-life from a few minutes to 5–7 h (10). A common problem with insulin treatment is weight gain; patients having insulin doses titrated to achieve glycemic targets often gain from 2 to 10 kg. The reason is unclear but is likely some combination of reduced wasting of calories from glycosuria and anabolic actions of insulin on adipose tissue (11). Several clinical trials have indicated that use of DET as a basal insulin replacement leads to less weight gain than other long-acting formulations (12–14). In fact, some trials have reported weight loss in patients switching to DET from other long-acting insulin analogs (15,16).

It has been proposed that the differential effects on weight loss of DET compared with other insulin

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formulations are due to an increase of insulin action in the CNS (17). Insulin signaling in the brain is catabolic, causing reduced food intake and increased energy expenditure (18–20). However, the degree to which DET penetrates the BBB is controversial, with one report indicating that DET transport is comparable with that of regular insulin (21) and another suggesting that transport of DET through the BBB does not occur (22). The current studies were designed to assess DET transport into the CNS by measuring its appearance in the CSF, compare its entry relative to other formulations of insulin, and compare the effect of acute central administration and chronic peripheral administration of DET with other formulations of insulin on parameters of energy homeostasis.

RESEARCH DESIGN AND METHODS

Animals and Housing

Adult male Wistar rats (400–550g) were bred at the Metabolic Diseases Institute of the University of Cincinnati, and adult male C57Bl6/J mice (22–25 g) were purchased from Charles River Laboratories (Wilmington, MA). All animals were maintained in Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal facilities and maintained on 12:12-h light:dark schedule. Rats were housed in tub cages, with those animals used for dose-response and time course studies being pair housed and those used for food intake and central infusion studies being single housed. Mice were single housed in standard Static Micro Isolator cages. Unless otherwise specified, animals had ad libitum access to water and pelleted chow diet (LM-485; Harlan Teklad, Madison, WI). All protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

CSF Sampling

CSF samples were taken from rats after an overnight fast or mice after a 4-h fast. Animals were placed in the chamber of an anesthetic isoflurane machine (VetEquip, Pleasanton, CA). After anesthetization, the area from between the shoulders to below the skull was shaved. The animal was positioned securely into a stereotaxic instrument using the ear bars and an isoflurane nose cone. The incisor bar was positioned so that the head was ventroflexed. CSF sampling was performed using our previously published methods for rats and mice (23,24). Briefly, in rats a semiblunted 25-gauge (1 1/2-inch) needle was connected to microrenathane tubing and mounted on an electrode holder that was then positioned perpendicular to the ear bars in the horizontal plane. A 1.0-mL syringe containing 0.9% saline was attached, using a 25-gauge needle, to the distal end of the microrenathane tubing. The tubing was flushed with saline, and slight negative pressure was applied, creating a small air pocket in the needle and tubing. The incision site was swabbed with ethanol, and a mid-sagittal incision of the skin (~20 mm) was made inferior to the occipital crest. Muscle was separated by blunt dissection using forceps to expose the atlanto-occipital membrane, and the region was cleaned

with sterile saline-soaked cotton swabs. The tip of the needle was advanced horizontally toward the membrane using the controls of the stereotaxic instrument until it penetrated the dura mater and entered the cisternum magnum. Clear CSF flow was spontaneously initiated with very slight negative pressure and when the air bubble entered the syringe, the needle was removed, and CSF was collected via gravity flow into a 500- μ L tube on ice. On average, ~150 μ L CSF was collected within 2 min and immediately frozen on dry ice in rats, and ~15 μ L CSF was obtained in mice using a glass capillary tube. Animals were immediately killed by decapitation, and blood was collected in chilled EDTA-coated tubes and stored on ice prior to centrifugation. The CSF and plasma samples were stored at -80°C prior to analysis. We have previously observed that <6% of the samples are contaminated with blood using this method, and any contaminated samples were discarded (23).

Identification of DET in CSF After Peripheral Injection

Dose-Response Experiment

Thirty minutes prior to CSF sampling, rats were injected with insulin (0, 0.1, 0.3, 1, 3, or 10 units/kg i.p. NPH insulin or DET) and returned to their home cage. Animals ($n = 8\text{--}10/\text{dose}$) were anesthetized after 25 min, and CSF was sampled precisely at 30 min postinjection.

Time Course Experiment

Rats ($n = 6\text{--}8/\text{time point}$) were administered NPH insulin or DET (0.5 units/kg i.p.) and returned to their home cage and had CSF sampled 5, 10, 20, 40, 80, 160, or 320 min postinjection.

Comparison of the Appearance of Long-Acting Formulations of Insulin, DET, and Glargine in Blood and CSF Over Time in Rats and Mice

Rats ($n = 7\text{--}8/\text{group}$) were injected with glargine (GLAR) (0.5 units/kg s.c.) or DET (0.5 units/kg s.c.). The change in dosing route was required, as GLAR is only long-acting when injected subcutaneously. After the injection, the rats were returned to their home cage, and CSF was sampled at baseline (0) and 6, 12, 24, 48, or 72 h postinjection. Mice ($n = 7\text{--}8/\text{group}$) were injected with GLAR (1 unit/kg s.c.) or DET (1 unit/kg s.c.). After the injection, mice were returned to their home cage, and CSF was sampled at baseline (0) and 6, 12, 24, or 48 h postinjection.

Third-Ventricular Cannulation

Each rat was anesthetized with ketamine (70 mg/kg i.p.) and xylazine (6 mg/kg i.p.), its head was shaved, and the skull was positioned in a stereotaxic instrument as previously described (25). Surgery was performed using sterile techniques. A small hole was drilled through the skull at 2.2 mm posterior to bregma on the midline. The sagittal sinus was then displaced laterally, and a stainless-steel guide cannula (Plastics One, Roanoke, VA) was lowered ventrally into the 3rd ventricle, 7.5 mm ventral to the dura. The cannula placement was fixed with dental

acrylic that was anchored to the skull with three to four screws. An obturator was inserted that extended 0.5 mm below the cannula. At least 5 days prior to food intake experiments, cannula placement was confirmed by injecting 10 ng angiotensin II (American Peptides, Sunnyvale, CA) in 1 μ L saline. Rats that consumed >5 mL water within 30 min were deemed to have a viable cannula. Four rats were excluded based on this criterion.

Food Intake and Body Weight After Third-Ventricular Cannulation of Insulin in Rats

Measurements of food intake and body weight were based on an established paradigm (26). Briefly, on each of the 4 days prior to an experiment, each animal ($n = 16$) was individually acclimated to handling for 4–5-min/day and had food removed for the final 4 h of light. All animals underwent this protocol on three occasions, 1 week apart, in a randomized crossover manner to receive DET (8 mU), NPH insulin (8 mU), or 2 μ L vehicle (sterile 0.9% saline) via third-ventricular cannulation (3VT) on the experimental days. Injections occurred 1 h before dark onset. Food hoppers were returned at the onset of dark, and intake and body weight were assessed after 24 and 48 h.

Food Intake and Body Composition of Mice Chronically Treated With Long-Acting Formulations of Insulin

Prior to commencement of treatment, mice were grouped based on fat mass as assessed by nuclear magnetic resonance (NMR) in conscious animals as previously described

(27) (EchoMRI, Waco, TX). Mice ($n = 12$ /group) then received daily subcutaneous injections of normal saline, DET (1 unit/kg), or GLAR (1 unit/kg). Animals were weighed, and food intake was monitored weekly for 6 weeks, after which time body composition was reassessed. In a follow-up experiment, mice ($n = 7$ –9/group) were injected subcutaneously with normal saline, GLAR (1 unit/kg), or DET (1 unit/kg) daily for 6 weeks. Twenty-four hours after their final injection, animals had CSF and plasma sampled.

Insulin ELISA and Glucose Measurement

Insulin levels in CSF and plasma were measured using insulin ELISA (Crystal Chem, Downers Grove, IL). Briefly, a sample of CSF, plasma, or insulin standard (5 μ L/sample of plasma, 10 μ L/sample of CSF) was added to an antibody-coated microplate. After incubation for 2 h at 4°C, 100 μ L anti-insulin enzyme conjugate was dispensed into each well and incubated at room temperature for 30 min. The plate was washed, and 100 μ L enzyme substrate solution was added to each well and incubated for 40 min. The stop solution was then added, and insulin concentrations were determined by subtracting the absorbance at 630 nm from the absorbance at 450 nm. Owing to differential detection of different insulin isoforms with the antibody, standard curves were produced using NPH insulin, DET, and GLAR diluted from the injected solutions. Glucose was measured in plasma in duplicate using glucometers (Accucheck; Roche Diagnostics, Indianapolis, IN) (27).

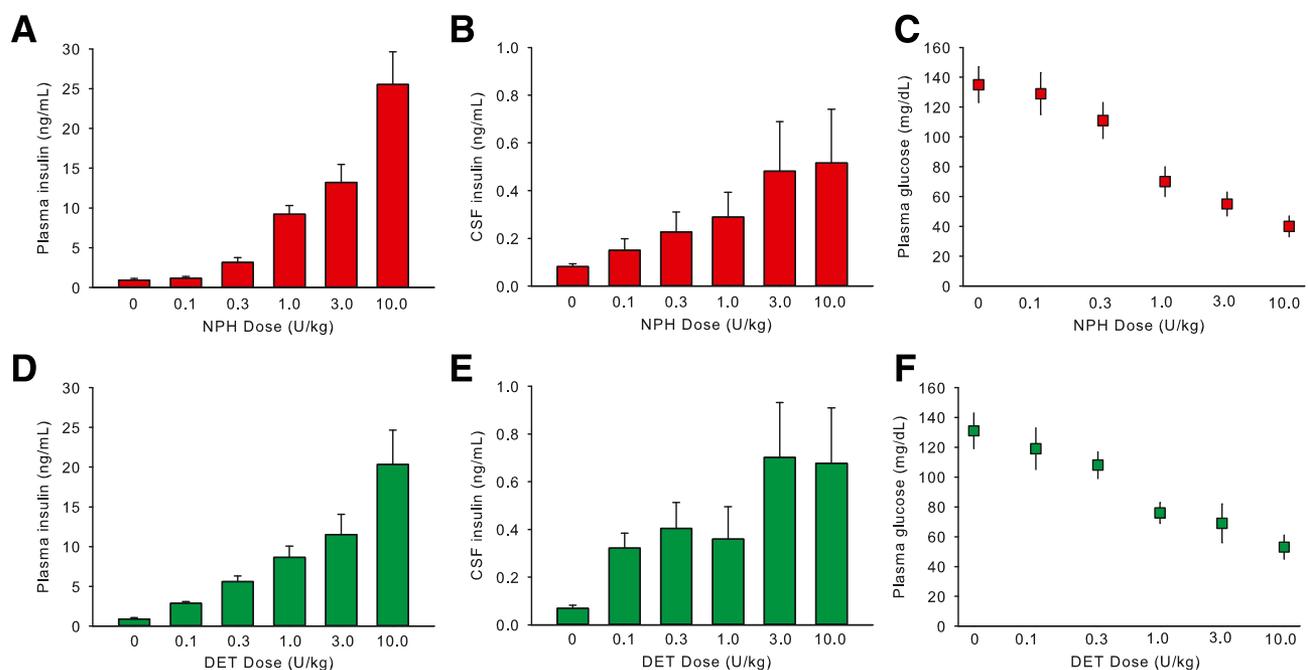


Figure 1—Mean \pm SEM concentration of insulin in plasma and CSF and glucose in plasma 30 min after the intraperitoneal administration of DET or NPH insulin at different doses in rats. Intraperitoneal NPH insulin and DET produced similar levels as a function of dose in plasma (A and D). Likewise, there were dose-dependent increases of CSF insulin with both NPH insulin and DET, with apparent saturation of the CSF insulin levels occurring by the 3 units/kg dose (B and E). Plasma glucose decreased in a dose-dependent manner after intraperitoneal NPH insulin or DET (C and F). Significant differences are described in the text.

Statistical Analyses

Plasma insulin, glucose, and CSF insulin were analyzed using factorial ANOVA. Food intake, body weight, and NMR data were analyzed using repeated-measures ANOVA. Post hoc Tukey tests were performed after observations of significant interaction effects (Statistica 7.1; Statsoft). Significance was accepted at $P < 0.05$, with data reported as means \pm SEM.

RESULTS

Appearance of DET and NPH Insulin in Plasma and CSF After Intraperitoneal Administration

Intraperitoneal NPH insulin produced a dose-dependent increase in plasma insulin after 30 min, with a significant increase first occurring at 0.3 units/kg ($P < 0.05$) (Fig. 1A). Likewise, there was a dose-dependent increase of CSF insulin 30 min after the injections of NPH insulin, with apparent saturation of the CSF insulin levels occurring by the 3 unit/kg dose (Fig. 1B). There was a dose-dependent decrease in plasma glucose reflecting the increased plasma insulin (Fig. 1C).

Plasma insulin was increased at all doses of DET relative to the 0 units/kg group ($P < 0.05$) (Fig. 1D). The magnitude was dose dependent, with the 10 units/kg DET group having significantly elevated plasma insulin relative to all other groups ($P < 0.05$). CSF insulin was

also elevated after all doses relative to the saline group in a dose-dependent manner ($P < 0.05$). Apparent saturation of CSF insulin occurred by the 3 units/kg dose, with no difference between 3 and 10 units/kg (Fig. 1E). There was a dose-dependent decrease in plasma glucose after DET similar to that observed after NPH insulin (Fig. 1F). No significant interaction between NPH and DET treatment was observed; i.e., the two insulin formulations resulted in comparable plasma and CSF insulin profiles.

Appearance of DET and NPH Insulin in Plasma and CSF at Different Time Points After Intraperitoneal Administration

Injection of 0.5 units/kg i.p. NPH insulin produced an immediate spike in plasma insulin that peaked by the 5-min time point ($P < 0.05$) and then decreased. By 160 min, plasma levels were no longer elevated relative to baseline and remained at this level at 320 min (Fig. 2A). CSF insulin levels also rose rapidly after intraperitoneal NPH insulin, with the increase apparent at 5 min ($P < 0.05$). Peak CSF insulin occurred at approximately the 40-min time point, and the level was subsequently significantly reduced from the peak at 80 and 160 min and reached baseline at 320 min (Fig. 2B). Plasma glucose levels closely tracked plasma insulin levels, with an immediate reduction at 5 min and a return to baseline by 80 min (Fig. 2C) ($P < 0.05$).

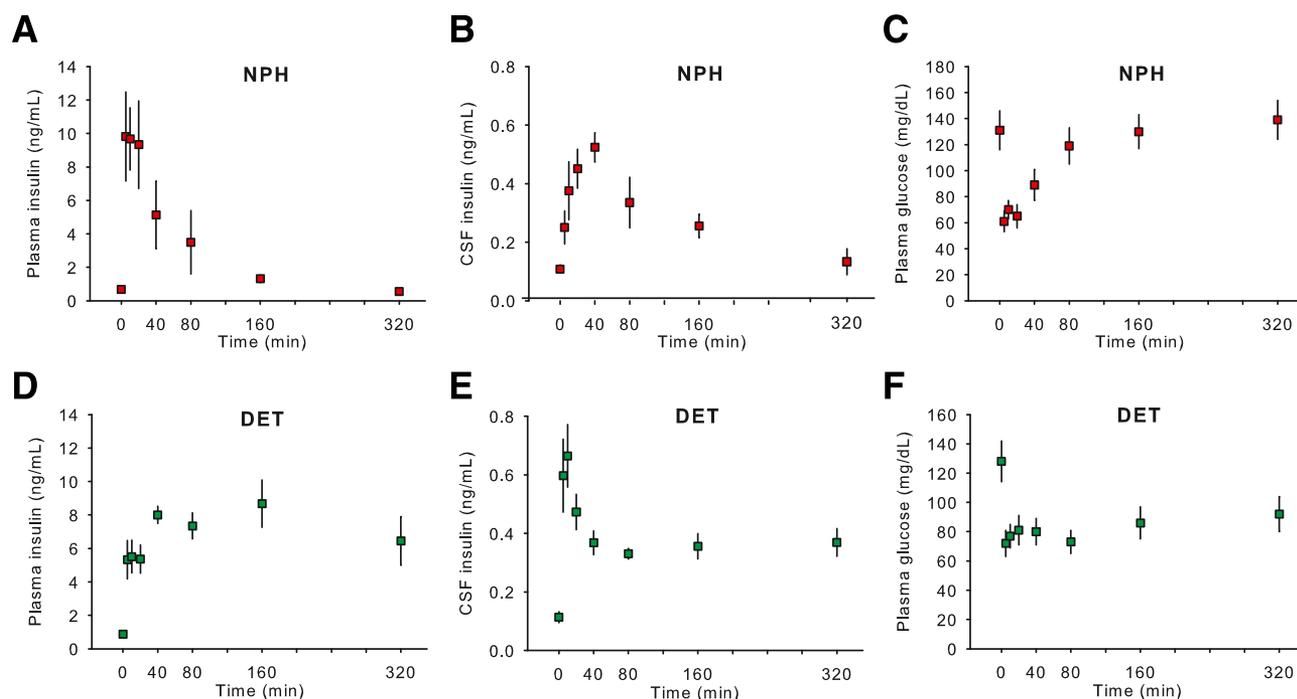


Figure 2—Mean \pm SEM concentration of insulin in plasma and CSF and glucose in plasma at different time points after 0.5 units/kg i.p. DET or NPH insulin in rats. Plasma insulin rose rapidly after the intraperitoneal injection of NPH insulin, peaking at 5 min and then decreasing (A). CSF insulin levels also rose rapidly after intraperitoneal NPH insulin, the peak occurring at around 40 min and levels returning to baseline by 320 min (B). Plasma glucose dropped sharply after intraperitoneal NPH insulin, returning to baseline by 80 min (C). Intraperitoneal DET also increased plasma insulin after 5 min, and it remained elevated at a relatively constant level throughout the 320 min (D). CSF insulin peaked rapidly after intraperitoneal DET at the 5- and 10-min time points before reducing to a stable but elevated level between 40 and 320 min (E). Plasma glucose was reduced after DET to a constant level for the duration of the experiment (F). Significant differences are described in the text.

Injection of 0.5 units/kg i.p. DET also resulted in increased plasma insulin after 5 min relative to baseline ($P < 0.05$), and levels remained similar at 10 and 20 min. Unlike what occurred after NPH insulin, plasma insulin levels remained elevated 320 min after DET treatment (Fig. 2D) ($P < 0.05$). There was a rapid peak in CSF insulin levels apparent at the 5- and 10-min time points ($P < 0.05$). CSF insulin became stable by the 40-min time point and remained at that level through to the 320-min time point, with all points remaining elevated relative to baseline ($P < 0.05$) (Fig. 2E). Plasma glucose levels were reduced by DET at a relatively constant level for the duration of the experiment (Fig. 2F) ($P < 0.05$).

Planned comparisons between NPH and DET treatment revealed a more rapid and higher increase of insulin in plasma in NPH relative to DET-treated animals at 5, 10, and 20 min ($P_s < 0.05$) and a more prolonged elevation of insulin in both plasma (160 and 320 min, $P_s < 0.05$) and CSF (320 min, $P < 0.05$) after DET. DET also produced a sustained hypoglycemia relative to NPH (80, 160, and 320 min, $P_s < 0.05$) (Fig. 2).

i3vT DET and NPH Insulin Reduce Food Intake and Body Weight

After i3vT injection of either DET or NPH insulin, rats ate significantly less food over the subsequent 24 h than when saline was administered ($P < 0.05$). There was no difference in the 24-h intake between DET or NPH insulin treatment (Fig. 3A). After 48 h, intake by the DET-treated

animals remained significantly reduced ($P < 0.05$), whereas it had returned to saline-control levels in the NPH insulin group (Fig. 3B). Body weight loss was consistent with the food intake data, with both NPH insulin and DET producing significant weight loss after 24 h ($P < 0.05$) and with only DET rats maintaining this weight loss after 48 h ($P < 0.05$) (Fig. 3C and D).

Comparison of the Appearance of Insulin in Plasma and CSF After Administration of Different Long-Acting Formulations of Insulin, DET, and GLAR

Injection of either DET or GLAR (0.5 units/kg s.c.) led to an increase in plasma insulin. At the first measured time point after baseline (6 h), plasma insulin was elevated more in DET- than in GLAR-treated animals (Fig. 4A) ($P < 0.05$), and by 12 h this effect was reversed ($P < 0.05$), with GLAR-treated animals having higher plasma insulin. By 24 h through to 72 h, plasma insulin had returned to baseline levels in DET-treated animals. Plasma insulin of GLAR-treated animals remained slightly elevated at 24 h ($P < 0.05$) but returned to baseline by 48 h. CSF levels of insulin were comparably increased in both groups at 6, 12, and 24 h (Fig. 4B) ($P_s < 0.05$), but at 48 h DET-treated animals continued to have elevated CSF insulin ($P < 0.05$), whereas levels in GLAR-treated animals had returned to baseline. Plasma glucose levels were reduced at both 6 h and 12 h by both DET and GLAR (Fig. 4C) ($P_s < 0.05$) but, consistent with plasma insulin, remained lower at 24 h only in GLAR-treated animals.

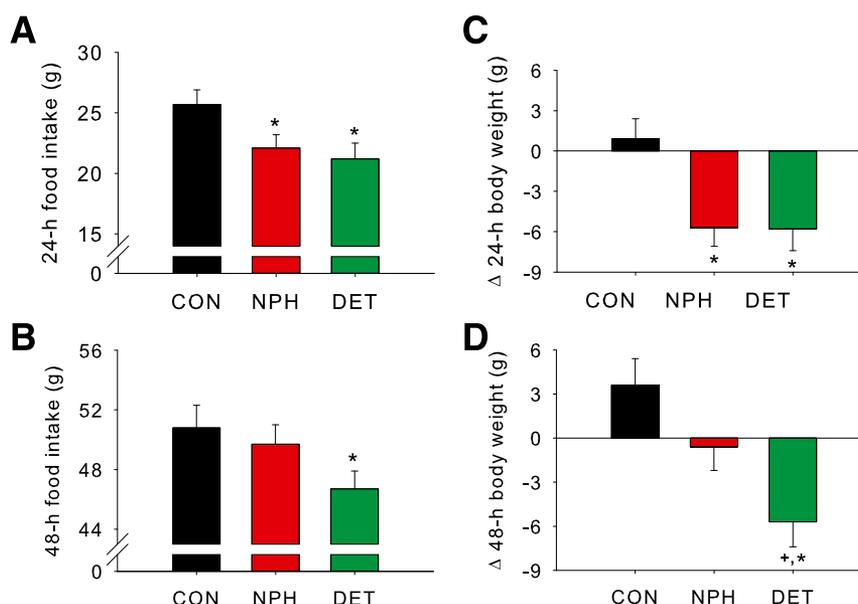


Figure 3—i3vT DET and NPH insulin reduce food intake and body weight in rats. i3vT DET and NPH insulin each reduced food intake over a 24-h period (A) relative to control (CON). After 48 h, intake by the DET group remained reduced, whereas that of the NPH group had returned to CON levels (B). Both NPH insulin and DET rats lost weight in the 24-h postinfusion (C); however, only the DET group maintained the weight loss after 48 h (D). Data are means \pm SEM. * $P < 0.05$ vs. control, + $P < 0.05$ vs. NPH insulin.

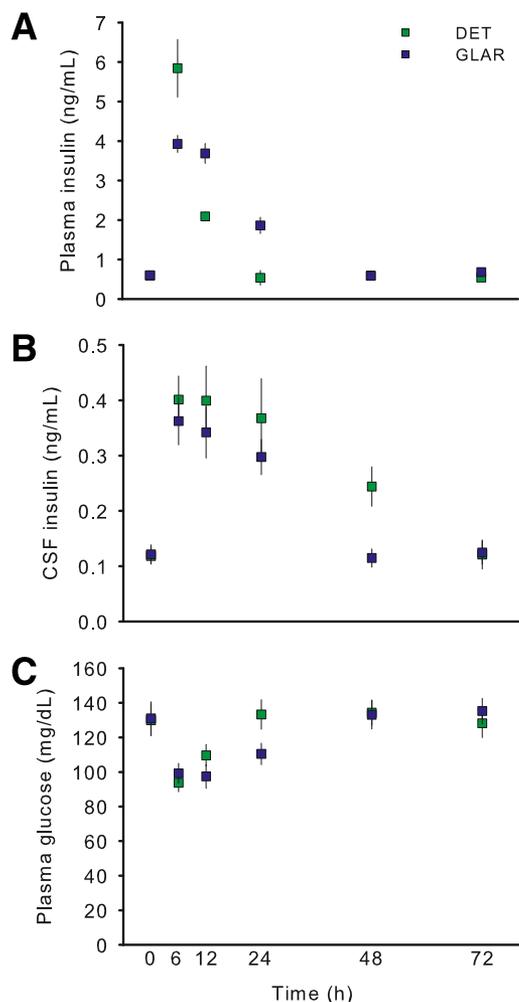


Figure 4—Comparison of the appearance of insulin in the plasma and CSF after the subcutaneous administration of two long-acting formulations of insulin, DET, and GLAR in rats. Injection of either DET or GLAR at 0.5 units/kg s.c. led to increased plasma insulin at 6–12 h. By 24 h, only GLAR-treated rats continued to have elevated insulin (A). In contrast, CSF insulin levels were similarly increased in both groups at 6, 12, and 24 h; but at 48 h, DET-treated animals had elevated CSF insulin levels, while CSF insulin of GLAR-treated animals had returned to baseline (B). Plasma glucose levels were reduced at both 6 and 12 h by both DET and GLAR but were lower at 24 h only in GLAR-treated animals (C). Data are means \pm SEM.

Effect of Chronic Subcutaneous Administration of Long-Acting Formulations of Insulin on Body Weight and Body Composition in Mice

Daily subcutaneous injection of DET reduced weight gain compared with both saline and GLAR treatment after 3 weeks ($P < 0.05$), and this difference was maintained for the remainder of the 6-week treatment period ($P < 0.05$) (Fig. 5A). Body weight of the GLAR group was actually increased relative to that of saline-treated animals by week 6 of treatment ($P < 0.05$). Cumulative food intake was significantly lower in the DET group than in either the GLAR or saline groups ($P < 0.05$) (Fig. 5B). Similar to

the effects observed on body weight, body fat was significantly lower in DET animals and higher in GLAR animals compared with the saline-control group ($P < 0.05$) (Fig. 5C). Lean mass did not differ among groups (Fig. 5D).

Appearance of Insulin in Plasma and CSF of Mice After Administration of 1 Unit/kg s.c. DET and GLAR

In mice, subcutaneous DET or GLAR led to an increase in plasma insulin similar to what occurred in rats. At the first measured time point after baseline (6 h), plasma insulin was elevated to a greater extent in DET- than in GLAR-treated mice (Fig. 6A) ($P < 0.05$), and by 12 h this effect was reversed ($P < 0.05$), with GLAR-treated animals having higher plasma concentrations. At 24 and 48 h, plasma insulin had returned to baseline in DET-treated animals, whereas plasma insulin of GLAR-treated animals remained elevated at 24 h ($P < 0.05$) and returned to baseline by 48 h. CSF insulin was comparably increased in both groups at 6 and 12 h ($P_s < 0.05$). After 24 and 48 h, DET-treated mice had elevated CSF insulin levels ($P < 0.05$), whereas levels in GLAR-treated animals were lower at 24 h and had returned to baseline by 48 h (Fig. 6B). After 6 weeks of treatment with DET or GLAR, with sampling occurring 24 h after the final injection, animals treated with GLAR but not DET continued to have elevated plasma insulin relative to controls (Fig. 6C) ($P < 0.05$). However, the reverse was observed in the CSF, where DET-treated, but not GLAR-treated, mice had increased insulin (Fig. 6D) ($P < 0.05$).

DISCUSSION

Long-acting insulin formulations are an important part of a treatment regimen for diabetic patients because of the importance of basal insulin to control fasting and intermeal glycemia (12,28). A common clinical side effect of treatment with insulin is weight gain (29). Based on recent data from clinical trials, this side effect seems to be reduced with DET (12–14), which is less anabolic than other long-acting insulins (15,16). Importantly, these effects appear to be independent of glycemic control (16). We hypothesized that the weight reduction observed with DET treatment is due to increased presence or activity of insulin in the CNS, where it reduces food intake and has a net catabolic effect. In the experiments reported here, insulin was detected in the CSF after presumably passing through the brain neuropil. Overall, the CSF appearance of insulin after DET and NPH was comparable, and this was true in rats and mice. The appearance of insulin in CSF was dose dependent and saturable at higher doses—pharmacologic characteristics previously reported for regular insulin (30). Based on these findings, we propose that insulin appearance in the CSF after DET administration peripherally is the result of active transport from the blood, similar to what occurs for other insulin formulations. The observation that the appearance of insulin in the CSF after NPH and DET was comparable implies that the altered physicochemical properties of DET, such as a higher binding to albumin in the plasma and the presence

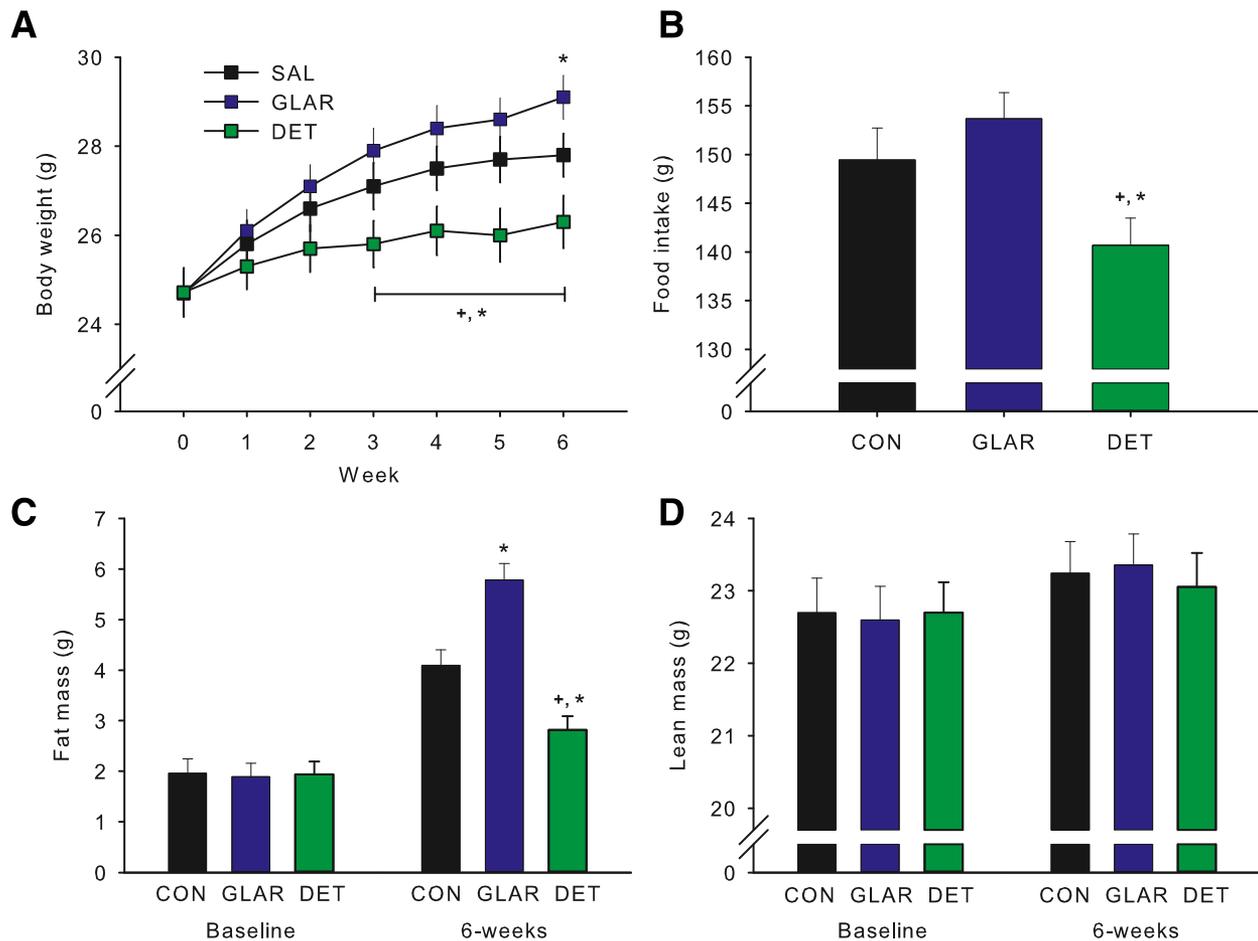


Figure 5—Chronic subcutaneous administration of DET reduces food intake and body weight relative to subcutaneous GLAR or vehicle in mice. DET reduced weight gain compared with both saline control (CON) and GLAR treatment by week 3. GLAR resulted in increased body weight relative to control at week 6 (A). Total food intake was reduced in the DET group compared with that in both GLAR and control (B). Fat mass was lower in DET and higher in GLAR compared with control (C). Lean mass was not altered by any treatment (D). Data are means \pm SEM. * $P < 0.05$ vs. control, + $P < 0.05$ vs. GLAR.

of an attached fatty acid, do not interfere with its transport into the brain; the data are consistent, however, with IR-mediated transport. Interestingly, CSF insulin levels were elevated longer in DET than GLAR-treated animals, despite a shorter plasma half-life.

Previous reports on DET and the BBB have been contradictory, with one report suggesting that DET is unable to cross the BBB (22) and another reporting that transport is not inhibited (21). Banks et al. (22) reported that DET does not enter the brain from the blood in mice. However, rather than measuring insulin directly, their group administered labeled DET systemically and measured radioactive counts in trichloroacetic acid-precipitable protein in brain extracts, where they found none. While we have no explanation for the difference between our results and those of Banks, it is important to note that our assay measures immunoreactive insulin (as opposed to a radioactive label), and we sampled CSF insulin, which comes from brain interstitial fluid (8). Further, our results are consistent with those of Hennige et al. (21), who

found preferential uptake of DET into the brain of mice as assessed by disproportionately elevated insulin in brain tissue extracts and selectively increased phosphorylation of the insulin receptor in mouse hypothalamus. Likewise, overweight humans administered DET intravenously had increased cerebrocortical activity, whereas those administered regular insulin did not (31), and Hallschmid et al. (32) reported that, compared with regular insulin and with plasma glucose comparably clamped, DET elicited a significant change in electroencephalogram activity in the frontal cortex of men and also caused a significant reduction of food intake. Endogenous insulin is believed to cross the BBB via a saturable, IR-mediated process in capillary endothelial cells, and it is probable that DET is transported in the same manner. Thus, our data are consistent with DET being actively transported into the brain.

After peripheral administration in our rats, insulin in the CSF peaked sooner after intraperitoneal DET and remained higher longer than treatment with NPH insulin.

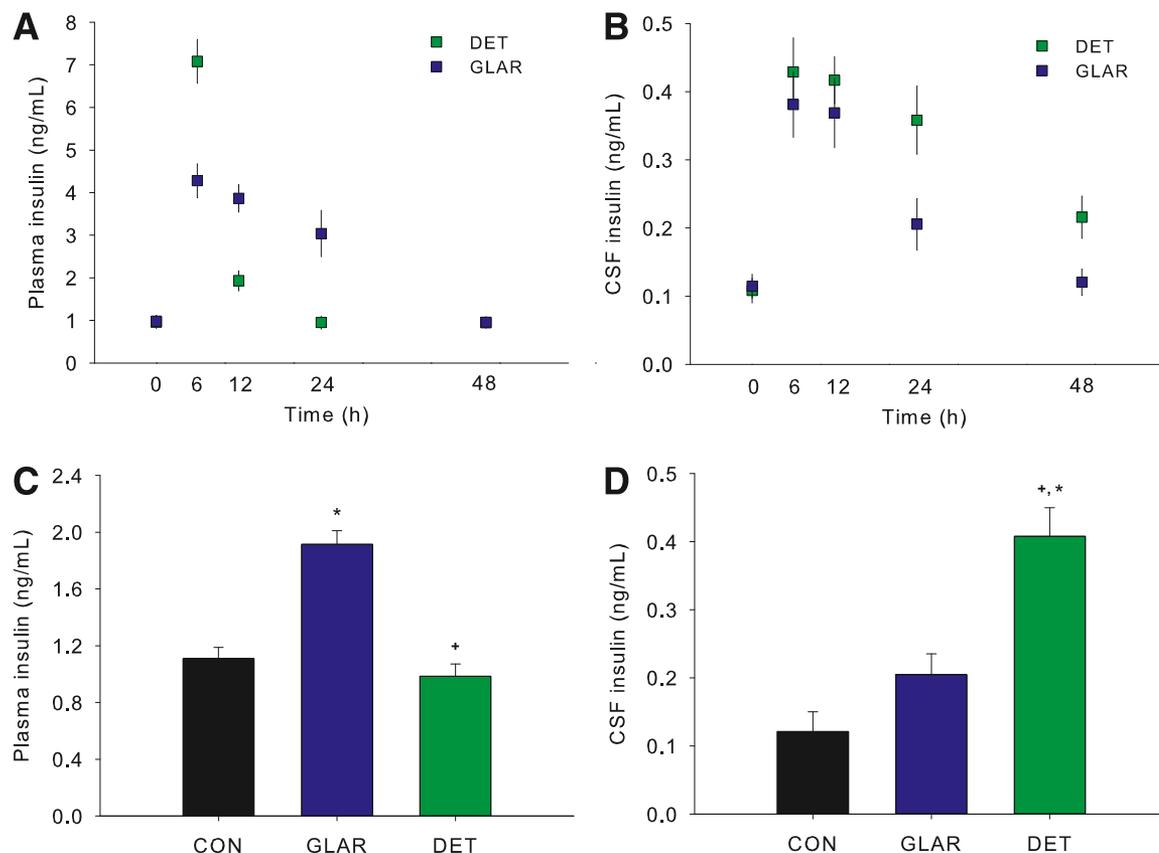


Figure 6—Appearance of insulin in plasma and CSF at different times after the administration of subcutaneous DET and GLAR in mice and the effect of chronic DET and GLAR treatment on CSF insulin. Injection of either DET or GLAR (1 unit/kg s.c.) led to increased plasma insulin at 6–12 h. By 24 h, only GLAR-treated animals continued to have elevated plasma insulin (A). In contrast, CSF insulin levels were increased in both groups at 6, 12, and 24 h; but at 48 h, DET-treated animals had elevated CSF insulin levels, whereas CSF insulin of GLAR-treated animals had returned to baseline (B). Twenty-four hours after cessation of chronic treatment with DET or GLAR, mice treated with GLAR continued to have elevated plasma insulin (C), but the reverse occurred in CSF, where DET-treated, but not GLAR-treated, mice had elevated insulin. Data are means \pm SEM. * $P < 0.05$ vs. control, + $P < 0.05$ vs. GLAR. CON, control.

In fact, insulin remained significantly elevated in the CSF of DET-treated animals for the duration of the study. The prolonged increase of CSF insulin after DET is likely due to its long peripheral half-life (10) and consequent continued transport into brain after its systemic administration, possibly coupled with slower CNS breakdown (21). The important point is that DET is transported into the CNS and has an earlier peak and prolonged elevated concentration when assessed in CSF, and these phenomena may be responsible for the reduced food intake and weight loss that can occur with DET treatment clinically.

Because DET is used clinically to augment basal insulin, we compared it with another insulin formulation, GLAR, since it is also used for the same purpose but lacks the attached fatty acid side chain of DET. Further, whereas chronic treatment of diabetic patients with GLAR leads to weight gain, treatment with DET at doses that cause the same degree of glucose lowering minimizes weight gain (15,16,33). Similar to previous work in rats (34), the present results demonstrate that whereas 6-week treatment with GLAR caused weight gain relative to controls, treatment

with DET caused weight loss in mice. When GLAR and DET were administered more acutely, GLAR had an apparent longer half-life in plasma but shorter half-life in CSF in both rats and mice. The prolonged half-life in the brain likely contributes to the weight-lowering action of chronic DET.

Insulin in the CNS promotes a catabolic state (18,19,35), and administration of insulin directly into the CNS reduces food intake and body weight across diverse species from rodents to humans (36–38). Because we have found that male rats are more sensitive to insulin's hypophagic action than females (20,39) and because men are analogously more sensitive to the ability of intranasal insulin to reduce body fat than are women (36,40), we utilized male rats in the present experiments. It will be important in future experiments to determine whether there is a sex difference in the transport of insulin into the brain. The current experiment is the first to demonstrate that centrally administered DET is as effective as NPH insulin at acutely reducing food intake. In fact, the effects of a single 13VT bolus of DET on food

intake are longer lasting than occur with regular insulin. Return of food intake to normal 48 h after NPH insulin is consistent with previous reports demonstrating rapid return of food intake after cessation of regular insulin infusion (18), and the longer duration of action of DET when it is administered directly into the CNS is presumably the result of a longer functional half-life in the brain.

One limitation of the current study is that we assessed central insulin sensitivity by administering pharmacological doses of insulin into the CSF. These large doses are typically used in such experiments because in order to reach insulin-sensitive sites in the mediobasal hypothalamus, the administered insulin must move against the bulk flow of interstitial fluid out of the brain parenchyma. Baskin et al. (41) found that similar doses of insulin administered directly into the CSF penetrate into the hypothalamic parenchyma sufficiently far to reach the arcuate nucleus, where insulin's catabolic action is thought to occur (42).

Overall, the current studies demonstrate that DET crosses the BBB and reduces food intake. This provides support for the hypothesis that DET improves weight management by an enhanced and prolonged centrally mediated reduction of energy intake. Insulin receptors are found in many areas of the brain in addition to the hypothalamus, including the hippocampus and several limbic areas (42), and because insulin entering the brain via the blood-brain barrier presumably accesses all brain insulin receptors as opposed to mainly those in the hypothalamus as occurs after its i3VT administration, the catabolic action of insulin from the brain likely results from both homeostatic and nonhomeostatic circuits (43–45). Greater understanding of insulin penetration into the brain will allow studies to incorporate this process as a novel strategy for the prevention and treatment of obesity and type 2 diabetes (46–48).

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Author Contributions. D.P.B., D.A.D., R.J.S., and S.C.W. designed the experiments. D.P.B., A.A.M., J.D.M., and M.L. collected and analyzed data. D.P.B., D.A.D., and S.C.W. wrote the manuscript. A.A.M., J.D.M., M.L., and R.J.S. reviewed and edited the manuscript. D.P.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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